Tetrahedron 64 (2008) 11738–11744

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Biooxidation of methyl group: application to the preparation of alcohol and acid metabolites of terfenadine, ebastine and analogues

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ARTICLE INFO

Article history:
Received 23 June 2008
Received in revised form
23 September 2008
Accepted 29 September 2008
Available online 8 October 2008

ABSTRACT

The aim of this study was to found the best conditions to prepare metabolites of terfenadine, ebastine and analogues. For that purpose we investigated the structural substrate requirements needed for the oxidative whole cell activity and selected the most efficient conditions to obtain each compound. Our results showed that either alcohol or acid derivative arising from the oxidation of a methyl group is the main product, ratio depending on the microorganism used and on the culture conditions of cells. The oxidized metabolites were synthesized at preparative scale and isolated in 35–88% yield before characterization.

1. Introduction

Non-sedating H_1-histamine receptor antagonists, terfenadine **1a** and ebastine **2a** undergo extensive first-pass metabolism[1–4] (hepatic CYP3A4) resulting in extremely low plasma concentration and the release of the pharmacologically active acid metabolites (major metabolite), fexofenadine **1c** (from **1a**) and carebastine **2c** (from **2a**). However, the concomitant administration of terfenadine and antibacterial or antifungal agent[5,6] leads to accumulation of **1a** in plasma, causing severe ventricular arrhythmia, *torsades de pointes*.[7] Thereby **1a** was taken off market and replaced by fexofenadine that is devoid of side effects.[8]

In contrast to their structural similarities, the synthetic route for terfenadine, which employs the Friedel–Crafts reaction to introduce the *para* substitution of the *tert*-butylphenyl ring, is slightly efficient for the synthesis of fexofenadine (Scheme 1). The acylation of ethyl 2-methyl-2-phenylpropanoate with 4-chlorobutanoyl chloride was reported and displayed the predominance of *meta* to *para* regio-isomer (2 *meta*/1 *para*)[9] and the required separation of isomers involves a low yield (15%).[10] Other approaches were used to circumvent this lack of regioselectivity. Use of 1,4-disubstituted phenyl ring like 2-(4-bromophenyl)acetic acid,[11] 2-(4-bromophenyl)-acetonitrile[12] or ethyl 2-methyl-2-*p*-tolylpropanoate[13] allowed to avoid Friedel–Crafts reaction.

Another strategy was depicted by Di Giacomo et al.[14] from a precursor where carboxylic acid group was replaced by alcohol function, reducing strongly the electron withdrawing effect of acid. This approach ensured a better *para* regioselectivity of Friedel–Crafts reaction but, fexofenadine was obtained in only 7% yield (12 steps) from 2-methyl-2-phenylpropyl acetate.

Microorganisms are able to transform drugs into metabolites identical to those observed in mammalian metabolism whence these systems are considered as a model of mammalian metabolism.[15] Work of this field has been reviewed.[16,17] As chemical synthesis of **1c** appeared laborious, the biooxidation[18] of *tert*-butyl group of terfenadine, that is readily synthesized and commercially available, has been investigated as an alternative solution.[19–21] The same approach has been used for the synthesis of carebastine **2c**.[22,23] However, side-products[19,20] were observed in some cases, and expected products were obtained in low yields. In a previous communication, we compared the ability of microorganisms in the three-step oxidation of terfenadine and ebastine and described the preparation of acid derivatives in high yields.[24] More recently we showed that the terfenadine metabolites ratio (acid/alcohol) in biotransformation depended on culture conditions. Thence, hydroxyterfenadine **1b** was exclusively obtained in good yield with cells grown in culture medium without soybean peptones.[25]

To highlight the effectiveness of these microbial oxidations in organic synthesis, we studied the relationship between the structure of the substrates and the activity of selected microorganisms in the oxidation of one methyl of *tert*-butyl group present in terfenadine and ebastine analogues **1a–6a** (Scheme 2). We report herein a direct and efficient preparation of their alcohol **1b–5b** and acid **1c–4c** metabolites. In addition to the production of biological active compounds such as acids, the goal of these syntheses was the preparation of alcohol intermediates that are

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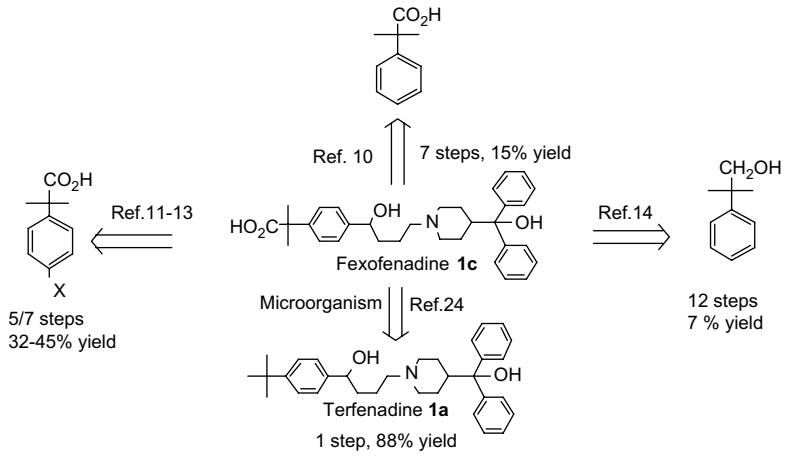
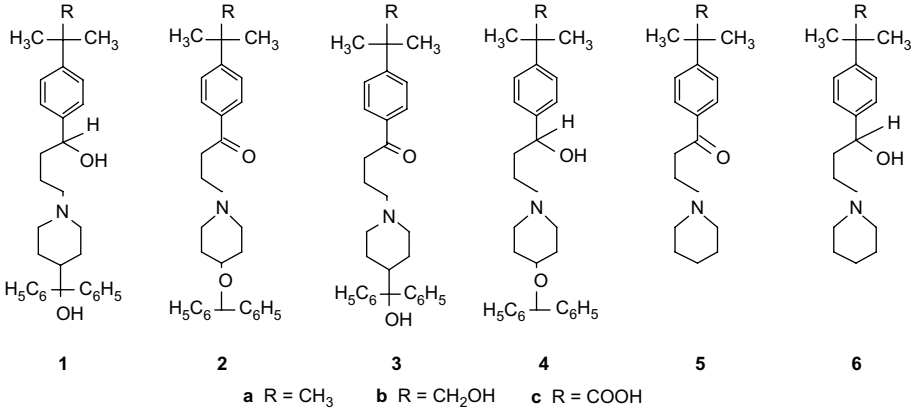
doi:10.1016/j.tet.2008.09.098



Scheme 1. Approaches towards fexofenadine.



