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Graphical Abstract

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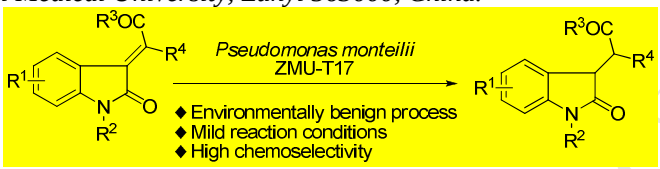

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Bioreduction of the C=C double bond with *Pseudomonas monteilii* ZMU-T17: One approach to 3-monosubstituted oxindoles

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ABSTRACT

An efficient whole cell-mediated bioreduction of 3-methylene-2-oxindoles has been developed, affording a range of 3-monosubstituted oxindoles in moderate to good yields (41–82%) with *Pseudomonas monteilii* ZMU-T17 as biocatalyst. Additionally, A possible reaction pathway for this bioreduction of C=C double bond was proposed.

1. Introduction

The oxindole core is represented in a wide variety of natural products and related compounds of biological and medicinal importance.[1,2] In recent years, 3,3-disubstituted oxindoles and spirocyclic oxindoles have emerged as attractive synthetic target owing to their potential applications in medicinal chemistry.[3] To the best of our knowledge, 3-monosubstituted oxindole has been served as one of the easily accessible precursors to 3,3-disubstituted oxindoles and spirocyclic oxindoles via all kinds of approaches.[4] Thus, numerous different methodologies for the synthesis of 3-monosubstituted oxindole scaffolds have been explored due to the potential applications in organic synthesis.[5,6] Notablely, the reduction of the C=C double bond of 3-methylene-2-oxindoles was a straightforward approach to 3-monosubstituted oxindoles.[6] For example, Shi reported the chemoselective reduction of isatin-derived electron-deficient alkenes using alkylphosphanes as reduction reagents [Scheme 1, (a)].[6b] In this process, water was the proton source and the corresponding reduction products were obtained in good to excellent yields (16–99% yield). However, most of these strategies accessing to 3-monosubstituted oxindoles were proceeded through chemical approaches. Despite with easy operating processes, chemical approachs need some toxic reagents and produce pollutions to environment. Accordingly, development of more “environment-friendly” methods under mild reaction conditions to 3-monosubstituted oxindoles for both laboratory and industrial applications is still desirable and valuable.

Biocatalytic processes have been intensely studied and have become a useful and ecologically sustainable manufacturing techniques in organic synthesis due to their multiple advantages.[7,8] Among them, bioreduction of C=C double bonds utilizing either whole-cell suspensions or isolated enzymes has been broadly applied in the manufacture of natural products, pharmaceuticals, fine chemicals etc.[9] In comparison with whole cell-mediated biotransformations that are often accompanied by some competing side reactions, isolated enzymes, such as the OYE (old yellow enzyme) family of oxidoreductases (EC1.6.99.1), need expensive coenzymes to carried out the reduction of C=C double bonds.[10,11] Therefore, it is valuable to excavate a microorganism with high chemoselectivity in the reduction of C=C double bonds.

In this context, as a continuation of our studies on the biocatalysis,[12] we found that 3-ylideneoxindoles could be chosen as a suitable substrate for the *Pseudomonas monteilii* ZMU-T17 mediated bioreduction of C=C double bonds, and the 3-ylideneoxindoles could be fully consumed as well as the corresponding 3-monosubstituted oxindoles were obtained in good isolated yields [Scheme 1, (b)]. Importantly, in this process, there was no side reaction, such as hydrolysis of an ester. Herein, we wish to report the results of our endeavors on this subject.

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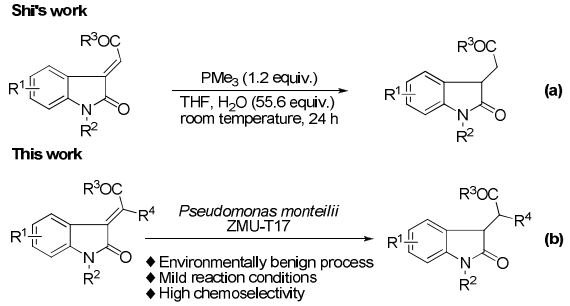
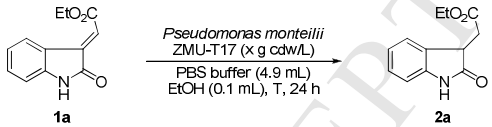


Scheme 1. Synthesis of 3-monosubstituted oxindoles via reduction processes.

2. Results and discussion

Initially, the bioreduction of (*E*)-ethyl 2-(2-oxoindolin-3-ylidene)acetate (**1a**) was tried by using *P. monteilii* ZMU-T17 (30 g cdw/L) prepared via cell growth and cultivation for the screening of reaction temperature. As shown in Table 1, the reactivities significantly depend on reaction temperature (entries 1–4), and 30 °C was the best choice for this transformation, in which **2a** was obtained in 74% yield (entry 2) (the X-ray structure of **2a** was shown in Figure 1).[13] Then, the cell density was examined, and either decreasing or increasing the density deteriorated the reaction efficiency to some extent (entries 5–8). However, there was no increasing in yield with increased cell density for the increased substrate concentration (entry 9). Furthermore, different pH values were also surveyed (Table 1, entries 10–13), it revealed that alkaline environment was favourable for the reaction, and the desired product **2a** was obtained in 81% yield at pH 9.0. (Table 1, entry 13). Surprisingly, reducing the substrate concentration from 6.0 to 2.0 mM led to **2a** in an improved yield (99%) (Table 1, entry 14). However, there was no improvement in the reaction performance by raising the concentration of the reaction (Table 1, entries 16–17). Afterwards, a survey of co-solvents indicated that MeOH, ^{i}PrOH, THF, acetone, MeCN, PhMe, DMF, and DMSO were inferior to EtOH for the bioreduction (Figure 2).

Table 1
Optimization of Reaction Conditions[a]



Entry	T (°C)	Cell density (g cdw/L)	pH	Subs. conc. (mM)	Yield (%)[b]
1	20	30	7	6	65
2	30	30	7	6	74
3	35	30	7	6	70
4	40	30	7	6	16
5	30	10	7	6	14
6	30	20	7	6	49
7	30	40	7	6	73
8	30	50	7	6	68
9	30	50	7	8	65
10	30	30	5	6	18
11	30	30	6	6	27
12	30	30	8	6	78
13	30	30	9	6	81
14[c]	30	30	9	2	99(66)[d]
15	30	30	9	4	80
16	30	30	9	8	73
17	30	30	9	10	36

[a] Unless otherwise noted, all reactions (5.0 mL) were performed in different pH values (50.0 mM Na_2HPO_4/KH_2PO_4) containing **1a**, *P. monteilii* ZMU-T17 (x g cdw/L) and EtOH (0.1 mL) at stated temperature and 250 rpm for 24 h.
[b] Determined by HPLC analysis of the crude reaction mixture using *p*-xylene as internal standard.
[c] 30 Parallel experiments were proceed for collecting products.
[d] Isolated yield in the parentheses.

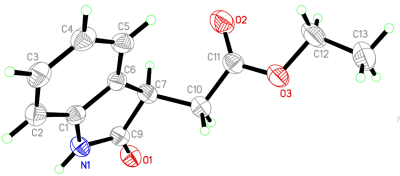


Fig. 1. The X-ray structure of compound **2a**.

