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Bioreduction of 2-azido-1-arylethanones mediated by *Geotrichum candidum* and *Rhodotorula glutinis*

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Abstract

Enantioselective reductions of *p*-X-$C_6H_4C(=O)CH_2N_3$ (X = H, Cl, Br, CH_3, OCH_3) mediated by *Rhodotorula glutinis* and *Geotrichum candidum* afforded the corresponding alcohols with complementary *R* and *S* configurations, respectively, in excellent yield and enantiomeric excesses. The obtained (*R*)-azidoalcohols are important starting materials for preparation of natural products and valuable pharmaceutical compounds such as (*R*)-Tembamide and (*R*)-Aegeline.

Keywords: Tembamide; Aegeline; (*R*)- and (*S*)-2-azido-1-arylethanol

1. Introduction

The enantioselective bioreduction of acetophenones bearing functional substituents, like the azido group, attached to the α-carbon gives valuable intermediates that can be used as chiral building blocks in organic synthesis [1]. Among the target pharmaceutical compounds that can be synthesized using these intermediates are (*R*)-phenyl- and (*R*)-aryl-ethanolamines, that are part of a class of compounds with biological activity acting on the nervous system [2]. (*R*)-(−)-Tembamide and (*R*)-(−)-Aegeline are used in traditional Indian medicines and have been shown to have good hypoglycemic activity (Fig. 1) [3].

Bioreductions of 2-azido-1-phenyl-1-ethanones [4], 2-azido-1-(4-substituted-phenyl)-1-ethanones [5] and 2-azido-1-phenyl-1-propanones [6] mediated by baker's yeast (*Saccharomyces cerevisiae*) have been accomplished affording the corresponding (*R*)-azidoalcohols in good yield and enantiomeric excesses. Reduction of 2-azido-1-(4-substituted-phenyl)-1-ethanones by *Daucas carota* root also gives (*R*)-azidoalcohols in good yield and enantiomeric excesses [7].

The performance of *Rhodotorula glutinis* and *Geotrichum candidum* to reduce aryl methyl ketones has been calling our attention, due to their efficiency and complementary enantioselectivity that can provide both enantiomers of corresponding alcohols in high ee. Recently, these 2 microorganisms were elected among other 12 in a screening involving 300 microorganisms to reduce 4-substituted-acetophenones [8]. We use *R. glutinis* to reduce 2-azido-1-(1,3-benzodioxol-5-yl)-1-ethanones affording the corresponding (*R*)-azidoalcohol in excellent yield and ee [9].

Herein, we report the enantioselective preparation of synthetically useful intermediates through bioreduction of 2-azido-1-(4-substituted-phenyl)-1-ethanones mediated by *R. glutinis* and *G. candidum* as a microbiological alternative route for preparation of both enantiomers of Tembamide and Aegeline.

2. Results and discussion

The 2-azidoketones **1a–e** were obtained by reaction of corresponding 2-chloroketones with NaN_3 in DMF at rt. The 2-chloroketones were prepared applying the Kaufman and Wyman methodology [10] by chlorination of corresponding 4-substituted-acetophenones with sulfuryl chloride in CH_2Cl_2 at 0 °C.

The bioreduction of ketones **1a–e**, mediated by *R. glutinis* in nutrient broth (YM) at temperature of 30 °C gave azidoalcohols (−)-(*R*)-**2a–e** in 88–98% yield and excellent ee > 99% (see Scheme 1 and Table 1). These results are similar to those obtained with *S. cerevisiae* as reported by the authors of reference [5]. However, the bioreduction of ketones **1b–e**, mediated by *G. candidum* in nutrient broth (ME) at temperature of 28 °C gave azidoalcohols (+)-(*S*)-**2b–e** in 93–99% yield and

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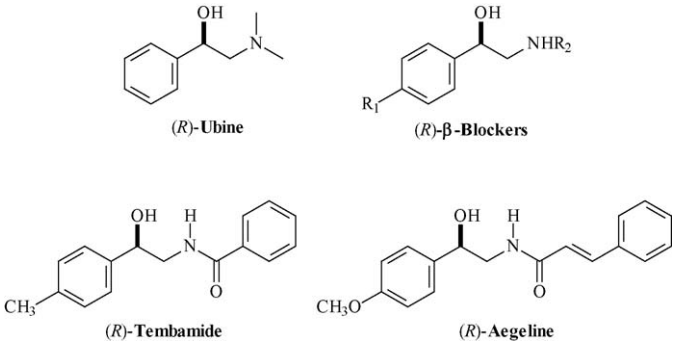
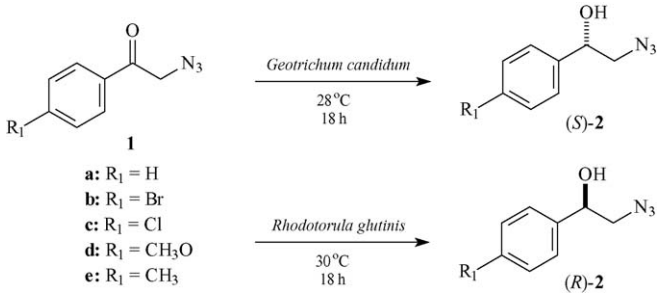


