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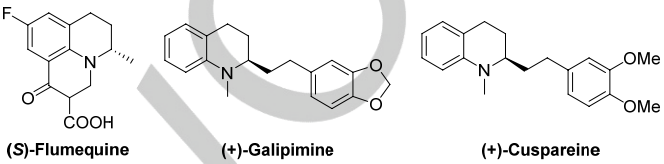
Deracemisation of phenyl substituted 2-methyl-1,2,3,4-tetrahydroquinolines by a recombinant monoamine oxidase from *Pseudomonas monteilii* ZMU-T01

Guozhong Deng,[a,b] Nanwei Wan,[a,b] Lei Qin,[a,b] Baodong Cui,[a,b] Miao An,[a,b] Wenyong Han,[a,b] and Yongzheng Chen*[a,b]

Abstract: A monoamine oxidase (MAO5) from *Pseudomonas monteilii* ZMU-T01 was firstly heterologously expressed in *Escherichia coli* BL21(DE3), and used as an biocatalyst for deracemisation of racemic 2-methyl-1,2,3,4-tetrahdroquinoline derivatives to yield unreacted (*R*)-enantiomer with up to >99% *ee*. Sequence alignment revealed MAO5 shared 14.7% identity toward the well-studied MAO-N.

Enantiomerically pure amines are important scaffolds in pharmaceutical compounds and widely used as chiral auxiliaries and resolving agents in organic synthesis. In the past decades, considerable efforts have been devoted to developing asymmetric routes to produce chiral amine derivatives with both highly chemical and enantiomeric purity. The chemocatalytic methods such as reductive amination and imine reduction have been well discussed in several reviews.[1] An alternative methodology for the synthesis of chiral amines via the toolkit of biocatalysts has been developed involving different classes of enzymes, including ω-transaminases, ammonia lyases, amine dehydrogenases, NADPH-dependent imine reductases, Pictet-Spenglerase and flavin-containing monoamine oxidases (MAOs).[2] MAOs could catalyse oxygen-dependent conversion of one enantiomer of amines into imines, thereby providing the corresponding chiral amines. MAO enzyme family include monoamine oxidase N (MAO-N) from *Aspergillus niger*, bacterial cyclohexylamine oxidase from *Brevibacterium oxydans* IH-35A (CHAO)[3] and 6-hydroxy nicotine oxidase (6-HNO).[4] The MAO-N is usually *S* selective and always has a broad substrate scope that has been demonstrated by Turner'swork, involving directed evolution forgenerating many genetic variants for the deracemisation of primary, secondary and tertiary amines.[5] Optically pure tetrahydroquinoline (THQ) derivatives are an important class of chiral amines, which serve as building blocks for asymmetric synthesis of pharmaceuticals, agrochemicals, and natural products,[6] such as antibacterialdrug (*S*)-flumequine and bioactive alkaloids (+)-Galipimine and (+)-Cuspareine (Figure1).[7] The transition- metal catalysed asymmetric

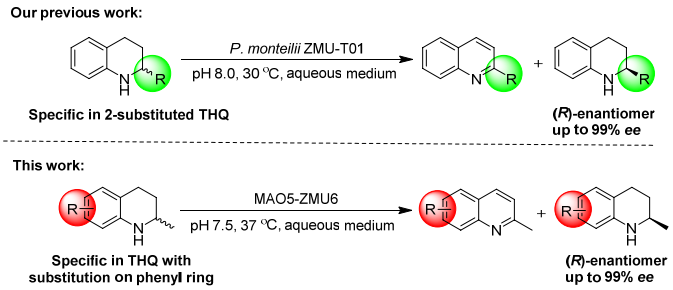


(*S*)-Flumequine (+)-Galipimine (+)-Cuspareine

Figure1. Representative examples of pharmaceuticals and natural products containing chiral tetrahydroquinolinescaffold.

hydrogenation and asymmetric transfer hydrogenations of quinolines are the usually efficient and atom-economic methods for synthesis of the chiral tetrahydroquinolines.[8]Recently, we have exploring the biocatalytic routes for the synthesis of chiral THQ derivatives. [9] The previous study revealed that strain *Pseudomonas monteilii* ZMU-T01 could specifically mediate oxidative resolution of racemic 2-substituted-THQ derivatives, but non activity were found toward the substrates with substitution on phenyl ring. [9c] In this work, we cloned and heterologously expressed a monoamine oxidase (MAO5) from the strain *P.monteilii* ZMU-T01, and further explored its application in deracemisation of racemic 2-methyl-1,2,3,4-tetrahdroquinoline (2-MTHQ) with different substitution on phenyl ring (Scheme 1). Herein, we wish to disclose our research progress on this subject.