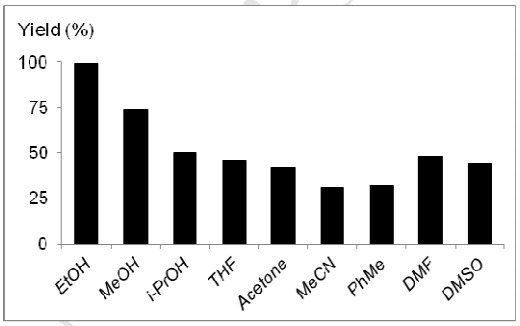
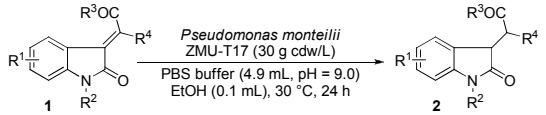


Figure 2. The effects of co-solvents (2%, v/v) on yield. The yield was determined by HPLC analysis of the crude reaction mixture using *p*-xylene as internal standard.

With the optimized conditions in hand, (Table 1, entry 12), the generality of this whole cell-mediated reactions was subsequently investigated. As illustrated in Table 2, all substrates could be completely converted (>99% conv.) and the corresponding 3-monosubstituted oxindoles were obtained in moderate to good yields (41-82%). For the substrate 3-ylideneoxindoles bearing electron-rich substitutes at the C5 position, the corresponding reductive products **2b** and **2c** were obtained in good yields (52–62%) (Table 2, entries 2 and 3), whereas the electron-deficient groups led to a slightly lower yields (43–55%) (Table 2, entries 4–6). Therefore, the electronic nature of R at the C5 position have a dramatic effect on the yield. Whether substituent group is an electron–donating or –withdrawing at the C7 position, the reactions proceeded smoothly and gave the corresponding products **2g**–**2j** in good yields (52–82%) (Table 2, entries 7–10). Compound **1k** and **1l** were also good substrates for this reaction, the desired products **2k** and **2l** were obtained in 64 and 60% yields, respectively (Table 2, entries 11 and 12). On the other hand, upon protecting the *N*1 with a methyl-group or acetyl-group, the corresponding bioreduction also provided the expected products with 62% yield for **2m** and 41% yield for **2n** (entries 13 and 14). These data suggest that the substituent groups incorporated in *N*1 of 3-ylideneoxindoles have a dramatic effect on the yield. To our delight, the method was compatible with 3-ylideneoxindoles with sterically bulky *n*-propyl ester or *t*-butyl ester group, affording the corresponding product **2o** and **2p** in 78% and 65% yields, respectively (entries 15 and 16). Unfortunately, when we further expanded the substrate scope to 3-(2-oxo-2-phenylethylidene)indolin-2-one (**1q**), the corresponding products were not obtained (Table 2, entry 17).

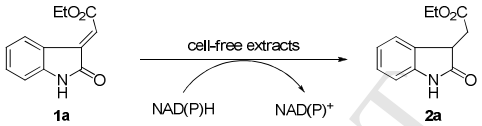
Table 2
Substrate scope[a]



Entry	Substrate	Product	Conv. (%)[b]	Yield(%)[c]
1	**1a**, R = H	**2a**	>99	66
2	**1b**, R = Me	**2b**	>99	52
3	**1c**, R = OMe	**2c**	>99	62
4	**1d**, R = F	**2d**	>99	54
5	**1e**, R = Cl	**2e**	>99	45
6	**1f**, R = OCF_3	**2f**	>99	43
7	**1g**, R = OMe	**2g**	>99	67
8	**1h**, R = F	**2h**	>99	82
9	**1i**, R = Cl	**2i**	>99	64
10	**1j**, R = CF_3	**2j**	>99	52
11	**1k**	**2k**	>99	64
12	**1l**	**2l**	>99	60
13	**1m**, R = Me	**2m**	>99	62
14	**1n**, R = Ac	**2n**	>99	41
15	**1o**, R = OMe	**2o**	>99	61
16	**1p**, R = O^{n}Pr	**2p**	>99	78
17	**1q**, R = O^{t}Bu	**2q**	>99	65
18	**1r**, R = Ph	**2r**, NR[d]	–	–
19	**1s**, R = CO_2Et	**2s** (57:43 dr)[e]	>99	57
20	**1t**, R = CN	**2t**, NR[d]	–	–

[a] The reactions (5.0 mL) were performed in buffer (50.0 mM Na_2HPO_4/KH_2PO_4, pH = 9.0) containing **1** (1.0×10^{-2} mmol, 2.0 mM), *P. monteilii* ZMU-T17 (30 g cdw/L) and EtOH (0.1 mL) at 30 °C and 250 rpm for 24 h, 30 parallel experiments were proceed for collecting products.
[b] Conversion (Conv.) was determined by HPLC analysis of the crude reaction mixture using *p*-xylene as internal standard.
[c] Isolated yields.
[d] NR = No reaction.
[e] The diastereoselectivity was determined by the ^{1}H NMR of compound **2s**.

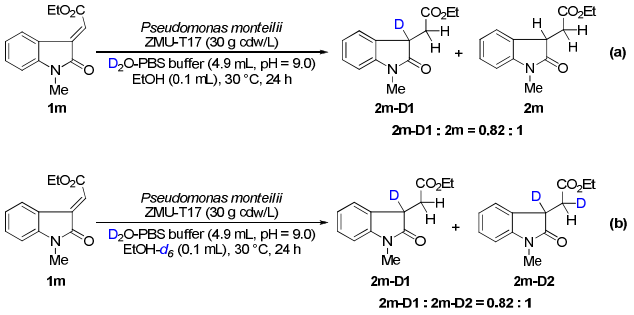
Table 3
Bioreduction of (*E*)-ethyl 2-(2-oxoindolin-3-ylidene)acetate with soluble cell-free extracts of *Pseudomonas monteilii* ZMU-T17



Entry	Coenzyme	Coenzyme conc. (mM)	Yield (%)[b]
1[c]	–	–	19
2	NADPH	2	44
3	NADH	2	94

[a] Unless otherwise noted, all reactions (5.0 mL) were performed in buffer (50.0 mM Na_2HPO_4/KH_2PO_4, pH = 9.0) containing **1a** (5.0×10^{-3} mmol, 1.0 mM), cell density (60 g cdw/L), coenzyme (2.0 mM), and EtOH (0.1 mL) at 30 °C and 250 rpm for 24 h.
[b] Determined by HPLC analysis of the crude reaction mixture using *p*-xylene as internal standard.
[c] The reaction was carried out in the absence of coenzyme.

To explore some information on the enzyme in *Pseudomonas monteilii* ZMU-T17 that is responsible for the bioreduction of the C=C double bond, bioreduction with cell-free extracts of strain ZMU-T17 was studied. As shown in Table 3, bioreduction of **1a** and the NADH with the soluble cell-free extracts gave the best result (94% yield). In comparison, bioreduction of **1a** with the soluble cell-free extracts without coenzymes gave only 19% yield. These results indicated that *Pseudomonas monteilii* ZMU-T17 may contain a soluble NADPH- or NADH-dependent alcohol dehydrogenases for the reduction of the C=C double bond. Obviously, NADH is the more preferred cofactor than NADPH.



Scheme 2. Deuterium labeling experiments.