Fig. 1. Pharmaceutical useful ethanolamines.



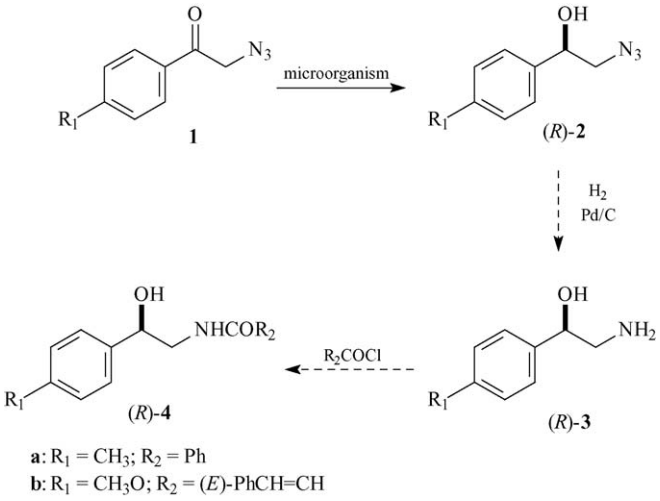
Scheme 1.

excellent ee > 99%. Only the azidoalcohol **2a** was obtained in poor ee (40%). This complementary enantioselectivity has been also observed in reduction of α-haloacetophenones mediated by these two microorganisms [11], and it is very convenient, allowing synthetic organic chemists to choose the best microorganisms for use in their synthesis projects. The (*R*)- and (*S*)-azidoalcohols were also obtained by enzymatic resolution of azidoalcohols mediated by lipase *P. cepacia* under ultrasonic conditions [12]. In addition, an interesting protocol was proposed using the same lipase immobilized on ceramic, for resolution of these azidoalcohols that were obtained in situ by $NaBH_4$ reduction of the corresponding ketoazides, giving (*R*)- and (*S*)-azidoalcohols in excellent ee [13]. The attribution of the absolute configuration to the obtained azidoalcohols **2a–e** was done by comparing $[\alpha]_D^{25}$ values found in literature [4,5].

Table 1
Bioreduction of 2-azido-1-arylethanones **1a–e** mediated by *Geotrichum candidum* CCT 1205 and *Rhodotorula glutinis* CCT 2182[a]

Ketone	Microorganism	*T* (°C)	Alcohol	Yield (%)	$[\alpha]_D^{25}$[b]	ee[c] (%)
1a	*Geotrichum candidum*	28	(*S*)-**2a**	93	+34.6	40 (*S*)
1b	*Geotrichum candidum*	28	(*S*)-**2b**	95	+38.7	>99 (*S*)
1c	*Geotrichum candidum*	28	(*S*)-**2c**	99	+79.3	>99 (*S*)
1d	*Geotrichum candidum*	28	(*S*)-**2d**	94	+39.1	>99 (*S*)
1e	*Geotrichum candidum*	28	(*S*)-**2e**	96	+28.3	>99 (*S*)
1a	*Rhodotorula glutinis*	30	(*R*)-**2a**	88	−82.1	>99 (*R*)
1b	*Rhodotorula glutinis*	30	(*R*)-**2b**	97	−38.7	>99 (*R*)
1c	*Rhodotorula glutinis*	30	(*R*)-**2c**	98	−79.3	>99 (*R*)
1d	*Rhodotorula glutinis*	30	(*R*)-**2d**	96	−39.1	>99 (*R*)
1e	*Rhodotorula glutinis*	30	(*R*)-**2e**	98	−28.3	>99 (*R*)

[a] 18 h, 2 mmol of ketone/1.5 mL of EtOH was added to 15 g of yeast (wet weight)/400 mL of malt extract (ME) nutrient broth—*Geotrichum candidum* or yeast-malt extract (YM) nutrient broth—*Rhodotorula glutinis*.
[b] *c*, 1 $CHCl_3$.
[c] Determined by GC–MS analysis (capillary chiral column CHIRASIL-DEX).