Scheme 2.

needed to study multi-step oxidation reactions. Furthermore such pure intermediates are of major interest to the biological community studying drugs metabolism in providing authentic samples.

2. Results and discussion

2.1. Substrates

Two structural differences appear in terfenadine and ebastine: first the aliphatic chain bears an hydroxyl group in terfenadine **1a** and a ketone in ebastine **2a**, second, the substituent in the 4-position of piperidinyl cycle is an hydroxydiphenylmethyl substituent in terfenadine and diphenylmethoxy substituent in ebastine. Then ebastine is more hydrophobe than terfenadine. In order to compare the ability of microorganisms to oxidize the methyl group, we studied the biotransformation of **1a**, **2a**, the oxidized terfenadine **3a** (terfenadone), the reduced ebastine **4a**, and the unsubstituted piperidinyl derivatives **5a** and **6a** (Scheme 2).

Terfenadone was obtained from 4′-*tert*-butyl-4-chlorobutyrophenone and azacyclonol by N-alkylation in 69% yield following the procedure described[14] to prepare fexofenadine. Compound **5a** was prepared by following a modified procedure[26] in 60% yield (after recrystallization) and using piperidine instead of azacyclonol. Reduction of ebastine **2a** and compound **5a** by sodium borohydride afforded the corresponding ebastol **4a** (95% yield) and compound **6a** (51% yield), respectively.

2.2. Microorganisms studies

It was known that the ability of microorganism to transform organic compounds depended on the culture medium composition.[27,28] Indeed, Sariaslani demonstrated that one cytochrome P450 involved in *Streptomyces griseus* hydroxylation of aromatic compounds was induced by genistein (5,7,4′-trihydroxyisoflavone), a soybean peptone component. Therefore, screenings of microorganisms able to produce fexofenadine or carebastine from terfenadine **1a** or ebastine **2a**, respectively, were carried out with cells grown in culture medium containing soybean peptones. However, hydroxylation of terfenadine **1a** into hydroxyterfenadine **1b** by *Cunninghamella blakesleeana*[29] and by *Streptomyces platensis*[25] cultured in medium without soybean peptones was described. So, in the hope to synthesize alcohol and acid metabolites, we investigated the biotransformation with microorganisms grown in culture medium containing (YMS) or without (YM) soybean peptones. Metabolites were identified by LC/MS analysis, and their structure was confirmed by NMR techniques (^{1}H, ^{13}C, DEPT, COSY, HMQC) after synthesis at preparative scales.

In analytical assays, incubations were carried out in culture medium. Substrates in DMF solution were added to 60 h-old

cultures (final substrate concentration 0.424 mM) and biotransformations were monitored during 96 h. However, concerning **5a** and **6a** this molar concentration was low for preparative scale, that is why we also tested the biotransformation of these compounds in higher concentration (0.7 mM, 0.2 g/L). The kinetic study of the oxidation of terfenadine showed[25] the fast disappearance of substrate from the incubation medium came from the adsorption of the hydrophobic compounds onto cells. After biotransformation, more polar metabolites were slowly released in medium, which complicated the accurately quantification of products formed during incubations. The results in Table 1 indicated the products detected after 96 h of incubation time. At this time, cells suspensions were extracted as described in Section 4, and crude extracts were analyzed by HPLC. Two main metabolites accumulated in culture medium, alcohol and acid derivatives. Nevertheless, presence in very small amount of aldehyde intermediate was attested by LC/MS. The absence of accumulation could be explained by a slow formation following by a fast transformation.

The biotransformation of terfenadine **1a** by *C. blakesleeana* grown in YM-culture medium gave the alcohol **1b** as described by Schmitz[29] whereas cells grown in the presence of soybean peptones gave fexofenadine **1c** in addition to **1b**, as described by Meiwes.[19] Similar results were observed for the biotransformation of ebastine **2a** and terfenadone **3a**, whereas ebastol **4a** was only oxidized into alcohol derivative **4b** whatever culture conditions. In case of compound **5a**, oxidation to alcohol was important without formation of acid metabolite. It seems that *C. blakesleeana* was more efficient in the oxidation of ketone analogues. But in all cases except **5a**, metabolites were obtained in low rate. The bacterium *Streptomyces rimosus*[21] and the fungus *C. echinulata*[22,23] had been selected for their abilities to oxidize terfenadine **1a** and ebastine **2a**, respectively. In our conditions (YM and YMS), we did not observe the same activity. The substituted piperidinyl compounds **1a**–**4a** were not (or in very small amount) oxidized by *C. echinulata*. However, the cells grown in YMS-culture medium were able to metabolize the unsubstituted ketone **5a** to give the alcohol **5b** as sole product. In contrast, the unsubstituted alcohol **6a** was not biotransformed. *S. rimosus* presented a very low oxidative activity towards **1a**–**3a**, **5a** and **6a**, and no activity towards ebastol **4a**.

The fungus *Absidia corymbifera* cultured in YM-culture medium was able to oxidize terfenadine **1a**, ebastine **2a**, terfenadone **3a** and ebastol **4a** leading to a mixture of alcohol and acid metabolites. In the experiments conducted with cells produced in YMS-culture medium, fexofenadine **1c**, carebastine **2c** and carebastol **4c** were obtained as sole product after 96 h of incubation of **1a**, **2a** and **4a**, respectively. However, *A. corymbifera* turned out to be less efficient in the oxidation of **3a** since a mixture of alcohol **3b** and acid **3c** was observed. In case of compound **5a**, oxidation to alcohol was only detected during incubation with cells grown in presence of soybean peptones. Unsubstituted substrate **6a** was not metabolized.

As previously described, *S. platensis* cells produced in YM-culture medium were able to transform terfenadine **1a** into hydroxyterfenadine **1b**. Whereas ebastine **2a** and ebastol **4a** were also hydroxylated, metabolites **2b** and **4b** were formed in lower amount than **1b**. Incubations of cells of *S. platensis* obtained in YMS-culture medium furnished a mixture of alcohol and acid metabolites from terfenadine, ebastine and ebastol. In contrast, terfenadone **3a** was very well oxidized since cells grown in YM-culture medium produced a mixture of alcohol **3b** and acid **3c**, and cells grown in YMS-culture medium gave the acid **3c** as unique product. *S. platensis* grown in YMS-culture medium seemed to be able to oxidize **5a** and **6a** to give corresponding alcohols in low yields.

These microbial oxidations are completely regioselective with transformation of *tert*-butyl group only. Contrary to human metabolism, no N-dealkylation as the result of the hydroxylation in 4-position of *n*-butyl chain was detected. Side-reaction took place only during biotransformation of terfenadine **1a** by *S. platensis* grown in YM-culture medium with the formation of **3b** in low amount. The absence of **3a** suggested that **3b** was the result of the oxidation of **1b**. In other case, no transformation of hydroxyl or ketone group was observed.