In order to understand the hydride source for this bioreduction, two isotopic labeling experiments were carried out as shown in Scheme 2. Performing the reaction in D_2O-PBS buffer (pH = 9.0) under the standard reaction conditions, **2m-D1** and **2m** were obtained in a 0.82:1 ratio [Scheme 2, eq (a)][14]. The reaction was carried out in D_2O-PBS buffer using EtOH-d_6 as the co-solvent, delivering **2m-D1** and **2m-D2** in a 0.82:1 ratio [Scheme 2, eq (b)][14]. Accordingly, it is possible that ethanol is the proton source for this reduction.

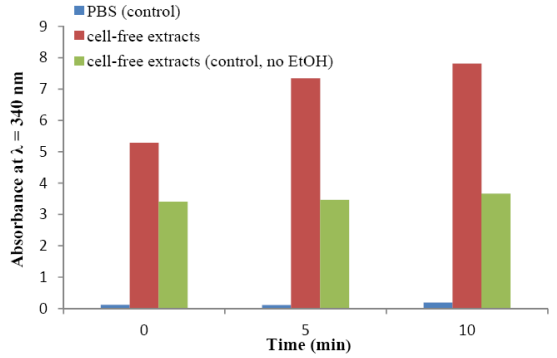
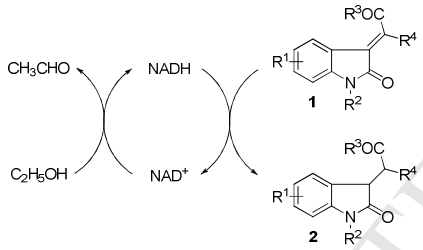


Figure 3. The absorbance of NADH at 340 nm.

Furthermore, it's revealed that there were alcohol dehydrogenases in *P. monteilii* ZMU-T17. Analysis of the alcohol dehydrogenase activity of *P. monteilii* ZMU-T17 were carried out as following: 5 mL cell-free extracts, 2 mM NAD^+ and 100 μL alcohol were added into a 10 mL flask. In one control experiment, 5 mL PBS, 2 mM NAD^+ and 100 μL ethanol were added into a 10 mL flask. In another control experiment, 5 mL cell-free extracts and 2 mM NAD^+ were added into a 10 mL flask in the absence of alcohol. These three reactions were performed at 30 °C and 150 rpm. At 0, 5 and 10 min, 300 μL samples were taken to dilute 10 times using water and determine the absorbance at 340 nm.[15] As shown in Figure 3, *P. monteilii* ZMU-T17 could convert alcohol generating NADH. However, there were no obviously changes of absorbance at 340 nm in the two control experiments. Based on the above results, it could be speculated that there were alcohol dehydrogenases in *P. monteilii* ZMU-T17 to generate NADH using the alcohol as substrate.



Scheme 3. Proposed catalytic cycle.

Based on the above results, a possible mechanism of bioreduction by *P. monteilii* ZMU-T17 was proposed. As shown in Scheme 3, ethanol joined in the alcohol dehydrogenases-mediated reaction besides being used as cosolvent. In this process, ethanol was converted to acetaldehyde and donated a proton to NAD^+ affording NADH. Afterwards, NADH transfer protons to **1**, leading to products **2** and regenerating NAD^+ into the next reaction cycle.

3. Conclusion

In summary, we have developed an efficient whole cell-mediated bioreduction, providing a series of 3-monosubstituted oxindoles in moderate to good yields (41–82%) with *Pseudomonas monteilii* ZMU-T17 as biocatalyst. Mechanism studies revealed that *P. monteilii* ZMU-T17 contained a soluble NADH-dependent dehydrogenase or reductase for the bioreduction of the C=C double bond. Deuterium labeling experiments indicted that ethanol maybe the hydride source for the reduction of 3-methylene-2-oxindoles. Additionally, there were alcohol dehydrogenases in *P. monteilii* ZMU-T17 to generate NADH using the alcohol as substrate. Further investigations for the asymmetric bioreduction of 3-methylene-2-oxindoles to chiral 3-monosubstituted oxindoles are currently underway.

4. Experimental section

4.1. General

All commercially available reagents were used without further purification. Column chromatography was performed on silica gel (200-400 mesh). 1H NMR (400 MHz) chemical shifts were reported in ppm (δ) relative to tetramethylsilane (TMS) with the solvent resonance employed as the internal standard. Data were reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, td = triplet of doublets, dd = doublet of doublets, ddd = doublet of doublet of doublets, m = multiplet), coupling constants (Hz) and integration. 13C NMR (100 MHz) chemical shifts were reported in ppm (δ) from tetramethylsilane (TMS) with the solvent resonance as the internal standard. Melting points were uncorrected.

4.2. Representative procedure for the synthesis of 3-ylideneoxindoles 1a.[16]

To a stirred solution of ethyl 2-(triphenylphosphoranylidene)acetate (3.83 g, 11.0 mmol) in CH_2Cl_2 (30.0 mL) was added isatin (1.47 g, 10.0 mmol) at 0 °C. After stirring for 8 h at 0 °C, the mixture was concentrated by rotary evaporation. The residue was purified by flash column chromatography on silica gel (petroleum ether/ethyl acetate = 3:1~10:1) to afford the compound **1a** as a red solid (1.78 g, 82%).

4.3. Cell growth and cultivation of *P. monteilii* ZMU-T17.

The cells of *P. monteilii* ZMU-T17 were grown in M9-agar plate and then inoculated to 10.0 mL LB medium (10.0 g tryptone, 5.0 g yeast extract and 5.0 g NaCl in 1.0 L deionized water) in 20 mL glass bottle with screw cap. The culture was shaken at 250 rpm at 30 ºC for 12 h and then added to 50.0 mL M9 liquid medium (17.09 g $NaHPO_4 \cdot 12H_2O$, 3.00 g KH_2PO_4, 0.50 g NaCl, 1.00 g NH_4Cl in 1.0 L deionized water) containing trace elements (1.00 mol/L HCl, 4.87 g/L $FeSO_4 \cdot 7H_2O$, 4.12 g/L $CaCl_2 \cdot 2H_2O$, 1.50 g/L $MnCl_2 \cdot 4H_2O$, 1.05 g/L $ZnSO_4$, 0.30 g/L H_3BO_3, 0.25 g/L $Na_2MoO_4 \cdot 2H_2O$, 0.15 g/L $CuCl_2 \cdot 2H_2O$, 0.84 g/L $Na_2EDTA \cdot 2H_2O$) 50.0 μL and 1.00 mol/L Mg_2SO_4 100.0 μL in a 250 mL shaking flask with plastic stopper to reach initial cell density of 0.1 g dcw/L (dry cell weight/liter). 15.0 mL tube containing 0.5 mL toluene was put in the flask and the vapor of toluene was used as carbon source. The culture was incubated at 250 rpm at 30 ºC. After 22 h, the cells were harvested by centrifugation.

4.4. General procedure for the synthesis of 3-monosubstituted oxindoles 2.

Cell of *P. monteilii* ZMU-T17 was suspended in 4.9 mL of 50.0 mM KH_2PO_4–Na_2HPO_4 buffer solution (pH = 9.0) to a cell density of 30 g dcw/L, **1** (0.01 mmol) was added with 0.1 mL EtOH, and the mixture was shaken at 250 rpm at 30 ºC for 24 h. Then the mixture was extracted with EtOAc (5 × 4 mL), and the combined organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated, followed by the addition of *p*-xylene (0.30 mL of 0.1 mol/L solution in *i*-PrOH) as internal standard. The resulting solution was diluted with *i*-PrOH to give a total volume of 3.0 mL. The crude reaction mixture was taken for the HPLC analysis to determine the conversion of substrate. Simultaneously, other 30 parallel experiments were carried out for collecting products. These 30 parallel reactions were combined and the reaction mixtures were extracted with EtOAc (5 × 120 mL), and the combined organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by flash

chromatography on silica gel (petroleum ether/ethyl acetate = 5:1~10:1) to afford the corresponding 3-monosubstituted oxindoles **2**.