Scheme 2.

It is known that the rate of hydride transfer from NADH or NADPH mediated by a dehydrogenase present in the *S. cerevisiae* cells, to the carbonyl carbon of 4-substituted-acetophenones is decreased by electron donating groups and therefore 1-(4-methoxyphenyl)-1-ethanone is not reduced by *S. cerevisiae* [14]. This relationship has been observed with other microorganisms [8] and some isolated ketoreductases [15]. On the other hand, electronegative groups, such as N_3, attached to α-carbon, seems to be responsible for the activation of the carbonyl carbon to undergo reduction mediated by *R. glutinis* or *G. candidum* in spite of the above effect of 4-methoxyphenyl group. In fact, it is known that the azido group was able to activate the bioreduction even when a more deactivated group such as 3,4-methylenedioxy-phenyl group is attached to the carbonyl carbon of the ketone [9].

The hydride transfer from NADH or NADPH to the carbonyl carbon, mediated by the oxidoreductase of *R. glutinis* responsible for reduction of **1a–e**, occurs by *Si*-face of those ketones, following the Prelog rule [16], taking into account that the bulk of 4-substituted-phenyl group is bigger than the $-CH_2N_3$ group. However, *G. candidum* gives anti-Prelog alcohols due to the hydride transfer occurring preferably by *Re*-face of these ketones.

The relevance of obtaining azidoalcohols (*R*)- or (*S*)-**2a–e** in excellent yield and ee is that it may be used as important starting material for preparation of (*R*)- or (*S*)-aminoalcohols as Tembamide and Aegeline from (*R*)-**2d** (Scheme 2) [3,5,17].

3. Conclusions

R. glutinis and *G. candidum* are excellent biocatalysts for reduction of 2-azido-1-(4-substituted-phenyl)-1-ethanones affording the corresponding alcohols with complementary *R* and *S* configurations, respectively, in excellent yield and

enantiomeric excesses that can be used as intermediates for enantioselective synthesis of chiral aminoalcohols.

4. Experimental

IR spectra were recorded on a Bomem MB Series spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer in $CDCl_3$. Gas chromatographic analyses were performed using a Shimadzu GC/MS Class 5000 and with helium as carrier gas, with a chiral GC-column CHIRASIL-DEX (30 m × 0.25 mm × 0.25 μm). Optical rotation was measured with a J-720, VRDM306 JASCO, 589.3 nm spectropolarimeter. The azidoacetophenones were obtained by reaction of 2-haloketones with NaN_3 in DMF at rt. The racemics **2a–c**, used as reference for determination of ee in a GC provided with a chiral column, were obtained by reacting the corresponding **1a–c** with $NaBH_4$ in water/methanol at rt. The 2-chloroketones were prepared applying the Kaufman and Wyman methodology [10] by chlorination of corresponding 4-substituted-acetophenones with sulfuryl chloride in CH_2Cl_2 at 0 °C or by bromination of 4-substituted-acetophenones in CH_2Cl_2 at 0 °C. All other reagents and solvents were reagent grade.

5. Growth conditions for yeast culture

The microorganisms *G. candidum* CCT 1205 and *R. glutinis* CCT 2182 are stored at Fundação André Tosello Pesquisa e Tecnologia [18]. *G. candidum* was cultivated in malt extract (ME) nutrient broth (400 mL) at 28 °C and *R. glutinis* was cultivated in yeast-malt (YM extract) nutrient broth (400 mL) at 30 °C. Both yeasts were incubated for 2 days on an orbital shaker (200 rpm) before use.

6. General procedure for bioreduction of α-azidoacetophenones

Compound **1** (2 mmol), dissolved in 1.5 mL of ethanol, was added to a slurry of growing microorganism (400 mL). The resulting suspension was stirred in an orbital shaker (200 rpm) at 28 °C for *G. candidum* and at 30 °C for *R. glutinis* until full conversion of **1** (18 h). The product was extracted with CH_2Cl_2 and was purified by column chromatography using hexane/ethyl acetate (7:3).