The present data documented the structural substrate requirements needed to perform the microbial activity. The presence of hydrophobic group (substitution of piperidine ring) appeared to be required to hydroxylation with *A. corymbifera* and with *S. platensis*. Moreover, ketone analogues appeared better substrates than alcohol derivatives for oxidation by *S. platensis*, and an opposite specificity was observed for *A. corymbifera*.

Whatever culture condition and microorganisms, the oxidation of unsubstituted substrates **5a** and **6a** stopped at alcohol step and corresponding acids were never detected. The soybean peptones-induced oxidative activity did not allow further oxidation, but only to improve the rate of alcohols. In order to verify the inability in further oxidation, we tested the biotransformation of **5b** by *C. blakesleeana*, *C. echinulata* and *A. corymbifera*, and no formation of acid **5c** was observed. Except for experiment with *C. echinulata* grown in YMS-culture medium, the increase of concentration of **5a** to 0.696 mM led to a collapse of alcohol production. A similar inhibition by substrate had been observed in terfenadine oxidation by *S. platenis*.[25]

The enhancement of oxidative activity in cells grown in presence of soybean peptones could be explained by an induction of new enzymatic activity and/or by an increase of biomass weight. Induction of an oxidative activity in *S. platensis* was demonstrated by study of terfenadine oxidation with cells grown in YM-culture medium supplemented with genistein (final concentration of 50 μg mL^{-1}). Cells obtained in these conditions produced alcohol and acid

Table 1
Microbial oxidation products from **1a** to **6a** after 96 h incubation

Microorganisms[a]		**1a**		**2a**		**3a**		**4a**		**5a**		**6a**	
		1b	**1c**	**2b**	**2c**	**3b**	**3c**	**4b**	**4c**	**5b**	**5c**	**6b**	**6c**
Cunninghamella blakesleeana ATCC 8688a	YM	+[b]	−	+	−	+	−	+	−	+++ (−)[c]	−	+ (+)[c]	−
Cunninghamella blakesleeana ATCC 8688a	YMS	+	+	++	+	++	+	++	−	+++ (++)	−	+ (+)	−
Cunninghamella echinulata ATCC 9245	YM	−	−	+	−	−	−	−	−	++ (+)	−	+ (−)	−
Cunninghamella echinulata ATCC 9245	YMS	+	−	+	−	+	−	+	−	+++ (+++)	−	+ (+)	−
Streptomyces rimosus NRRL 2234	YM	−	−	−	−	+	−	−	−	− (−)	−	+ (−)	−
Streptomyces rimosus NRRL 2234	YMS	+	−	+	−	+	−	−	−	++ (+)	−	+ (+)	−
Absidia corymbifera LCP 63-1800	YM	++	++	++	++	++	+	++	++	+ (−)	−	− (−)	−
Absidia corymbifera LCP 63-1800	YMS	−	+++	−	+++	++	++	−	+++	+++ (−)	−	+ (−)	−
Streptomyces platensis NRRL 2364	YM	+++	−	++	−	++	++	++	+	++ (+)	−	− (−)	−
Streptomyces platensis NRRL 2364	YMS	++	++	++	++	−	+++	++	++	++ (++)	−	++ (−)	−

[a] Microorganisms were grown in culture medium without (YM) or with (YMS) soybean peptones.
[b] − no product formed, + product observed (<20%), ++ product present (20–50%) with other (substrate or metabolite), +++ unique product observed (>75%).
[c] Results obtained from experiments performed at 0.7 mM are given in parentheses.

Table 2
Preparative scale of alcohols and acids metabolites

Substrate[a]	Microorganisms	Culture medium	Incubation conditions		Product	Yield (%)
			[Biomass][b]	Time (h)		
1a	*Streptomyces platensis*	YM	2.7	72	Alcohol **1b**	51
1a	*Absidia corymbifera*	YMS	7.04	48	Acid **1c**	88
2a	*Streptomyces platensis*	YM	2.7	16	Alcohol **2b**	63
2a	*Absidia corymbifera*	YMS	7.04	48	Acid **2c**	80
3a	*Absidia corymbifera*	YM	6.9	18	Alcohol **3b**	49
3a	*Streptomyces platensis*	YMS	4.66	35	Acid **3c**	50
4a	*Streptomyces platensis*	YM	2.7	72	Alcohol **4b**	77
4a	*Absidia corymbifera*	YMS	7.04	90	Acid **4c**	52
5a	*Cunninghamella echinulata*	YMS	7.9	90	Alcohol **5b**	35

[a] The reactions were performed with 0.2 g/L substrate concentration (0.7 mM for **5a** and 0.424 mM for **1a**–**4a**).
[b] Dry weight (g/L).

metabolites. Their formation took place in similar rates of those observed with cells grown in YMS-culture medium.

2.3. Preparative scales of metabolites

Recently, we reported[25] the optimization of terfenadine hydroxylation by *S. platensis*. The best conditions for preparative scale of hydroxyterfenadine were obtained by incubation of cells in citrate buffer (pH 5, 0.1 M). These conditions were applied to the other microorganisms with success. Thereby the reactions were faster and/or work-up was easier (no or few emulsion during extraction) compared to incubations performed in culture medium. Results of preparative experiments were summarized in Table 2.

The preparations of substituted alcohols were performed by incubation of substrates with cells grown in YM-culture medium. Hydroxylation of **1a**, **2a** and **4a** by *S. platensis* furnished alcohols **1b**, **2b** and **4b** in 51%, 63% and 77% yield, respectively, and hydroxylation of **3a** by *A. corymbifera* gave alcohol **3b** in 49% yield. Preparation of unsubstituted alcohol metabolite **5b** was achieved using *C. echinulata* cells grown in YMS-culture medium with 35% yield. Syntheses of substituted acids were carried out with cells grown in culture medium containing soybean peptones using *A. corymbifera* except for the preparation of **3c**, which was obtained using *S. platensis*. Acids **1c**–**4c** were produced in three-step oxidation with 88% (lit.[20] 61%), 80% (lit.[23] 43%), 50% and 52% yield, respectively.