4.5. Deuterium labeling experiments, ESI-MS and NMR study.

(a) Freeze-dried cells of *P. monteilii* ZMU-T17 (0.15 g) were suspended in 4.9 mL of 50.0 mM KH_2PO_4–Na_2HPO_4 buffer solution (D_2O, pH = 9.0) to a cell density of 30 g dcw/L, **1m** (2.3 mg, 0.01 mmol) was added with 0.1 mL EtOH, and the mixture was shaken at 250 rpm at 30 ºC for 24 h. Then the mixture was extracted with EtOAc (5 × 4 mL), and the combined organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated. Afterward, the residue (**2m-D1** + **2m**) was characterized by ^{1}H NMR to determine the position of deuterium atom. Simultaneously, another parallel experiment was carried out for ESI-MS study. After the same procedure, the residue (**2m-D1** + **2m**) was dissolved in 2.0 mL of MeOH, this solution was directly infused into the ESI-HRMS with positive electronspray ionization mode (ESI^+, Interface voltages: 4.5 kV, CDL Temperature: 180 °C, heat block temperature: 180 ºC).

(b) Freeze-dried cells of *P. monteilii* ZMU-T17 (0.15 g) were suspended in 4.9 mL of 50.0 mM KH_2PO_4–Na_2HPO_4 buffer solution (D_2O, pH = 9.0) to a cell density of 30 g dcw/L, **1m** (2.3 mg, 0.01 mmol) was added with 0.1 mL EtOH-d_6, and the mixture was shaken at 250 rpm at 30 ºC for 24 h. Then the mixture was extracted with EtOAc (5 × 4 mL), and the combined organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated. Afterward, the residue (**2m-D1** + **2m-D2**) was characterized by ^{1}H NMR to determine the position of deuterium atom. Simultaneously, another parallel experiment was carried out for ESI-MS study. After the same procedure, the residue (**2m-D1** + **2m-D2**) was dissolved in 2.0 mL of MeOH, this solution was directly infused into the ESI-HRMS with positive electronspray ionization mode (ESI^+, Interface voltages: 4.5 kV, CDL Temperature: 180 °C, heat block temperature: 180 ºC).

4.6. Bioreduction of 3-methylene-2-oxindole 1a with soluble cell-free extracts of *P. monteilii* ZMU-T17

Cells of *P. monteilii* ZMU-T17 were prepared from a 22 h culture as described above. The cells were resuspended in buffer (50 mM Na_2HPO_4/KH_2PO_4, pH = 9.0) to a density of 60 g cdw/L. The cell suspension was disrupted using cell disrupter for 1 h. The cell debris was removed by centrifugation at 10,000 rpm at 4 °C for 10 min and the cell-free extract was collected. Three parallel reactions were performed for 24 h: 4.9 mL cell-free extract, 0.1 mL EtOH and **1a** (1.2 mg, 5.0×10^{-3} mmol, 1.0 mM); 4.9 mL cell-free extract, 0.1 mL EtOH, NADPH (8.33 mg, 0.01 mmol, 2.0 mM) and **1a** (1.2 mg, 5.0×10^{-3} mmol, 1.0 mM); 4.9 mL cell-free extract, 0.1 mL EtOH, NADH (7.09 mg, 0.01 mmol, 2.0 mM) and **1a** (1.2 mg, 5.0×10^{-3} mmol, 1.0 mM) in three different flasks. Samples were taken and analyzed by HPLC, and the results are listed in Table 3.

4.7. Characterization data of compounds 2a−2p.

*4.7.1. Ethyl 2-(2-oxoindolin-3-yl)acetate (**2a**).* Light yellow solid, yield 66%; mp 121.0-122.6 °C (lit.[6b] mp 118-120 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (br s, 1H), 7.24-7.20 (m, 2H), 7.01 (td, *J* = 0.8, 7.6 Hz, 1H), 6.89 (d, *J* = 7.6 Hz, 1H), 4.20-4.08 (m, 2H), 3.81 (dd, *J* = 4.4, 7.6 Hz, 1H), 3.08 (dd, *J* = 3.6, 16.8 Hz, 1H), 2.84 (ddd, *J* = 0.8, 8.0, 16.8 Hz, 1H), 1.20 (tt, *J* = 0.8, 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.2, 171.1, 141.6, 128.9, 128.4, 124.3, 122.6, 109.9, 61.2, 42.4, 34.9, 14.2; HRMS (ESI-TOF) Calcd for $C_{12}H_{13}NNaO_3$ $[M+Na]^+$: 242.0788; found: 242.0784.

*4.7.2. Ethyl 2-(5-methyl-2-oxoindolin-3-yl)acetate (**2b**).* Light yellow solid, yield 52%; mp 138.6-139.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (br s, 1H), 7.04 (s, 1H), 7.02 (d, *J* = 8.0 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 4.21-4.09 (m, 2H), 3.77 (dd, *J* = 4.4, 8.0 Hz, 1H), 3.06 (dd, *J* = 4.4, 16.8 Hz, 1H), 2.82 (dd, *J* = 8.0, 16.8 Hz, 1H), 2.30 (s, 3H), 1.22 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.8, 171.2, 138.9, 132.2, 129.0, 128.7, 125.1, 109.4, 61.1, 42.4, 35.0, 21.3, 14.2; HRMS (ESI-TOF) Calcd For $C_{13}H_{15}NNaO_3$ $[M+Na]^+$: 256.0944; found: 256.0941.

*4.7.3. Ethyl 2-(5-methoxy-2-oxoindolin-3-yl)acetate (**2c**).* White solid, yield 62%; mp 170.0-170.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 20.4 Hz, 1H), 6.85 (s, 1H), 6.80 (d, *J* = 8.4 Hz, 1H),6.74 (dd, *J* = 2.4, 8.4 Hz, 1H), 4.21-4.09 (m, 2H), 3.79 (dd, *J* = 4.4, 8.0 Hz, 1H), 3.76 (s, 3H), 3.06 (dd, *J* = 4.4, 16.8 Hz, 1H),2.82 (dd, *J* = 8.0, 16.8 Hz, 1H), 1.22 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.2, 171.2, 155.9, 135.1, 130.3, 112.8, 111.6, 110.2, 61.2, 55.9, 42.9, 34.9, 14.2; HRMS (ESI-TOF) Calcd for $C_{13}H_{15}NNaO_4$ $[M+Na]^+$: 272.0893; found: 272.0886.