6.1. (R)-(−)-2-Azido-1-phenylethanol *2a*

When 0.32 g (2 mmol) of **1a** was subjected to the general procedure for bioreduction using *R. glutinis* CCT 2182, the isolated product was **2a** (0.31 g, 96%) as an oil; $[\alpha]_D^{25}$ −82.1 (*c* 1, $CHCl_3$), $[\alpha]_D^{25}$ −80.1 literature [4,5] (*c* 0.78, $CHCl_3$, (*R*)), ee > 99% determined by CG using a chiral column. IR (film) 3377, 3107, 3086, 3063, 3031, 2972, 2922, 2106, 1600, 1494, 1452, 1076, 900, 760, 700 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.41 (brs, 1H); 3.46 (m, 2H); 4.87 (m, 1H); 7.25–7.38 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.98; 73.30; 125.62; 128.10; 140.22; 139.64. MS *m/z* (rel. int.%): 149 (13), 106 (52), 107 (80), 105 (59), 77 (100).

6.2. (S)-(+)-2-Azido-1-phenylethanol *2a*

When 0.32 g (2 mmol) of **1a** was subjected to the general procedure for bioreduction using *G. candidum* CCT 1205, the isolated product was **2a** (0.30 g, 93%) as an oil; $[\alpha]_D^{25}$ +34.6 (*c* 1, $CHCl_3$), $[\alpha]_D^{25}$ −80.1 literature [4,5] (*c* 0.78, $CHCl_3$, (*R*)), ee 40% determined by CG using a chiral column; NMR and IV spectra were identical to those observed with its (*R*) enantiomer.

6.3. (R)-(−)-2-Azido-1-(4-bromophenyl)ethanol *2b*

When 0.48 g (2 mmol) of **1b** was subjected to the general procedure for bioreduction using *R. glutinis* CCT 2182, the isolated product was **2b** (0.47 g, 97%) as white crystalline solid: mp 67 °C (literature [5] 66–67.5 °C); $[\alpha]_D^{25}$ −38.7 (*c* 1, $CHCl_3$), $[\alpha]_D^{25}$ −36.4 literature [5] (*c* 0.95, $CHCl_3$, (*R*)), ee > 99% determined by CG using a chiral column. IR (KBr) 3411, 3028, 2974, 2920, 2856, 2107, 1592, 1488, 1302, 1074, 883, 776, 716 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.62 (brs, 1H); 3.38–3.47 (m, 2H); 4.80–4.83 (m, 1H); 7.22 (d, 2H, *J* = 8.7 Hz); 7.47 (d, 2H, *J* = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.91; 72.76; 122.24; 127.64; 131.81; 139.50. MS *m/z* (rel. int.%): 187 (54), 185 (54), 157 (14), 159 (10), 78 (46), 77 (100).

6.4. (S)-(+)-2-Azido-1-(4-bromophenyl)ethanol *2b*

When 0.48 g (2 mmol) of **1b** was subjected to the general procedure for bioreduction using *G. candidum* CCT 1205, the isolated product was **2b** (0.53 g, 95%) as white crystalline solid: mp 67 °C (literature [5] 66–67.5 °C); $[\alpha]_D^{25}$ +38.7 (*c* 1, $CHCl_3$), $[\alpha]_D^{25}$ −36.4 literature [5] (*c* 0.95, $CHCl_3$, (*R*)), ee > 99% determined by CG using a chiral column; NMR and IV spectra were identical to those observed with its (*R*) enantiomer.

6.5. (R)-(−)-2-Azido-1-(4-chlorophenyl)ethanol *2c*

When 0.40 g (2 mmol) of **1c** was subjected to the general procedure for bioreduction using *R. glutinis* CCT 2182, the isolated product was **2c** (0.38 g, 98%) as white crystalline solid: mp 48 °C (literature [5] 47–48.5 °C); $[\alpha]_D^{25}$ −79.3 (*c* 1, $CHCl_3$), $[\alpha]_D^{25}$ −79.1 literature [5] (*c* 1.25, $CHCl_3$, (*R*)), ee > 99% determined by CG using a chiral column. IR (KBr) 3401, 3075, 2979, 2900, 2780, 2102, 1504, 1487, 1246, 1098, 875, 785, 727, 717 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.67 (brs, 1H); 3.42 (m, 2H); 4.84 (dd, 1H, *J* = 5.5 and 7.5 Hz); 7.25–7.35 m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.84; 72.73; 127.00; 128.55; 133.78; 138.72. MS *m/z* (rel. int.%): 141 (38), 139 (100), 113 (12), 111 (32), 89 (7), 75 (29), 74 (12).

