Enzymatic Hydrolysis of Esters Containing a Tetrazole Ring

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ABSTRACT The lipase-catalyzed enantioselective hydrolysis of acetates containing tetrazole moiety was studied. Among all tested lipases, Novozyme SP 435 allowed to obtain optically active 4-(5-aryl-2*H*-tetrazol-2yl)butan-2-ol and 1-(5-aryl-2*H*-tetrazol-2yl)-propan-2-ol and their acetates with the highest optical purities (ee = 95%-99%) and excellent enantioselectivity (E>100). Some of the synthesized tetrazole derivatives were screened for their antifungal activity. Racemic mixtures of 4-[5-(4-chlorophenyl)-2*H*-tetrazol-2-yl)butan-2-ol as well as pure enantiomers of this compound showed promising antifungal activity against *F. sambucinum*, *F. oxysporum*, *C. coccodes*, and *A. niger*. *Chirality 26:811–816, 2014*.

KEY WORDS: azole; enantioseparation; lipase; antifungal activity; kinetic resolution

INTRODUCTION

Tetrazole derivatives exhibit diverse biological and pharmaceutical activity. This ring system is present in drugs[1–4] and in many inhibitors of enzymes.[5–12] Moreover, some tetrazole derivatives possess analgesic,[13] antiinflammatory,[14–16] antiallergic,[17,18] antibacterial,[19–24] and antifungal activity.[20–31] The last one is extremely valuable, because of the growing number of systemic fungal infections caused by *Aspergillus sp*., *Candida sp*., *Cryptococcus sp*., and other species.[32] This kind of infection is especially dangerous for populations with altered immunity. There are known tetrazole derivatives with activity higher or comparable with common antifungal agents like intraconazole, ketoconazole, fluconazole, and griseofulvin.[20–31] An interesting group of these compounds constitute 5-(phenylselanylmethyl)-1*H*-tetrazole and 1-(2,4-dihydroxy-thiobenzoyl) tetrazole with high activity against various *Candida* species.[25,31] Other useful antifungal agents are 5-thio-substituded tetrazole derivatives exhibiting high activity against some *Aspergillus* and *Penicillium* species and 3-aryl-1-(5-phenyl-1*H*-tetrazol-1-yl)prop-2-en-1-ones which appeared to be the most active against *Candida albicans* and *Aspergillus niger*.[20,30] Many newly designed tetrazole derivatives possess common azole substructures seen in other antifungals. Among the noteworthy structures are (2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol and (2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol, inhibiting growth of *Candida*, *Aspergillus*, and *Cryptococcus* species.[26] Moreover, antifungal tetrazoles containing a benzotriazole and benzimidazole moiety are known.[22–24] A broad spectrum of biological activities of compounds containing a tetrazole ring in the molecule prompted us to synthesize and evaluate activities of 5-(aryltetrazolyl-2)-alkanols. Recently,[33] we described a chemoenzymatic procedure for the preparation of enantiomerically enriched 4-(5-aryl-2*H*-tetrazol-2-yl)butan-2-ol and 1-(5-aryl-2*H*-tetrazol-2-yl)propan-2-ol and their acetates. The enzymatic step involves enantioselective acetylation of appropriate alcohols catalyzed by commercially available lipases. Screening the biological activities of the synthesized racemic mixtures of alcohols containing a tetrazole ring, we found interesting properties in some of them. To develop more precise investigation, single enantiomers of sufficiently high enantiomeric purity are required, and we need better than the described[33] procedure for enantiomers separation.

Herein, we report a lipase-catalyzed hydrolysis as an alternative method for the preparation of optically active alcohols containing a tetrazole ring. Additionally, the evaluation of the antifungal properties of synthesized tetrazole derivatives is described.