*4.7.4. Ethyl 2-(5-fluoro-2-oxoindolin-3-yl)acetate (**2d**).* Yellow solid, yield 55%; mp 154.8-156.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (br s, 1H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.91 (td, *J* = 0.8, 8.8 Hz, 1H), 6.85-6.82 (m, 1H), 4.18-4.11 (m, 2H), 3.80 (dd, *J* = 4.4, 7.6 Hz, 1H), 3.07 (ddd, *J* = 0.8, 4.4, 17.2 Hz, 1H), 2.82 (ddd, *J* = 1.2, 8.0, 17.2 Hz, 1H), 1.21 (td, *J* = 1.2, 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.6, 171.0, 159.1 (d, *J* = 240 Hz, 1C), 137.8, 130.5 (d, *J* = 9.0 Hz, 1C), 114.7(d, *J* = 23.4 Hz, 1C), 112.3 (d, *J* = 24.9 Hz, 1C), 110.6 (d, *J* = 8.1 Hz, 1C), 61.3, 43.0, 34.7, 14.2.; HRMS (ESI-TOF) Calcd for $C_{12}H_{12}FNNaO_3$ $[M+Na]^+$: 260.0693; found: 260.0685.

*4.7.5. Ethyl 2-(5-chloro-2-oxoindolin-3-yl)acetate (**2e**).* Yellow solid, yield 45%; mp 171.6-172.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (br s, 1H), 7.23 (s, 1H), 7.20 (dd, *J* = 0.8, 8.4 Hz, 1H),6.82 (d, *J* = 8.0 Hz, 1H), 4.20-4.12 (m, 2H), 3.78 (dd, *J* = 4.0, 7.6 Hz, 1H), 3.07 (dd, *J* = 4.4, 17.2 Hz, 1H), 2.85 (dd, *J* = 7.6, 17.2 Hz, 1H), 1.23 (td, *J* = 0.8, 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.7, 170.9, 140.2, 130.6, 128.4, 128.0, 124.8, 110.8, 61.4, 42.5, 34.6, 14.2; HRMS (ESI-TOF) Calcd for $C_{12}H_{12}ClNNaO_3$ $[M+Na]^+$: 276.0398; found: 276.0394.

*4.7.6. Ethyl 2-(2-oxo-5-(trifluoromethoxy)indolin-3-yl)acetate (**2f**).* Yellow solid, yield 43%; mp 135.8-137.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.28-9.20 (m, 1H), 7.14 (s, 1H), 7.09 (d, *J* = 8.4 Hz, 1H),6.90 (d, *J* = 8.4 Hz, 1H), 4.20-4.09 (m, 2H), 3.82 (dd, *J* = 4.4, 8.0 Hz, 1H), 3.08 (dd, *J* = 4.4, 17.2 Hz, 1H),2.86 (dd, *J* = 8.0, 17.2 Hz, 1H), 1.21 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.6, 170.9, 144.7, 140.6, 130.3, 121.7, 120.6 (q, *J* = 255.0 Hz, 1C), 118.3, 110.5, 61.4, 42.8, 34.6, 14.2; HRMS (ESI-TOF) Calcd for $C_{13}H_{12}F_3NNaO_4$ $[M+Na]^+$: 326.0611; found: 326.0611.

*4.7.7. Ethyl 2-(7-methoxy-2-oxoindolin-3-yl)acetate (**2g**).* White solid, yield 67%; mp 158.8-159.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (br s, 1H), 6.98 (t, *J* = 8.4 Hz, 1H), 6.85 (d, *J* = 7.6 Hz, 1H),6.82 (t, *J* = 8.4 Hz, 1H), 4.21-4.09 (m, 2H), 3.86 (d, *J* = 1.2, 3H), 3.83 (dd, *J* = 4.4, 8.0 Hz, 1H), 3.07 (dd, *J* = 4.4, 16.8 Hz, 1H), 2.81 (dd, *J* = 8.0, 16.8 Hz, 1H), 1.21 (td, *J* = 1.2, 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 171.1, 143.8, 130.3, 129.6, 123.1, 116.6, 110.7, 61.1, 55.8, 43.0, 34.9, 14.2; HRMS (ESI-TOF) Calcd for $C_{13}H_{15}NNaO_4$ $[M+Na]^+$: 272.0893; found: 272.0889

*4.7.8. Ethyl 2-(7-fluoro-2-oxoindolin-3-yl)acetate (**2h**).* White solid, yield 82%; mp 181.2-183.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (br s, 1H), 7.04-6.94 (m, 3H), 4.20-4.08 (m, 2H),

3.84 (t, *J* = 5.6 Hz, 1H), 3.09 (dd, *J* = 4.0, 17.2 Hz, 1H), 2.88 (dd, *J* = 7.6, 17.2 Hz, 1H), 1.21 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2, 170.9, 147.1 (d, *J* = 242.5 Hz, 1C), 131.5 (d, *J* = 3.3 Hz, 1C), 128.9 (d, *J* = 2.2 Hz, 1C), 123.3 (d, J = 5.8 Hz, 1C), 119.9 (d, *J* = 3.3 Hz, 1C), 115.7 (d, *J* = 17.0 Hz, 1C), 61.3, 42.7, 34.8, 14.2; HRMS (ESI-TOF) Calcd for $C_{12}H_{12}FNNaO_3$ $[M+Na]^+$: 260.0693; found: 260.0693.

*4.7.9. Ethyl 2-(7-chloro-2-oxoindolin-3-yl)acetate (**2i**).* Light yellow solid, yield 64%; mp 158.1-159.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (br s, 1H), 7.20 (d, *J* = 8.0 Hz, 1H), 7.13 (d, *J* = 7.2 Hz, 1H),6.95 (t, *J* = 8.0 Hz, 1H), 4.19-4.07 (m, 2H), 3.88 (dd, *J* = 4.4, 7.6 Hz, 1H), 3.09 (dd, *J* = 4.4, 17.2 Hz, 1H), 2.85 (dd, *J* = 8.0, 17.2 Hz, 1H), 1.20 (td, *J* = 1.2, 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2, 170.8, 139.5, 130.2, 128.4, 123.4, 122.5, 115.2, 61.2, 43.4, 34.8, 14.2; HRMS (ESI-TOF) Calcd for $C_{12}H_{12}ClNNaO_3$ $[M+Na]^+$: 276.0398; found: 276.0395

*4.7.10. Ethyl 2-(2-oxo-7-(trifluoromethyl)indolin-3-yl)acetate (**2j**).* Yellow solid, yield 52%; mp 194.3-195.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (br s, 1H), 7.43 (t, *J* = 8.8 Hz, 2H), 7.11 (t, *J* = 7.6 Hz, 1H), 4.20-4.07 (m, 2H), 3.81 (dd, *J* = 4.4, 7.6 Hz, 1H), 3.11 (dd, *J* = 4.4, 17.2 Hz, 1H), 2.89 (dd, *J* = 7.6, 17.2 Hz, 1H), 1.19 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 170.70, 139.0, 130.4, 127.7, 125.2 (q, *J* = 4.0 Hz, 1C), 124.0 (q, *J* = 270.0 Hz, 1C), 122.5, 112.2 (q, *J* = 33.0 Hz, 1C), 61.3, 41.4, 34.6, 14.1; HRMS (ESI-TOF) Calcd for $C_{13}H_{12}F_3NNaO_3$ $[M+Na]^+$: 310.0661; found: 310.0660.