6.6. (S)-(+)-2-Azido-1-(4-chlorophenyl)ethanol *2c*

When 0.40 g (2 mmol) of **1c** was subjected to the general procedure for bioreduction using *G. candidum* CCT 1205, the isolated product was **2c** (0.39 g, 99%) as white crystalline solid: mp 48 °C (literature [5] 47–48.5 °C); $[\alpha]_D^{25}$ +79.3 (*c* 1, $CHCl_3$), $[\alpha]_D^{25}$ −79.1 literature [5] (*c* 1.25, $CHCl_3$, (*R*)), ee > 99% determined

by CG using a chiral column; NMR and IV spectra were identical to those observed with its (*R*) enantiomer.

6.7. (R)-(−)-2-Azido-1-(4-methoxyphenyl)ethanol **2d**

When 0.38 g (2 mmol) of **1d** was subjected to the general procedure for bioreduction using *R. glutinis* CCT 2182, the isolated product was **2d** (0.37 g, 96%) as an oil; $[\alpha]_D^{25}$ −39.1 (*c* 1, $CHCl_3$), $[\alpha]_D^{25}$ −39.0 literature [5] (*c* 1, $CHCl_3$, (*R*)), ee > 99% determined by CG using a chiral column. IR (film) 3443, 2927, 2861, 2101, 1599, 1596, 1510, 1300, 1091, 883, 769 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.24 (brs, 1H); 3.45 (m, 2H); 3.81 (s, 3H); 4.86 (dd, 1H, *J* = 4.1 and 7.7 Hz); 6.82 (d, 2H, *J* = 6.8 Hz); 7.25 (d, 2H, *J* = 6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.6; 58.4; 73.5; 114.6; 127.5; 132.7; 159.9. MS *m/z* (rel. int.%): 152 (21), 137 (93), 135 (13), 134 (45), 119 (27), 109 (49), 94 (34), 92 (9), 91 (38), 89 (5), 79 (9), 78 (8), 77 (42), 65 (38), 63 (13), 51 (30), 50 (19), 43 (100).

6.8. (S)-(+)-2-Azido-1-(p-methoxyphenyl)ethanol **2d**

When 0.38 g (2 mmol) of **1d** was subjected to the general procedure for bioreduction using *G. candidum* CCT 1205, the isolated product was **2d** (0.36 g, 94%) as an oil; $[\alpha]_D^{25}$ +39.1 (*c* 1, $CHCl_3$), $[\alpha]_D^{25}$ −39.0 literature [5] (*c* 1, $CHCl_3$, (*R*)), ee > 99% determined by CG using a chiral column; NMR and IV spectra were identical to those observed with its (*R*) enantiomer.

6.9. (R)-(−)-2-Azido-1-(4-methylphenyl)ethanol **2e**

When 0.35 g (2 mmol) of **1e** was subjected to the general procedure for bioreduction using *R. glutinis* CCT 2182, the isolated product was **2e** (0.38 g, 98%) as an oil; $[\alpha]_D^{25}$ −28.3 (*c* 1, $CHCl_3$), $[\alpha]_D^{25}$ −28.2 literature [5] (*c* 1.2, $CHCl_3$, (*R*)), ee > 99% determined by CG using a chiral column. IR (film) 3406, 2921, 2854, 2105, 1595, 1493, 1302, 1264, 1090, 1014, 826, 783 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H); 2.42 (brs, 1H); 3.43 (m, 2H); 4.82 (m, 1H); 7.18 (d, 2H, *J* = 8.5 Hz); 7.25 (d, 2H, *J* = 8.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.1; 57.9; 73.1; 125.6; 129.1; 137.4; 137.8. MS *m/z* (rel. int.%): 141 (96), 140 (65), 139 (87), 136 (16), 121 (50), 117 (8), 113 (29), 111 (61), 93 (49), 91 (45), 89 (4), 78 (5), 77 (87), 76 (8), 75 (39), 74 (22), 65 (20), 51 (53), 50 (70), 44 (27), 43 (30), 40 (100).

6.10. (S)-(+)-2-Azido-1-(4-methylphenyl)ethanol **2e**

When 0.35 g (2 mmol) of **1e** was subjected to the general procedure for bioreduction using *G. candidum* CCT 1205, the isolated product was **2e** (0.34 g, 96%) as an oil; $[\alpha]_D^{25}$ +28.3 (*c* 1, $CHCl_3$), $[\alpha]_D^{25}$ −28.2 literature [5] (*c* 1.2, $CHCl_3$, (*R*)), ee > 99% determined by CG using a chiral column; NMR and IV spectra were identical to those observed with its (*R*) enantiomer.

Acknowledgements

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