Thus, these purified metabolites were used as authentic samples in the study of the active site of CYP 2J2.[30,31]

3. Conclusion

We demonstrated the efficacy of biotransformation in synthetic purpose. First, because oxidation step of methyl group could be modulated following microorganism used and culture conditions, thus it was possible to prepare the alcohol or the acid metabolites. Besides, use of buffer permitted to shorten the incubation times, and the three-step oxidations gave the acid derivatives, fexofenadine and carebastine, in high yield. Secondly, microbial biotransformation of drugs was an efficient tool in the synthesis of intermediate and final metabolites. It presented a great interest in analytical chemistry to provide authentic samples and in preparative scale insofar as preparation of all drug metabolites is necessary to study their biological properties.[32,33]

4. Experimental section

4.1. Materials

Terfenadine was purchased from Sigma. Ebastine and fexofenadine were kindly provided from Pharmafarm Almirall Prodesfarma and Aventis Pharma, respectively. All organic solvents were purchased from SDS (Peyrin, France) and were of the highest purity available. Column chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). Thin-layer chromatography (TLC) was done on silica gel $60F_{254}$ (0.26 mm thickness). ^1H and ^{13}C NMR spectra were recorded on a Brucker ARX-250 spectrometer at 250.13 and 62.9 MHz, respectively. Chemical shifts are reported in parts per million using tetramethylsilane as an internal standard and the coupling constants *J* are given in hertz.

An HPLC analysis system was developed for separation of all substrates and their metabolites. The system used was Gilson with pump 305, pump 306, gradient dynamic mixer 811B and auto-injector 234. The system was controlled and the results were analyzed by Unipoint Gilson software. The column (Agilent, C18, 5 μm (250×4.6)) was in oven (Shimadzu CTO-10A model) at 40 °C and eluted with a appropriate gradient solvent system: isocratic solvent A (70% NH_4OAc 0.1 M/30% CH_3CN) for **1a**, **2a**, **3a** and **4a** or solvent A′ (90% NH_4OAc 0.1 M/10% CH_3CN) for **5a** and **6a** for 7 min followed by gradient to 100% solvent B (40% NH_4OAc 0.1 M/60% CH_3CN) in 5 min and held at 100% (B) 18 min. The detection was at UV 230 nm for substrates **1a**, **4a** and **6a**, and 250 nm for substrates **2a**, **3a** and **5a** and sample volumes were 20 μL.

LC-MS data were recorded with a Surveyor-LCQ Advantage mass spectrometer, using same conditions except a flow rate of 0.5 mL/min and UV detection. The MS ionization was carried out using an ESI source in positive mode with a capillary temperature of 275 °C, a capillary voltage of 41 V, and a spray voltage of 5 kV. MS2 analyses were performed with a fragmentation power set to 30–40%, depending on the compound analyzed.

4.2. Chemical synthesis of terfenadine and ebastine analogues

*4.2.1. 1-(4-tert-Butylphenyl)-4-(4-(hydroxydiphenylmethyl) piperidin-1-yl)butanone (**3a**)*

A mixture of 1-(4-*tert*-butylphenyl)-4-chlorobutanone (942 mg, 4 mmol), azacyclonol (1.28 g, 4.8 mmol), sodium carbonate (840 mg, 7.93 mmol), sodium iodide (100 mg, 0.66 mmol) in dry *N*,*N*-dimethylformamide (10 mL) was refluxed under nitrogen for 24 h. After cooling, the reaction mixture was diluted with dichloromethane (50 mL), washed with water and brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The crude product was purified by chromatography on silica gel and eluted with dichloromethane/methanol (90:10) to give **3a** (1.32 g, 69%) as a white solid. Mp 139–141 °C; ^1H NMR (250.13 MHz, $CDCl_3$): δ=7.95 (d, *J*=8.28 Hz, 2H, *H*ar), 7.54–7.18 (m, 12H, *H*ar), 3.02–2.96 (m, 4H, C*H*NC*H*′, CH_2CO), 2.46–2.40 (m, 3H, $CH_2(CH_2)_2CO$, C*H*), 2.08–1.90 (m, 4H, C*H*NC*H*′, CH_2CH_2CO), 1.54–1.46 (m, 4H, CH_2CH_2N), 1.39 (s, 9H, CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=200.2

(CO), 156.9, 146.5, 135.1, 128.5, 126.9, 126.2, 125.9, 79.9, 58.4, 54.4, 44.6, 36.7, 35.5, 31.5, 26.7, 22.4. Anal. Calcd for $C_{32}H_{39}NO_2 \cdot 0.5H_2O$: C 80.29, H 8.42, N 2.93. Found: C 80.46, H 8.26, N 2.95. HRMS *m*/*z* calcd for $C_{32}H_{40}NO_2$ $[M+H]^+$: 470.3054. Found: 470.3059.

4.2.2. 4-(4-(Benzhydryloxy)piperidin-1-yl)-1-(4-tert-butylphenyl)butanol (**4a**)

Sodium borohydride (40.3 mg, 1.065 mmol) was added in small portion to a cooled (0 °C) and stirred solution of ebastine **2a** (200 mg, 0.426 mmol) in methanol (4 mL). After 6 h, the excess hydride was destroyed using HCl (1 N), and water was added. The product was extracted with ethyl acetate (three times) and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel (dichloromethane/methanol: 95:5) to afford **4a** (190.6 mg, 95%) as a white solid. Mp 124–126 °C; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=7.39–7.24 (m, 14H, *H*ar), 5.50 (s, 1H, C*H*(Ar)$_2$O), 4.71–4.69 (m, 1H, C*H*OH), 3.71–3.62 (m, 1H, C*H*O), 3.02–2.88 (m, 2H, C*H*NC*H*′), 2.82–2.62 (m, 4H, C*H*NC*H*′, C*H*$_2$(CH$_2$)$_2$CHOH), 2.19–2.10 (m, 2H, C*H*CH$_2$NCH$_2$C*H*′), 2.00–1.81 (m, 6H, C*H*CH$_2$NCH$_2$C*H*′, C*H*$_2$C*H*$_2$CHOH), 1.34 (s, 9H, C*H*$_3$); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=150.1, 143.3, 128.9, 128.0, 127.6, 126.0, 125.6, 80.8, 73.9 (CHOH), 71.4, 59.2, 50.9, 39.8, 35.0, 32.0 (CH_3), 30.9, 24.3. MS (ESI): *m*/*z* 472 $(M+H^+)$; Anal. Calcd for $C_{32}H_{41}NO_2 \cdot 0.5H_2O$: C 79.96, H 8.81, N 2.91. Found: C 80.01, H 9.06, N 2.67. HRMS *m*/*z* calcd for $C_{32}H_{42}NO_2$ $[M+H]^+$: 472.3198. Found: 472.3216.