*4.7.11. Ethyl 2-(6-chloro-2-oxoindolin-3-yl)acetate (**2k**).* Light yellow solid, yield 64%; mp 153.7-154.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (d, *J* = 9.6 Hz, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 6.97 (dd, *J* = 2.0, 8.0 Hz, 1H),6.92 (d, *J* = 2.0 Hz, 1H), 4.19-4.09 (m, 2H), 3.75 (dd, *J* = 4.4, 7.2 Hz, 1H), 3.06 (dd, *J* = 4.4, 17.2 Hz, 1H), 2.85 (dd, *J* = 8.0, 17.2 Hz, 1H), 1.21 (td, *J* = 1.6, 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.7, 171.0, 143.0, 134.1, 127.2, 125.0, 122.5, 110.8, 61.3, 42.1, 34.7, 14.2;HRMS (ESI-TOF) Calcd for $C_{12}H_{12}ClNNaO_3$ $[M+Na]^+$: 276.0398; found: 276.0389

*4.7.12. Ethyl 2-(4,7-dichloro-2-oxoindolin-3-yl)acetate (**2l**).* White solid, yield 60%; mp 199.3-200.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, *J* = 22.4 Hz, 1H), 7.16 (d, *J* = 8.8 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 1H), 4.11-3.97 (m, 2H), 3.81 (t, *J* = 4.8 Hz, 1H), 3.38 (dd, *J* = 5.6, 17.2 Hz, 1H), 3.24 (dd, *J* = 4.0, 17.2 Hz, 1H), 1.14 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 170.2, 141.1, 129.5, 128.8, 126.8, 123.8, 113.7, 61.2, 43.9, 32.6, 14.1; HRMS (ESI-TOF) Calcd for $C_{12}H_{11}Cl_2NNaO_3$ $[M+Na]^+$: 310.0008; found: 310.0006.

*4.7.13. Ethyl 2-(1-methyl-2-oxoindolin-3-yl)acetate (**2m**).* Light yellow oil (lit.[2] colourless liquid), yield 62%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 8.0 Hz,1H), 7.24 (d, *J* = 7.2 Hz, 1H), 7.02 (t, *J* = 7.6 Hz, 1H),6.83 (d, *J* = 8.0 Hz, 1H), 4.18-4.06 (m, 2H), 3.77 (dd, *J* = 4.4, 8.0 Hz, 1H), 3.22 (d, *J* = 1.2 Hz, 3H), 3.07 (ddd, *J* = 1.2, 4.4, 16.8 Hz, 1H), 2.78 (ddd, *J* = 1.2, 8.0, 16.8 Hz, 1H), 1.19 (td, *J* = 1.2, 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 171.2, 144.4, 128.4, 128.3, 123.9, 122.6, 108.2, 61.0, 41.9, 35.0, 26.4, 14.2; HRMS (ESI-TOF) Calcd for $C_{13}H_{15}NNaO_3$ $[M+Na]^+$: 256.0944; found: 256.0935.

*4.7.14. Ethyl 2-(1-acetyl-2-oxoindolin-3-yl)acetate (**2n**).* Light yellow oil, yield 41%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.0 Hz,1H), 7.32 (t, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 7.2 Hz, 1H),7.18 (t, *J* = 7.2 Hz, 1H), 4.11-4.03 (m, 2H), 3.91 (t, *J* = 5.6 Hz, 1H), 3.11-3.00 (m, 2H), 2.69 (d, *J* = 0.4 Hz, 3H), 1.15 (td, *J* = 0.4, 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.7, 171.0, 170.4, 140.8, 128.8, 127.1, 125.2, 123.2, 116.7, 61.3, 42.7, 35.3, 26.8, 14.1; HRMS (ESI-TOF) Calcd for $C_{13}H_{15}NNaO_3$ $[M+Na]^+$: 284.0893; found: 284.0885.

*4.7.15. Methyl 2-(2-oxoindolin-3-yl)acetate (**2o**).* White solid, yield 61%; mp 248.5-251.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (br s, 1H), 7.24-7.20 (m, 2H), 7.01 (d, *J* = 7.6 Hz, 1H), 6.91 (d, *J* = 8.0 Hz, 1H), 3.82 (dd, *J* = 4.8, 8.0 Hz, 1H), 3.70 (s, 3H), 3.09 (dd, *J* = 4.4, 16.8 Hz, 1H), 2.84 (ddd, *J* = 0.8, 8.0, 16.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.2, 171.6, 141.5, 128.6, 128.3, 124.1, 122.5, 109.9, 52.1, 42.3, 34.5; HRMS (ESI-TOF) Calcd for $C_{11}H_{11}NNaO_3$ $[M+Na]^+$: 228.0631; found: 228.0637.

*4.7.16. Propyl 2-(2-oxoindolin-3-yl)acetate (**2p**).* Light yellow solid, yield 78%; mp 97.3-98.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (d, *J* = 15.2Hz, 1H), 7.22-7.18 (m, 2H), 6.99 (t, *J* = 7.2 Hz, 1H), 6.91 (d, *J* = 7.6 Hz, 1H), 4.08-3.99 (m, 2H), 3.81 (dd, *J* = 4.8, 7.4 Hz, 1H), 3.09 (dd, *J* = 4.8, 17.2 Hz, 1H), 2.84 (dd, *J* = 8.0, 17.2 Hz, 1H), 1.63-1.54 (m, 2H), 0.88 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.8, 171.3, 141.9, 128.9, 128.4, 124.1, 122.5, 110.1, 66.7, 42.5, 34.8, 21.9, 10.4; HRMS (ESI-TOF) Calcd for $C_{13}H_{15}NNaO_3$ $[M+Na]^+$: 256.0944; found: 256.0938.

*4.7.17. Tert-butyl 2-(2-oxoindolin-3-yl)acetate (**2q**).* Light yellow solid, yield 65%; mp 146.3-147.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.42 (br s, 1H), 7.23 (d, *J* = 6.8 Hz, 1H), 7.20 (dd, *J* = 0.8, 7.6 Hz, 1H), 7.00 (t, *J* = 7.6 Hz, 1H), 6.92 (d, *J* = 7.6 Hz, 1H), 3.75 (t, *J* = 5.6 Hz, 1H), 2.98 (dd, *J* = 4.8, 16.4 Hz, 1H), 2.82 (dd, *J* = 7.2, 16.4 Hz, 1H), 1.33 (d, *J* = 0.8 Hz, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.2, 170.1, 141.9, 129.0, 128.3, 124.1, 122.5, 110.0, 81.5, 42.8, 36.0, 27.9; HRMS (ESI-TOF) Calcd for $C_{14}H_{17}NNaO_3$ $[M+Na]^+$: 270.1101; found: 270.1091.

*4.7.18. Ethyl 2-cyano-2-(2-oxoindolin-3-yl)acetate (**2s**).* Light brown oil, yield 57%; 57:43 dr; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (s, 0.85H), 8.99 (s, 1.15H), 7.51 (d, *J* = 7.6 Hz, 1.15H), 7.31-7.28 (m, 2H), 7.21 (d, *J* = 7.2 Hz, 0.85H), 7.06 (td, *J* = 2.8, 7.6 Hz, 2H), 6.93 (t, *J* = 8.4 Hz, 2H), 4.43-4.38 (m, 3H), 4.34 (d, *J* = 3.6 Hz, 1H), 4.17 (d, *J* = 3.6 Hz, 0.85H), 4.15-4.06 (m, 2H), 3.98 (d, *J* = 2.8 Hz, 1.15H), 1.38 (t, *J* = 7.2Hz, 2.56H), 1.09 (t, *J* = 7.2 Hz, 3.44H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 164.6, 163.3, 141.9, 141.8, 129.7, 129.6, 124.8, 124.2, 124.1, 124.1, 123.2, 123.0, 115.3, 113.4, 110.7, 110.4, 63.8, 63.3, 45.4, 45.3, 38.9, 37.7, 29.7, 14.0, 13.6; HRMS (ESI-TOF) Calcd for $C_{13}H_{12}N_2NaO_3$ $[M+Na]^+$: 267.0740; found: 267.0746.