We began our study by searching the complete genome sequence of *P. monteilii* SB3101.[10] Five MAO sequences were obtained, amplified and heterologously expressed in *E. coli* BL21(DE3) with the expression plasmid pET-32a(+) (see TableS1 and Figure S1). Activity assay was carried out using the whole cells of recombinant MAO strains. The results revealedthat only recombinant MAO5 showed the catalytic activity toward (*rac*)-2-MTHQ. Sequence alignment showed the amino acid sequence of MAO5 sharing low identity toward MAO-N[3] (14.7%) and CHAO[5] (25.2%) (see Figure S2).



Scheme 1. Deracemisation of 2-substituted THQ derivatives using *P.monteilii* ZMU-T01 or a recombinant monoamine oxidase strain (MAO5-ZMU6).

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The expression conditions of MAO-5 in *E. coli* were optimized, including the concentration of IPTG, induction temperature and induction time (see Figure S3-S5). To further improve the catalytic efficiency, several expression systems were applied to heterologous expression of MAO5. From the results of Figure 2, it could be found that strain MAO5-ZMU6, which expressed MAO5 using pET-28b(+) in *E. coli* BL21(DE3) along with a chaperone plasmid pG-KJE8, showed the relative highest activity. The relative activity of strain MAO5-ZMU6 was 14-fold higher than *P. monteilii* ZMU-T01 and 1.6-fold higher than strain MAO5-ZMU1. Unfortunately, expression of MAO5 in *Pichia pastoris* X-33 did not show activity with both shuttle plasmid pPICZα-A and pPICZ-A. SDS-PAGE analysis indicated *P. pastoris* X-33 was not applicable to heterologous express MAO5. Subsequently, the pH and temperature effects of strain MAO5-ZMU6 were investigated. The results indicated the relative highest activity was found at pH 7.5 in Tris-H_2SO_4 buffer and 37 ^oC (see Figure S6-S7).

Based on the optimal reaction conditions, the activity and enantioselectivity toward eight 2-substituted-THQ derivatives **1a-h** were investigated using the whole cells of strain MAO5-ZMU6 as biocatalyst (Table 1). Generally, the MAO5 was found to be slightly active towards **1a-f**, while inactive towards **1g** and **1h**. Among these eight substrates examined in Table 1, strain MAO5-ZMU6 showed specifically excellent resolution activity to **1a**, giving >99% *ee*of (*R*)-**1a**.

Subsequently, the substrate specificity in 2-methyl-THQ derivatives **1i-r** with different substitution on the phenyl ring wasfurther examined and the results were depicted in Table 2.In general, regardless of electronic property or position of the substitution on the phenyl ring of 2-methyl-THQ **1**, strain MAO5-ZMU6 showed overall excellent catalytic activity and the corresponding (*R*)-2-methyl-THQ **1** were obtained with97%->99% *ee* and up to>200 *E* value.

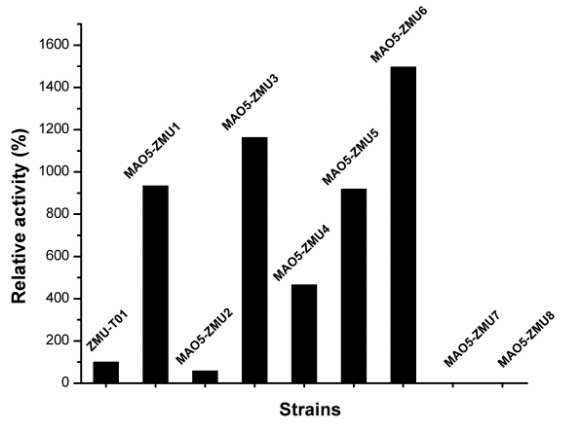
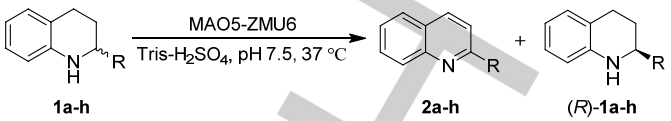
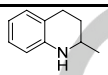
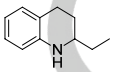
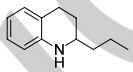
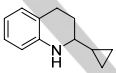
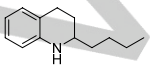
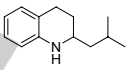
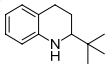
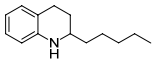


Figure 2. The relative activities toward (*rac*)-2-MTHQ of different strains. The information of strains was listed in Table S2.