4.2.3. 1-(4-tert-Butylphenyl)-4-(piperidin-1-yl)butanone (**5a**)

Piperidine (573.3 μL, 5.79 mmol) and anhydrous potassium carbonate (1.348 g, 9.75 mmol) were added to a solution of 1-(4-*tert*-butylphenyl)-4-chlorobutanone (800 mg, 3.35 mmol) in toluene (31 mL) and water (12 mL), and mixture was heated to 100 °C until TLC (eluent: dichloromethane/methanol/ammoniac: 93:7:0.5%) showed no or only traces of starting material (about six days). The solution was cooled to room temperature and diluted with ethyl acetate and water. The organic phase was separated then washed with brine, dried over sodium sulfate and evaporated under reduce pressure. Purification by chromatography on silica gel (dichloromethane/methanol: 97:3) yielded a thick brown oil (707 mg, 73%), which was recrystallized from acetone/pentane to give **5a** (577.7 mg, 60%) as a pale yellow powder. Mp 148 °C (decomp.); ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=7.81 (d, *J*=8.45 Hz, 2H, Har), 7.36 (d, *J*=8.45 Hz, 2H, Har), 2.89 (t, *J*=7.14 Hz, 2H, C*H*$_2$CO), 2.36–2.30 (m, 6H, 3C*H*$_2$N), 1.93–1.81 (m, 2H, C*H*$_2$CH$_2$CO), 1.54–1.45 (m, 4H, 2C*H*$_2$CH$_2$N), 1.35–1.33 (m, 2H, CH$_2$C*H*$_2$CH$_2$N), 1.24 (s, 9H, CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=198.6 (CO), 155.5, 133.5, 127.0, 124.4, 57.4, 53.3, 35.3, 34.0, 30.1 (CH_3), 24.6, 23.3, 20.4. HRMS *m*/*z* calcd for $C_{19}H_{30}NO$ $[M+H]^+$: 288.2327. Found: 288.2314.

4.2.4. 1-(4-tert-Butylphenyl)-4-(piperidin-1-yl)butanol (**6a**)

A solution of **5a** (250 mg, 0.87 mmol) in methanol (10 mL) was cooled to 0 °C and sodium borohydride (98.74 mg, 2.61 mmol) was added in small portions and mixture was stirred for 4 h. Aqueous HCl (1 M) was added then the solvent was evaporated in vacuo. The residue was diluted with ethyl acetate and water, the organic layer was separated, washed with brine, dried over sodium sulfate and concentrated. The crude product was chromatographed on silica gel (dichloromethane/methanol: 98:2) to afford a brown oil (201.4 mg, 80%), which was recrystallized from acetone/pentane to give **6a** (138.5 mg, 55%) as a white powder. Mp 94–96 °C; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=7.35 (d, *J*=8.21 Hz, 2H, *H*ar), 7.27 (d, *J*=8.21 Hz, 2H, *H*ar), 4.69–4.66 (m, 1H, C*H*OH), 2.65–2.59 (m, 2H, C*H*NC*H*′), 2.49–2.45 (m, 4H, C*H*NC*H*′, C*H*$_2$(CH$_2$)$_2$CHOH), 2.04–1.84 (m, 2H, C*H*$_2$CHOH), 1.75–1.71 (m, 6H, C*H*$_2$CH$_2$CHOH, 2C*H*$_2$CH$_2$N), 1.53–1.51 (m, 2H, C*H*$_2$CH$_2$CH$_2$N), 1.34 (s, 9H, C*H*$_3$); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=149.8, 143.2, 125.8, 125.4, 73.7 (CHOH), 59.6, 54.7, 39.9, 34.8, 31.8, 25.6, 24.4, 24.2. HRMS *m*/*z* calcd for $C_{19}H_{32}NO$ $[M+H]^+$: 290.2484. Found: 290.2473.

4.3. Biotransformations

Cultures were maintained on agar slants (filamentous fungi: bactopeptones 5 g/L, yeast extract 5 g/L, malt extract 5 g/L, glucose 20 g/L and agar 20 g/L; *Streptomyces* bacteria: ISP medium 2) and stored at 4 °C. Liquid culture media containing (g/L) glucose 16, yeast extract 4, malt extract 10 (YM medium) and glucose 16, yeast extract 4, malt extract 10 and soybean peptones 5 (YMS medium) were sterilized without glucose at 120 °C for 20 min. Separately sterilized glucose solution was added afterwards.

4.3.1. Analytical studies

Flasks (25 mL) containing 10 mL of YM or YMS-culture medium were inoculated with glycerol suspension of tested microorganism and incubated at 30 °C and 200 rpm (orbital shaker). After 60 h, the substrate dissolved in 20 μL of *N*,*N*-dimethylformamide was added to the culture medium (final concentration 0.424 mM or 0.696 mM). The biotransformations were performed in the same conditions and daily monitored by HPLC. Samples (800 μL) were diluted with methanol (700 μL), mixed vigorously and centrifuged at 10,000 g for 5 min. The resulting supernatants were micro-filtered (0.45 μm) before analysis. After 96 h of incubation, the biotransformation was stopped by addition of a mixture of AcOEt/Et_2O/MeOH (5:5:1; 20 mL), the suspension was stirred over 20 min and filtered through Celite. The organic layer was separated, dried over magnesium sulfate and concentrated. The residue was then taken up with methanol (700 μL), micro-filtered and analyzed by HPLC.

4.3.2. Preparative scales

Microorganisms were cultured 48 h in 0.5 L of culture medium (YM or YMS) at 30 °C in 2-L erlenmeyer and put in orbital shaker (200 rpm). For *Streptomyces* species, broth was inoculated by preculture (40 mL/L). Biomass was collected by centrifugation (6000 g, 25 min) for *Streptomyces* or by filtration for fungi. Cells were then suspended in citrate buffer (0.1 M, pH 5) and the substrate dissolved in DMF was added (final concentration 0.2 g/L). Biotransformations were performed at 30 °C in an orbital shaker for suitable time, depending on desired product (Table 2). Biomass was harvested by centrifugation or filtration, suspended in methanol (150 mL), stirred (1 h) and removed. Methanol was evaporated and the residue was taken up with ethyl acetate and washed with brine. Supernatant was saturated (NaCl) and pH was adjusted at 10 with concentrated NaOH in alcohol production or at 1–2 with HCl 5 N in acid production. Aqueous phase was extracted with ethyl acetate (three times) and organic phases were combined, dried over $NaSO_4$. After evaporation, the product was purified by flash chromatography, elution with CH_2Cl_2/CH_3OH 9:1 (alcohol) or CH_2Cl_2/CH_3OH 9:1 containing acetic acid 1% (acid).