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References and notes

1. (a) Kagata, T.; Saito, S.; Shigemori, H.; Ohsaki, A.; Ishiyama, H.; Kubota, T.; Kobayashi, J. *J. Nat. Prod.* **2006**, *69*, 1517−1521; (b) Wang, K.; Zhou, X.-Y.; Wang, Y.-Y.; Li, M.-M.; Li, Y.-S.; Peng, L.-Y.; Cheng, X.; Li, Y.; Wang, Y.-P.; Zhao, Q.-S. *J. Nat. Prod.* **2011**, *74*, 12−15; (c) Tan, S.-J.; Lim, J.-L.; Low, Y.-Y.; Sim, K.-S.; Lim, S.-H.; Kam, T.-S. *J. Nat. Prod.* **2014**, *77*, 2068−2080; (d) Tang, B.-Q.; Wang, W.-J.; Huang, X.-J.; Li, G.-Q.; Wang, L.; Jiang, R.-W.; Yang, T.-T.; Shi, L.; Zhang, X.-Q.; Ye, W.-C. *J. Nat. Prod.* **2014**, *77*, 1839−1846; (e) Chen, Y.-P.; Lu, M.-N.; Hao, J.-C.; Li, M.-H.; Hattori, M.; Wang, W. *J. Asian Nat. Prod. Res.* **2015**, *17*, 384−390.