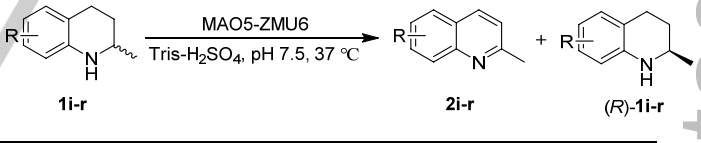
Table 1. Catalytic activity and enantioselectivity of the strain MAO5-ZMU6 on a series of 2-substituted THQ **1a-h.**[a]

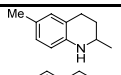
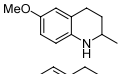
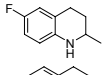
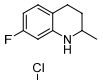
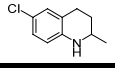


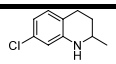
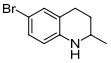
Entry	Substrate **1**	Conv.(%)[b]	*ee* (%)[b]/Config.[c]
1[d]	**1a** 	51	>99/(*R*)-**1a**
2	**1b** 	56	14/(*R*)-**1b**
3	**1c** 	64	18/(*R*)-**1c**
4	**1d** 	66	36/(*S*)-**1d**
5	**1e** 	39	27/(*R*)-**1e**
6	**1f** 	6	10/(*R*)-**1f**
7	**1g** 	Trace	N.D [e]
8	**1h** 	Trace	N.D.

[a]Unless otherwise noted, the reactions were performed in 5 mL Tris-H_2SO_4 buffer (50 mM, pH 7.5) containing 20 dcw g/L of MAO5-ZMU6 and 2 mM substrate.[b]The conversion of **1a-h** to **2a-h** was calculated by HPLC analysis.[c]Determined by comparison with the literature data or by analogue. [d]The reaction was conducted for 6 h. [e]N.D. = not detected.

Table 2. Deracemisation of 2-methyl-THQ derivatives **1i-r** using the MAO5-ZMU6 strain.[a]



Entry	Substrate**1**	Conv. (%)[b]	*ee*(%)[b] /Config.[c]	*E*[d]
1	**1i** 	64	99/(*R*)-**1i**	17
2	**1j** 	58	>99/(*R*)-**1j**	31
3	**1k** 	56	99/(*R*)-**1k**	39
4	**1l** 	53	>99/(*R*)-**1l**	80
5	**1m** 	51	>99/(*R*)-**1m**	>200
6	**1n** 	51	>99/(*R*)-**1n**	>200
7	**1o** 	58	99/(*R*)-**1o**	28
8	**1p** 	51	97/(*R*)-**1p**	>200

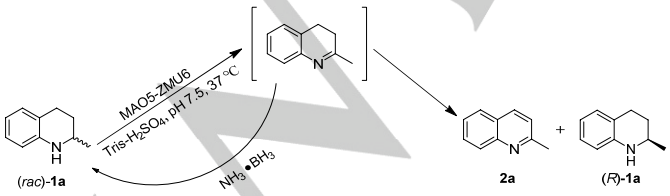
9	**1q** 	65	>99/(*R*)-**1q**	16
10	**1r** 	51	98/(*R*)-**1r**	122

[a]Unless otherwise noted, the reactions were performed in 5 mL Tris-H_2SO_4 buffer (50 mM, pH 7.5) containing 20 dcwg/L of MAO5-ZMU6 and substrate **1i-r** (1-4 mM).[b] The conversion of **1i-r** to **2i-r** was calculated by HPLC analysis.[c]Determined by comparison with the literature data or by analogue. [d]The *E* values were calculated by using the program selectivity.[11]

To understand the resolution process of 2-MTHQ derivatives, kinetic resolution experiment was performed using 4 mM of (*rac*)-**1a** with the cell pellet of strain MAO5-ZMU6 as biocatalyst. The reaction time courses were determined and showed in Figure 3A. In the kinetic resolution process, (*S*)-**1a** was quickly converted to **2a** for 5 h. At this moment, the *ee* of (*R*)-**1a** reached up to >99%, remaining 1.94 mM unreacted (*R*)-**1a**. As time went by, the concentration of (*R*)-**1a** was reduced slowly and accompanied by the increase of the yield of **2a**. The kinetic resolution results revealed that the (*R*)-**1a** could also be converted to **2a** when the (*S*)-**1a** was depleted. The highest 49% analytical yield and >99% *ee* of (*R*)-**1a** were obtained in this kinetic resolution process.

Dynamic kinetic resolution experiment toward 4 mM of (*rac*)-**1a** was also carried out using 4 mM of $NH_3{\cdot}BH_3$ as non-selective reductant (Scheme 2). The reaction course depicted in Figure 3B revealed the concentration of (*R*)-**1a** could reach highest to 3.54 mM with only 96% *ee* for 10 h. Furthermore, the *ee* of (*R*)-**1a** slowly increased to 97% for 18 h and 98% for 24 h, respectively, while the concentration reduced to about 3.20 mM. In the dynamic kinetic resolution process, up to 98% *ee* of (*R*)-**1a** was obtained with 83% analytical yield. Obviously, this was the highest yield to be obtained by deracemisation of (*rac*)-**1a**.