*4.3.2.1. 1-(4-(1-Hydroxy-2-methylpropan-2-yl)phenyl)-4-(4-(hydroxydiphenylmethyl)piperidin-1-yl) butan-1-ol (**1b**).* Incubation of terfenadine **1a** (200 mg, 0.424 mmol) with cells of *Streptomyces platensis* NRRL 2364 obtained in YM-culture medium (1 L) and suspended in buffer (1 L) afforded **1b** (105.4 mg, 51%) as a white powder. Mp 104–109 °C (decomp.); ^{1}H NMR ($CDCl_3$, 250.13 MHz): δ=7.60–7.13 (m, 14H, *H*ar), 4.67–4.53 (m, 1H, C*H*OH), 3.56 (s, 2H, C*H*$_2$OH), 3.26–2.97 (m, 2H, C*H*NC*H*′), 2.62–2.40 (m, 3H, C*H*$_2$(CH$_2$)$_2$CHOH, C*H*), 2.31–2.02 (m, 2H, C*H*NC*H*′), 1.97–1.60 (m, 6H, C*H*CH$_2$NCH$_2$C*H*′, C*H*$_2$C*H*$_2$CHOH), 1.58–1.44 (m, 2H, C*H*CH$_2$NCH$_2$C*H*′), 1.32 (s, 6H, 2C*H*$_3$); NMR ($CDCl_3$, 62.9 MHz) δ=146.6, 145.5, 143.7, 128.6, 126.8, 126.5, 126.2, 126.1, 79.6, 73.7 (CHOH), 73.3 (CH_2OH), 58.7, 53.4, 44.1, 40.2, 39.5, 25.9, 25.7, 24.0.

*4.3.2.2. 2-(4-(1-Hydroxy-4-(4-(hydroxydiphenylmethyl)piperidin-1-yl)butyl)phenyl)-2-methyl propanoic acid (**1c**).* Incubation of terfenadine **1a** (200 mg, 0.425 mmol) with mycelium of *A. corymbifera* LCP 63-1800 obtained in YMS-culture medium (1 L) and suspended in buffer (1 L), afforded fexofenadine **1c** (187 mg, 88%). Mp 193–195 °C; ^{1}H NMR (250.13 MHz, CD_3OD): δ=7.55 (m, 4H, *H*ar), 7.40 (m, 2H), 7.30 (m, 6H, *H*ar), 7.18 (d, 2H, *J*=7 Hz, *H*ar), 4.61 (dd, 1H, *J*=5.6, 6 Hz, C*H*OH), 3.32–3.30 (m, 2H, CH_2N), 2.90–2.75 (m, 5H), 1.82–1.55 (m, 8H), 1.50 (s, 6H, CH_3); ^{13}C NMR (62.9 MHz, CD_3OD): δ=186.3 (COOH), 149.8, 149.3, 145.5, 130.5, 129.0, 128.5, 81.5, 76.0 (CHOH), 59.0, 55.5, 51.3, 44.5, 39.5, 29.9, 27.3, 23.5.

*4.3.2.3. 4-(4-(Benzhydryloxy)piperidin-1-yl)-1-(4-(1-hydroxy-2-methylpropan-2-yl)phenyl)butan-1-one (**2b**).* Incubation of ebastine **2a** (100 mg, 0.213 mmol) with cells of *Streptomyces platensis* NRRL 2364 obtained in YM-culture medium (500 mL) and suspended in buffer (500 mL) afforded hydroxyebastine **2b** (65 mg, 63%). Mp 52 °C (decomp.); ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=7.97 (d, *J*=8.36 Hz, 2H, *H*ar), 7.51 (d, *J*=8.36 Hz, 2H, *H*ar), 7.37–7.25 (m, 10H, *H*ar), 5.55 (s, 1H, C*H*(Ar)$_2$O), 3.67 (s, 2H, CH_2OH), 3.48–3.41 (m, 1H, C*H*O), 3.00 (t, *J*=7.08 Hz, 2H, CH_2CO), 2.80–2.75 (m, 2H, C*H*NC*H*′), 2.40 (t, *J*=7.10 Hz, 2H, CH_2(CH_2)$_2$CO), 2.17–2.09 (m, 2H, C*H*NC*H*′), 2.00–1.82 (m, 4H, C*H*CH$_2$NCH$_2$C*H*′, CH_2CH$_2$CO), 1.74–1.62 (m, 2H, C*H*CH$_2$NCH$_2$C*H*′), 1.39 (s, 6H, CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=200.2 (CO), 152.5, 143.2, 135.5, 128.7, 128.6, 127.7, 127.5, 126.8, 80.4, 73.1 (CH$_2$OH), 72.2, 58.1, 51.5, 40.9, 36.7, 31.7, 25.6 (CH$_3$), 22.4; MS (ESI): *m*/*z* 486 (M+H$^+$). Anal. Calcd for $C_{32}H_{39}NO_3\cdot 0.5H_2O$: C 77.70, H 8.15, N 2.83. Found: C 77.81, H 8.41, N 2.66. HRMS *m*/*z* calcd for $C_{32}H_{40}NO_3$ [M+H]$^+$: 486.3008. Found: 486.2995.

*4.3.2.4. 2-(4-(4-(4-(Benzhydryloxy)piperidin-1-yl)butanoyl)phenyl)-2-methylpropanoic acid (**2c**).* Incubation of ebastine **2a** (100 mg, 0,21 mmol) with mycelium of *A. corymbifera* LCP 63-1800 obtained in YMS-culture medium (500 mL) and suspended in buffer (500 mL), afforded carebastine **2c** (84 mg, 80%). Mp 173–175 °C; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=7.75 (d, *J*=8.45 Hz, 2H, *H*ar), 7.57 (d, *J*=8.46 Hz, 2H, *H*ar), 7.36–7.24 (m, 10H, *H*ar), 5.48 (s, 1H, C*H*(Ar)$_2$O), 3.73 (m, 1H, C*H*O), 3.05–3.02 (m, 2H, C*H*NC*H*′), 2.77–2.66 (m, 4H, CH_2CH$_2$CH_2CO), 2.20–2.07 (m, 2H, C*H*CH$_2$NCH$_2$C*H*′), 2.00–1.81 (m, 4H, C*H*CH$_2$NCH$_2$C*H*′, CH_2CH$_2$CO), 1.58 (s, 6H, CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=198.5 (CO), 181.7 (COOH), 154.2, 142.6, 134.3, 128.9, 128.1, 128.0, 127.2, 126.7, 81.2, 69.0, 56.6, 48.6, 48.0, 36.0, 28.7, 27.1, 19.3.