2. Prasanna, P.; Balamurugan, K.; Perumal, S.; Yogeeswari, P.; Sriram, D. *Eur. J. Med. Chem.* **2010**, *45*, 5653−5661; (b) Monteiro, A.; Goncalves, L. M.; Santos, M. M. M. *Eur. J. Med. Chem.* **2014**, *79*, 266−272; (c) Yu, B.; Yu, D.-Q.; Liu, H.-M. *Eur. J. Med. Chem.* **2015**, *97*, 673−698; (d) Sampson, P. B.; Liu, Y.; Forrest, B.; Cumming, G.; Li, S.-W.; Patel, N. K.; Edwards, L.; Laufer, R.; Feher, M.; Ban, F.; Awrey, D. E.; Mao, G.; Plotnikova, O.; Hodgson, R.; Beletskaya, I.; Mason, J. M.; Luo, X.; Nadeem, V.; Wei, X.; Kiarash, R.; Madeira, B.; Huang, P.; Mak, T. W.; Pan, G.; Pauls, H. W. *J. Med. Chem.* **2015**, *58*, 147−169; (e) Zhao, Y.; Aguilar, A.; Bernard, D.; Wang, S. *J. Med. Chem.* **2015**, *58*, 1038−1052.
3. For selected reviews, see: (a) Marti, C.; Carreira, E. M. *Eur. J. Org. Chem.* **2003**, 2209−2219; (b) Trost, B. M.; Brennan, M. K. *Synthesis* **2009**, 3003−3025; (c) Zhou, F.; Liu, Y. L.; Zhou, J. *Adv. Synth. Catal.* **2010**, *352*, 1381−1407; (d) Shen, K.; Liu, X.; Lin, L.; Feng, X. *Chem. Sci.* **2012**, *3*, 327−334; (e) Ball-Jones, N. R.; Badillo, J. J.; Franz, A. K. *Org. Biomol. Chem.* **2012**, *10*, 5165−5181; (f) Hong, L.; Wang, R. *Adv. Synth. Catal.* **2013**, *355*, 1023−1052; (g) Cheng, D.; Ishihara, Y.; Tan, B.; Barbas, III C. F. *ACS Catal.* **2014**, *4*, 743−762; (h) Cao, Z.-Y.; Zhou, J. *Org. Chem. Front.* **2015**, *2*, 849−858; (i) Chen, J.-R.; Yu, X.-Y.; Xiao, W.-J. *Synthesis* **2015**, *47*, 604−629; (j) Han, W.-Y.; Zhao, J.-Q.; Zuo, J.; Xu, X.-Y.; Zhang, X.-M.; Yuan, W.-C. *Adv. Synth. Catal.* **2015**, *357*, 3007−3031.
4. For recent selected examples, see: (a) Zhao, J.-Q.; Wu, Z.-J.; Zhou, M.-Q.; Xu, X.-Y.; Zhang, X.-M.; Yuan, W.-C. *Org. Lett.* **2015**, *17*, 5020−5023; (b) Zhao, J.-Q.; Zhou, M.-Q.; Wu, Z.-J.; Wang, Z.-H.; Yue, D.-F.; Xu, X.-Y.; Zhang, X.-M.; Yuan, W.-C. *Org. Lett.* **2015**, *17*, 2238−2241; (c) You, Y.; Wu, Z.-J.; Wang, Z.-H.; Xu, X.-Y.; Zhang, X.-M.; Yuan, W.-C. *J. Org. Chem.* **2015**, *80*, 8470−8477; (d) Chen, L.; You, Y.; Zhang, M.-L.; Zhao, J.-Q.; Zuo, J.; Zhang, X.-M.; Yuan, W.-C. Xu, X.-Y. *Org. Biomol. Chem.* **2015**, *13*, 4413−4417; (e) Wang, L.; Yang, D.; Li, D.; Wang, R. *Org. Lett.* **2015** 17, 3004−3007; (f) Huang, J.-Z.; Zhang, C.-L.; Zhu, Y.-F.; Li, L.-L.; Chen, D.-F.; Han, Z.-Y.; Gong, L.-Z. *Chem. Eur. J.* **2015**, *21*, 8389−8393; (g) Babu, K. N.; Kinthada, L. K.; Ghosh, S.; Bisai, A. *Org. Biomol. Chem.* **2015**, *13*, 10641−10655.
5. For selected examples, see: (a) Bowman, W. R.; Heaney, H.; Jordan, B. M. *Tetrahedron Lett.* **1988**, *29*, 6657−6660; (b) Huang, A.; Kodanko, J. J.; Overman, L. E. *J. Am. Chem. Soc.* **2004**, *126*, 14043−14053; (c) Hamashima, Y.; Suzuki, T.; Takano, H.; Shimura, Y.; Sodeoka, M. *J. Am. Chem. Soc.* **2005**, *127*, 10164−10165; (d) Pugh, David S.; Klein, Johannes E. M. N.; Perry, Alexis; Taylor, Richard J. K. *Synlett*. **2010**, 934−938; (e) Trost, B. M.; Zhang, Y. *Chem.-Eur. J.* **2011**, *17*, 2916−2922; (f) Lv, J.; Zhang-Negrerie, D.; Deng, J.; Du, Y.; Zhao, K. *J. Org. Chem.* **2014**, *79*, 1111−1119; (g) Zhai, C.; Xing, D.; Jing, C.; Zhou, J.; Wang, C.; Wang, D.; Hu, W. *Org. Lett.* **2014**, *16*, 2934−2937; (h) Dong, W.; Liu, Y.; Hu, B.; Ren, K.; Li, Y.; Xie, X.; Jiang, Y.; Zhang, Z. *Chem. Commun.* **2015**, *51*, 4587−4590.
6. For selected examples, see: (a) Elmorsy, S. S.; El-Ahl, A.-A. S.; Soliman, H.; Amer, F. A. *Tetrahedron Lett.* **1996**, *37*, 2297−2298; (b) Cao, S.-H.; Zhang, X.-C.; Wei, Y.; Shi, M. *Eur. J. Org. Chem.* **2011**, 2668−2672; (c) Tan, B.; Candeias, N. R.; Barbas III C. F. *Nature Chem.* **2011**, *3*, 473−477; (d) Albertshofer, K.; Tan, B.; Barbas III C. F. *Org. Lett.* **2012**, *14*, 1834−1837; (e) Sun, J.; Xie, Y.-J.; Yan, C.-G. *J. Org. Chem.* **2013**, *78*, 8354−8365; (f) Irgashev, R. A.; Karmatsky, A. A.; Kozyukhin, S. A.; Ivanov, V. K.; Sadovnikov, A.; Kozik, V. V.; Grinberg, V. A.; Emets, V. V.; Rusinov, G. L.; Charushin, V. N. *Synthetic Met.* **2015**, *199*, 152−158.
7. For selected reviews, see: (a) Huisman, G. W.; Collier, S. J. *Curr. Opin. Chem. Biol.* **2013**, *17*, 284−292; (b) Tufvesson, P.; Lima-Ramos, J.; Haque, N. A.; Gernaey, K. V.; Woodley, J. M. *Org. Process Res. Dev.* **2013**, *17*, 1233−1238; (c) Lima-Ramos, J.; Neto, W.; Woodley, J. M. *Top. Catal.* **2014**, *57*, 301−320; (d) Groeger, H. *Angew. Chem. Int. Ed.* **2014**, *53*, 3067−3069; (e) Narancic, T.; Davis, R.; Nikodinovic-Runic, J.; O'Connor, K. E. *Biotechnol. Lett.* **2015**, *37*, 943−954; (f) Zhanga, Y.; Gea, J.; Zheng, L. *ACS Catal.* **2015**, *5*, 4503−4513
8. For selected examples, see: (a) Reeve, H. A.; Lauterbach, L.; Lenz, O.; Vincent, K. A. *ChemCatChem* **2015**, *7*, 3480−3487; (b) Halder, J.; Das, D.; Nanda, S. *Tetrahedron: Asymmetry* **2015**, *26*, 1197−1208; (c) Chung, J. Y. L.; Marcune, B.; Strotman, H. R.; Petrova, R. I.; Moore, J. C.; Dormer, P. G. *Org. Process Res. Dev.* **2015**, *19*, 1418−1423; (d) Weise, N. J.; Parmeggiani, F.; Ahmed, Syed T.; Turner, Nicholas J. *J. Am. Chem. Soc.* **2015**, *137*, 12977−12983.
9. For leading reviews, see: (a) Stuermer, R.; Hauer, B.; Hall, M.; Faber, K. *Curr. Opin. Chem.* Biol. **2007**, *11*, 203−213; (b) De Wildeman, S. M. A.; Sonke T.; Schoemaker, H. E.; May, O. *Acc. Chem. Res.* **2007**, *40*, 1260−1266; (c) *Enzyme Catalysis in Organic Synthesis*; 3rd, Completely Revised and Enlarged ed.; Drauz, K., Gröger, H., May, O., Eds.; Wiley-VCH: Weinheim, 2011; (d) *Comprehensive Chirality*; Carreira, E. M., Yamamoto, H., Eds.; Elsevier: Oxford, 2012; (e) Huang, M.; Hu, H.; Ma, L.; Zhou, Q.; Yu, L.; Zeng, S. *Drug Metab. Rev.* **2014**, *46*, 362−378.
10. For whole cell-mediated reduction of C=C double bonds, see: (a) Noma, Y.; Takahashi, H.; Asakawa, Y. *Phytochemistry* **1991**, *30*, 1147−1151; (b) Noma, Y.; Okajima, Y.; Takahashi, H.; Asakawa, Y. *Phytochemistry* **1991**, *30*, 2969−2972; (c) Fronza, G.; Fuganti, C.; Grasselli, P.; Lanati, S.; Rallo, R.; Tchilibon, S. *J. Chem. Soc. Perkin Trans. 1* **1994**, *20*, 2927−2930; (d) Matsumoto, K.; Kawabata, Y.; Takahashi, J.; Fujita, Y.; Hatanaka, M. *Chem. Lett.* **1998**, *27*, 283−284; (e) Goretti, M.; Ponzoni, C.; Caselli, E.; Marchigiani, E.; Cramarossa, M. R.; Turchetti, B.; Buzzini, P.; Forti, L. *Enzyme Microb. Technol.* **2009**, *45*, 463−468.
11. For the reduction of C=C double bonds with isolated enzymes, see: (a) Matsushima, A.; Sato, Y.; Otsuka, M.; Watanabe, T.; Yamamoto, H.; Hirata, T. *Bioorg. Chem.* **2008**, *36*, 23−28; (b) Hirata, T.; Matsushima, A.; Sato, Y.; Iwasaki, T.; Nomura, H.; Watanabe, T.; Toyoda, S.; Izumi, S. *J. Mol. Catal. B: Enzym.* **2009**, *59*, 158−162; (c) Wang, H.-B.; Pei, X.-Q.; Wu, Z.-L. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 705−715. (d) Liu, J.; Wu, J.; Li, Z. *Chem. Commun.* **2014**, *50*, 9729−9732.
12. (a) Chen, Y.; Xu, J.; Xu, X.; Xia, Y.; Lin, H.; Xia, S.; Wang, L. *Tetrahedron: Asymmetry* **2007**, *18*, 2537−2540; (b) Chen, Y.; Lin, H.; Xu, X.; Xia, S.; Wang, L. *Adv. Synth. Catal.* **2008**, *350*, 426−430; (c) Xia, S.; Chen, Y.; Zhuo, J.; Xu, H. *Biocatal. Biotransform.* **2013**, *31*, 66−70; (d) Chen, Y.; Zhuo, J.; Zheng, D.; Tian, S.; Li, Z. *J. Mol. Catal. B: Enzym.* **2014**, *106*, 100−104; (e) Zheng, D.; Yang, M.; Zhuo, J.; Li, K.; Zhang, H.; Yang, J.; Cui, B.; Chen, Y. *J. Mol. Catal. B: Enzym.* **2014**, *110*, 87−91; (f) Zhou, X.; Zheng, D.; Cui, B.; Han, W.; Chen, Y. *Tetrahedron* **2015**, *71*, 4738−4744.
13. See Supporting Information for the details of X-ray structure of product **2a**.
14. The ratios of the mixtures and the positions of deuterium atom were determined by the ESI-MS and NMR study, respectively. For details, see Supporting Information.
15. McComb, R. B.; Bond, L. W.; Burnett, R. W.; Keech, R. C.; Bowers, G. N. Jr. *Clin. Chem.* **1976**, *22*, 141−150.
16. (a) Palumbo, C.; Mazzeo, G.; Mazziotta, A.; Gambacorta, A.; Loreto, M. A.; Migliorini, A.; Superchi, S.; Tofani, D.; Gasperi, T. *Org. Lett.* **2011**, *13*, 6248–6251; (b) Li, T.-R.; Duan, S.-W.; Ding, W.; Liu, Y.-Y.; Chen, J.-R.; Lu, L.-Q.; Xiao, W.-J. *J. Org. Chem.* **2014**, *79*, 2296−2302.