In summary, we have developed an efficient biocatalytic procedure for specific deracemisation of 2-methyl-1,2,3,4-tetrahdroquinolines with different substitution on the phenyl ring. With the recombinant monoamine oxidase from *P. monteilii* ZMU-T01 as the biocatalyst, a series of (*R*)-2-methyl-1,2,3,4-tetrahdroquinolines were provided with up to >99% *ee*. Besides, dynamic kinetic resolution of (*rac*)-2-MTHQ **1a** with $NH_3{\cdot}BH_3$ improved the yield of (*R*)-2-MTHQ **1a** up to 83% with 98% *ee*. Further study focusing on soluble expression and directed evolution of MAO5 will be performed to increase substrate concentration.



Scheme 2. The process for dynamic kinetic resolution of (*rac*)-**1a** using MAO5-ZMU6.

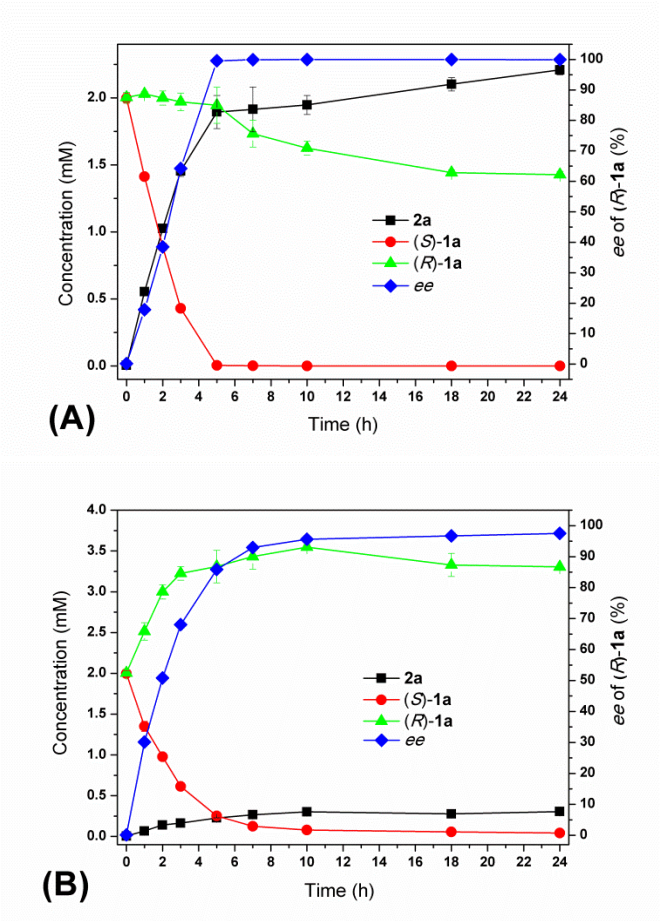


Figure 3. The time courses for kinetic resolution (A) and dynamic kinetic resolution (B) of (*rac*)-**1a** using the whole cells of strain MAO5-ZMU6.

Acknowledgements

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Keywords: Monoamine oxidase • Deracemisation• 2-methyl-1,2,3,4-tetrahydroquinoline• Biocatalysis

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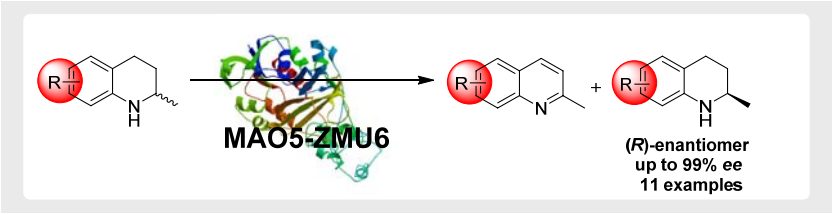

A novel monoamine oxidase (MAO5) was heterologously expressed in *Escherichia coli* BL21(DE3). Deracemisation of eleven 2-methyl-1,2,3,4-tetrahydroquinoline derivatives by the recombinant monoamine oxidase gave excellent *ee* values up to >99% of unreacted (*R*)-enantiomer.

*Guozhong Deng, Nanwei Wan,Lei Qin, Baodong Cui, Miao An,Wenyong Han, and Yongzheng Chen**

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Deracemisation of 2-methyl-1,2,3,4-tetrahydroquinoline derivatives by a recombinant monoamine oxidase from *Pseudomonas monteilii* ZMU-T01