*4.3.2.5. 1-(4-(1-Hydroxy-2-methylpropan-2-yl)phenyl)-4-(4-(hydroxydiphenylmethyl)piperidin-1-yl)butan-1-one (**3b**).* Incubation of terfenadone **3a** (200 mg, 0.426 mmol) with mycelium of *A. corymbifera* LCP 63-1800 obtained in YM-culture medium (1 L) and suspended in buffer (1 L), afforded **3b** (101.2 mg, 49%) as a white solid. Mp 149–151 °C (lit.[34] 135–137 °C); ^{1}H NMR (250.13 MHz, CD_3OD): δ=7.95 (d, *J*=8.38 Hz, 2H, *H*ar), 7.57–7.14 (m, 12H, *H*ar), 3.62 (s, 2H, CH_2OH), 3.24–3.19 (m, 2H, C*H*NC*H*′), 3.08 (t, *J*=6.60 Hz, 2H, CH_2CO), 2.72–2.66 (m, 3H, C*H*(CH_2)$_2$N, CH_2(CH_2)$_2$CO), 2.45–2.37 (m, 2H, C*H*NC*H*′), 2.04–1.92 (m, 2H, CH_2CH$_2$CO), 1.73–1.62 (m, 4H, CH_2CH$_2$N), 1.35 (s, 6H, CH_3); ^{13}C NMR (62.9 MHz, CD_3OD): δ=201.7 (CO), 155.3, 148.0, 136.2, 129.4, 128.1, 127.8, 127.6, 80.6, 73.0 (CH$_2$OH), 58.8, 55.1, 44.8, 41.8, 36.6, 27.0, 26.2 (CH$_3$), 21.7. MS (ESI) *m*/*z* 486 (M+H$^+$), 468, 219. HRMS calcd for $C_{32}H_{40}N0_3$ (MH$^+$): 486.3008. Found: 486.3012.

*4.3.2.6. 2-(4-(4-(4-(Hydroxydiphenylmethyl)piperidin-1-yl)butanoyl)phenyl)-2-methylpropanoic acid (**3c**).* Incubation of terfenadone **3a** (200 mg, 0.426 mmol) with cells of *Streptomyces platensis* NRRL 2364 obtained in YM-culture medium (1 L) and suspended in buffer (1 L) afforded **3c** (106 mg, 50%) as a pale yellow solid. Mp 164–166 °C; ^{1}H NMR (250.13 MHz, CD_3OD): δ=7.98 (d, *J*=8.56 Hz, 2H, *H*ar), 7.59–7.01 (m, 12H, *H*ar), 3.64–3.59 (m, 2H, C*H*NC*H*′), 3.22–3.04 (m, 6H, CH_2CO, C*H*NC*H*′, CH_2(CH_2)$_2$CO), 2.92–2.84 (m, 1H, C*H*), 2.11–2.09 (m, 2H, CH_2CH$_2$CO), 1.89–1.79 (m, 4H, CH_2CH$_2$N), 1.60 (s, 6H, CH_3); ^{13}C NMR (62.9 MHz, CD_3OD): δ=200.4 (CO), 180.3 (COOH), 152.9, 147.5, 136.6, 129.6, 128.0, 127.7, 127.5, 80.2, 57.8, 54.4, 46.0, 43.1, 36.5, 27.4 (CH$_3$), 25.9, 20.0. HRMS *m*/*z* calcd for $C_{32}H_{38}NO_4$ [M+H]$^+$: 500.2801. Found: 500.2805.

*4.3.2.7. 4-(4-(Benzhydryloxy)piperidin-1-yl)-1-(4-(1-hydroxy-2-methylpropan-2-yl)phenyl)butan-1-ol (**4b**).* Incubation of ebastol **4a** (70 mg, 0.148 mmol) with cells of *Streptomyces platensis* NRRL 2364 obtained in YM-culture medium (400 mL) and suspended in buffer (350 mL) afforded hydroxyebastol **4b** (56 mg, 77%) as a white solid. Mp 68 °C (decomp.); ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=7.36–7.24 (m, 14H, *H*ar), 5.50 (s, 1H, C*H*(Ar)$_2$O), 4.68–4.66 (m, 1H, C*H*OH), 3.65–3.61 (m, 1H, C*H*O), 3.59 (s, 2H, CH_2OH), 3.00–2.84 (m, 2H, C*H*NC*H*′), 2.75–2.60 (m, 4H, C*H*NC*H*′, CH_2(CH_2)$_2$CHOH), 2.17–2.00 (m, 2H, C*H*CH$_2$NCH$_2$C*H*′), 1.92–1.80 (m, 6H, C*H*CH$_2$NCH$_2$C*H*′, CH_2CH$_2$CHOH), 1.34 (s, 6H, CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=145.4, 143.6, 142.9, 128.8, 127.8, 127.4, 126.5, 126.2, 80.8 (CH(Ar)$_2$O), 73.5 (CHOH), 73.4 (CH$_2$OH), 70.7 (CHO), 58.7, 50.3, 40.2, 38.9, 30.2, 25.8 (CH$_3$), 23.5; MS (ESI): *m*/*z* 488 (M+H$^+$). Anal. Calcd for $C_{32}H_{41}NO_3\cdot H_2O$: C 76.00, H 8.57, N 2.77. Found: C 75.51, H 8.54, N 2.61. HRMS calcd for $C_{32}H_{42}N0_3$ (M+H$^+$): 488.3165. Found: 488.3165.

*4.3.2.8. 2-(4-(4-(4-(Benzhydryloxy)piperidin-1-yl)-1-hydroxybutyl)phenyl)-2-methylpropanoic acid (**4c**).* Incubation of ebastol **4a** (82 mg, 0.174 mmol) with mycelium of *A. corymbifera* LCP 63-1800 obtained in YMS-culture medium (500 mL) and suspended in buffer (410 mL), afforded **4c** (46.4 mg, 52%) as a slightly coloured solid. Mp 119–120 °C; ^{1}H NMR (250.13 MHz, CD_3OD): δ=7.41–7.26 (m, 14H, Har), 5.62 (s, 1H, C*H*(Ar)$_2$O), 4.71–4.67 (m, 1H, C*H*OH), 3.81–3.70 (m, 1H, C*H*O), 3.37–3.22 (m, 2H, C*H*NC*H*′), 3.14–3.02 (m, 4H, C*H*NC*H*′, CH_2(CH_2)$_2$CHOH), 2.02–1.96 (m, 4H, CH_2CH$_2$N), 1.85–1.76 (m, 4H, CH_2CH_2CHOH), 1.53 (s, 6H, CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=181.2 (COOH), 145.3, 142.9, 142.4, 128.9, 128.1, 127.2, 126.2, 126.1, 81.4 (CH(Ar)$_2$O), 72.9 (CHOH), 67.8 (CHO), 57.3, 48.8, 46.9, 36.1, 28.0, 27.1 (CH$_3$), 21.1; HRMS calcd for $C_{32}H_{40}N0_4$ (MH$^+$): 502.2957. Found: 502.2946.