MATERIALS AND METHODS

Chemicals and Reagents

The 5-substituted tetrazoles were prepared in high yields (64–91%) from commercially available nitriles, NaN_3 and NH_4Cl in dimethylformamide (DMF) according to the described[34] method. Amano AK (native lipase from *Pseudomonas fluorescens*), Amano PS (native lipase from *Burkholderia cepacia*, earlier *Pseudomonas* cepacia) were purchased from Sigma-Aldrich (Germany). Novozyme SP 435 (lipase from *C. antarctica*-B immobilized on a macroporous acrylic resin) was purchased from Novozymes (Denmark). All commercially available reagents were obtained from Sigma-Aldrich (Germany), Merck (Germany), and POCH (Poland), and were used without further purification.

Apparatus and Materials

^1H (400 MHz) and ^{13}C (100 MHz) nuclear magnetic resonance (NMR) spectra were recorded on Varian Mercury 400 MHz spectrometer (Palo Alto, CA) in $CDCl_3$ solution; chemical shifts (δ) are reported in ppm; coupling constants (*J*) are given in hertz. Ee's of the alcohols **2a-d**, **4b-d**. and esters **3c-d**, were determined on a Shimadzu high-performance liquid chromatography (HPLC) apparatus (Japan) consisting of an LC-20AD chromatograph, CTO-10AS oven, SPD-20A UV detector, and Chiralcel OD-H column (in hexane:ethanol 95:5; 0.8 mL/min for alcohols **2a-c**, hexane:*iso*-propanol 95:5; 0.8 mL/min for alcohols **2d, 4c-d** and esters **3c-d**, hexane:*iso*-propanol 97:3 for alcohol **4b**) using the corresponding racemic compounds as references. Optical rotations were measured with an ATAGO AP-300 automatic polarimeter (Japan). The reactions were monitored by thin-layer chromatography (TLC) aluminum plates with silica gel Kieselgel 60 F_{254} (0.2 mm thickness film, Merck, Germany) using UV light as visualizing agent and by gas chromatography (GC) with Hewlett-Packard Model 5890 II chromatograph with helium as the carrier gas. Column chromatography was performed using Kieselgel 60 (0.040-0.063 mm, Merck, Germany).

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General Procedure for the Enzyme-Catalyzed Hydrolysis of Esters (±)-1a-d and (±)-3a-d

Acetate (±)–**1a–d** or (±)-**3a-d** (1.5 mmol) was dissolved in 20 mL of TBME (*tert*-butyl methyl ether). The solution was mixed with 0.1 M phosphate buffer (50 mL, pH = 7.2) and 40 mg of enzyme was added. The mixture was stirred at room temperature (25°C) and the conversion monitored by TLC using toluene–ethyl acetate (5:1 v/v) as the eluent. After an appropriate time the reaction was stopped by filtering off the enzyme and the products extracted with CH_2Cl_2 (5 x 30 mL). The organic layers were combined, washed with water (3 x 50 mL), and dried over anhydrous $MgSO_4$. The crude mixture was purified by chromatography on a silica-gel column with toluene–ethyl acetate (5:1 v/v). NMR spectra of the enantiomerically enriched alcohols (–)-(*R*)-**2a-d**, (–)-(*R*)-**4a-d** and acetates (+)-(*S*)-**1a-d**, (–)-(*S*)-**3a-d** were identical to those given in the literature.[33]

(–)-(*R*)-4-(5-phenyl-2*H*-tetrazol-2-yl)butan-2-ol ((–)-(*R*)-2a). Oil, yield 45%. $[\alpha]_D^{24}$ = -31.01 (*c* 1.13 in CH_3OH, ee=97%). ^1H NMR (400 MHz, $CDCl_3$, δ): 8.11-8.13 (m, 2H, C_6H_5), 7.46-7.48 (m, 3H, C_6H_5), 4.78-4.85 (m, 2H, CH_2N), 3.80-3.90 (m, 1H, CH), 2.20-2.25 (m, 2H, CH_aH_b, OH), 2.20-2.25 (m, 2H, CH_aH_b, OH), 2.08-2.12 (m, 1H, CH_aH_b), 1.26 (d, 3H, CH_3, JCH_3CH=6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 165.02, 130.28, 128,81, 127.26, 126.74, 64.66, 50.14, 38.02, 23.61.