*4.3.2.9. 1-(4-(1-Hydroxy-2-methylpropan-2-yl)phenyl)-4-(piperidin-1-yl)butanone (**5b**).* Incubation of **5a** (200 mg, 0.696 mmol) with mycelium of *Cunninghamella echinulata* ATCC 9245 obtained in YMS-culture medium (1 L) and suspended in buffer (1 L) afforded **5b** (74 mg, 35%) as a yellow oil. ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=7.93 (d, *J*=8.35 Hz, 2H, *H*ar), 7.51 (d, *J*=8.36 Hz, 2H, *H*ar), 3.66 (s, 2H, CH_2OH), 3.12 (t, *J*=6.54 Hz, 2H, CH_2CO), 2.92–2.85 (m, 6H, CH_2N), 2.24–2.15 (m, 2H, CH_2CH$_2$CO), 1.91–1.88 (m, 4H, CH_2CH$_2$N), 1.59–1.57 (m, 2H, CH_2CH$_2$CH$_2$N), 1.36 (s, 6H, CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=199.1 (CO), 153.4, 134.7, 128.5, 127.0, 72.8 (CH$_2$OH), 57.4, 53.8, 40.9, 36.0, 25.6, 23.8, 23.0, 19.3; MS (ESI): *m*/*z* 304 (M+H$^+$); HRMS: calcd for $C_{19}H_{30}N0_2$ (MH$^+$): 304.2277. Found: 304.2268.

Acknowledgements

We thank Mrs. A. Triclin for technical assistance, and Drs. Pierre Lafite, Patrick Dansette and Dr. Chantal Blandin for fruitful discussions. Part of this work was financially supported by RDR2 '*Aller vers une Chimie écocompatible*'.

References and notes

1. Yun, C. H.; Okerholm, R. A.; Guengerich, F. P. *Drug Metab. Dispos.* **1993**, *21*, 403–409.

2. Ling, K. H. J.; Leeson, G. A.; Burmaster, S. D.; Hook, R. H.; Reith, M. K.; Cheng, L. K. *Drug Metab. Dispos.* **1995**, *23*, 631–636.
3. Jones, B. C.; Hyland, R.; Ackland, M.; Tyman, C. H.; Smith, D. A. *Drug Metab. Dispos.* **1998**, *29*, 875–882.
4. Hashizume, T.; Imaoka, S.; Mise, M.; Terauchi, Y.; Fujii, T.; Miyazaki, H.; Kamataki, T.; Funae, Y. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 298–304.
5. Pohjola-Sintonen, S.; Viitasalo, M.; Toivonen, L. *Eur. J. Clin. Pharm.* **1993**, *45*, 191–193.
6. Jurima-Romet, M.; Crawford, K.; Cyr, T.; Inaba, T. *Drug Metab. Dispos.* **1994**, *22*, 849–857.
7. Monahan, B. P.; Ferguson, C. L.; Killeavy, E. S.; Lloyd, B. K.; Troy, J.; Cantilena, L. R. J. *JAMA* **1991**, *266*, 2375–2376.
8. Markham, A.; Wagstaff, A. *Drugs* **1998**, *55*, 269–274.
9. D'Ambra, T. E. U.S. Patent 5,578,610, 1996.
10. Jaweed Mukarram, S. M.; Merwade, A. Y.; Kahan, A. R.; Solanki, P. V. WO Patent 007347, 2007.
11. Kawai, S. H.; Hambalek, R. J.; Just, G. *J. Org. Chem.* **1994**, *59*, 2620–2622.
12. Patel, S.; Waykole, L.; Repic, O.; Chen, K.-M. *Synth. Commun.* **1996**, *26*, 4699–4710.
13. Fang, Q. K.; Senanayake, C. H.; Wilkinson, H. S.; Wald, S. A.; Li, H. *Tetrahedron Lett.* **1998**, *39*, 2701–2704.
14. Di Giacomo, B.; Coletta, D.; Natalini, B.; Ni, M.-H.; Pellicciari, R. *Il Farmaco* **1999**, *54*, 600–610.
15. Smith, R. V.; Rosazza, J. P. *J. Pharm. Sci.* **1975**, *64*, 1737–1759.
16. Azerad, R. *Adv. Biochem. Eng./Biotechnol.* **1999**, *63*, 169–218.
17. Abourahed, E. A.; Clark, A. M.; Hofford, C. D. *Curr. Med. Chem.* **1999**, *6*, 359–374.
18. *Modern Biooxidation*; Schmid, R., Urlacher, V., Eds; Wiley-VCH Gmbh & Co: Weinheim, Germany, 2007.
19. Meiwes, J.; Worm, M. U.S. Patent 5,990,127, 1999.
20. Azerad, R.; Biton, J.; Lacroix, I. WO Patent 47693, 1999.
21. Michels, P. C.; Zirbes, E. L. U.S. Patent 2002/0087003, 2002.
22. Schwartz, H.; Böttcher, H. U.S. Patent 5,204,249, 1993.
23. Schwartz, H.; Liebig-Weber, A.; Hochstätter, H.; Böttcher, H. *Appl. Microbiol. Biotechnol.* **1996**, *44*, 731–735.
24. Mazier, C.; Jaouen, M.; Sari, M.-A.; Buisson, D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5423–5426.
25. Mazier, C.; Lombard, M.; Sari, M.-A.; Buisson, D. *Biocatal. Biotransform.* **2007**, *25*, 401–407.
26. Ayers, A.; Brown, W. U.S. Patent 0,022,919, 2003.
27. Sariaslani, F. S.; Kunz, D. A. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 405–410.
28. Schwartz, H.; Licht, R. E.; Radunz, H. E. *Appl. Microbiol. Biotechnol.* **1993**, *40*, 382–385.
29. Schmitz, G.; Franke, D.; Stevens, S.; Takors, R.; Weuster-Botz, D.; Wandrey, C. *J. Mol. Catal. B: Enzym.* **2000**, *10*, 313–324.
30. Lafite, P.; Dijols, S.; Buisson, D.; Macherey, A. C.; Zeldin, D. C.; Dansette, P. M.; Mansuy, D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2777–2780.
31. Lafite, P.; Dijols, S.; Zeldin, D. C.; Dansette, P. M.; Mansuy, D. *Arch. Biochem. Biophys.* **2007**, *464*, 155–168.
32. Raeissi, S. D.; Hidalgo, I. J.; Segura-Aguilar, J.; Artursson, P. *Pharm. Res.* **1999**, *16*, 625–632.
33. Imamura, Y.; Shimizu, K.; Yamashita, F.; Yamaoka, K.; Takakura, Y.; Hashida, M. *Biol. Pharm. Bull.* **2001**, *24*, 930–934.
34. King, C.-H.; Kaminski, M. WO Patent 93/21156, 1993.