(–)-(*R*)-4-[5-(4-methylphenyl)-2*H*-tetrazol-2-yl]butan-2-ol ((–)-(*R*)-2b). Colorless crystals, mp 48-49°C, yield 43%. $[\alpha]_D^{24}$ = -23.81 (*c* 0.97 in CH_3OH, ee=99%). ^1H NMR (400 MHz, $CDCl_3$, δ): 7.99-8.01 (m, 2H, C_6H_4), 7.26-7.29 (m, 2H, C_6H_4), 4.77-4.84 (m, 2H, CH_2N), 3.84-3.86 (m, 1H, CH), 2.40 (s, 3H, CH_3), 2.17-2.23 (m, 2H, CH_aH_b, OH), 2.07-2.13 (m, 1H, CH_aH_b), 1.26 (d, 3H, CH_3, JCH_3CH=6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 165.11, 140.44, 129.53, 126.67, 124.46, 64.67, 50.07, 38.02, 23.60, 21.43.

(–)-(*R*)-4-[5-(4-chlorophenyl)-2*H*-tetrazol-2-yl]butan-2-ol ((–)-(*R*)-2c). Colorless crystals, mp 70-72°C, yield 45%. $[\alpha]_D^{24}$ = -26.54 (*c* 1.22 in CH_3OH, ee=98%). ^1H NMR (400 MHz, $CDCl_3$, δ): 8.04-8.08 (m, 2H, C_6H_4), 7.43-7.46 (m, 2H, C_6H_4), 4.74-4.88 (m, 2H, CH_2N), 3.84-3.89 (m, 1H, CH), 2.19-2.23 (m, 1H, CH_aH_b), 2.06-2.12 (m, 2H, CH_aH_b, OH), 1.27 (d, 3H, CH_3, JCH_3CH=6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 164.18, 136.31, 129.16, 128.05, 125.80, 64.71, 50.22, 37.98, 23.68.

(–)-(*R*)-4-[5-(2-chlorophenyl)-2*H*-tetrazol-2-yl]butan-2-ol ((–)-(*R*)-2d). Oil, yield 44%. $[\alpha]_D^{24}$ = -23.88 (*c* 1.38 in CH_3OH, ee=99%). ^1H NMR (400 MHz, $CDCl_3$, δ): 7.92-7.95 (m, 1H, C_6H_4), 7.52-7.54 (m, 1H, C_6H_4), 7.37-7.41 (m, 2H, C_6H_4), 4.84-4.90 (m, 2H, CH_2N), 3.86-3.90 (m, 1H, CH), 2.20-2.28 (m, 1H, CH_aH_b), 2.06-2.15 (m, 1H, CH_aH_b), 2.02 (s, 1H, OH), 1.26 (d, 3H, CH_3, JCH_3CH=6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 163.23, 132.99, 131.29, 131.09, 130.80, 126.92, 126.40, 64.73, 50.29, 38.01, 23.62.

(–)-(*R*)-1-(5-phenyl-2*H*-tetrazol-2-yl)propan-2-ol ((–)-(*R*)-4a). Colorless crystals, mp 53-55°C, yield 45%. $[\alpha]_D^{24}$ = -58.48 (*c* 1.18 in CH_3OH, ee=nd). ^1H NMR (400 MHz, $CDCl_3$, δ): 8.06-8.09 (m, 2H, C_6H_5), 7.45-7.47 (m, 3H, C_6H_5), 4.66 and 4.69 (dd, 1H, CH_aH_bN, JH_bCH=3.6 Hz), 4.57 and 4.61 (dd, 1H, CH_aH_bN, JH_aCH=7.6 Hz, JH_aH_b=13.6 Hz), 4.43-4.47 (m, 1H, CH), 3.02 (s, 1H, OH), 1.34 (d, 3H, CH_3, JCH_3CH = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$,δ): 165.06, 130.41, 128.85, 126.93, 126.77, 66.18, 59.78, 20.22.

(–)-(*R*)-1-[5-(4-methylphenyl)-2*H*-tetrazol-2-yl)propan-2-ol ((–)-(*R*)-4b). Colorless crystals, mp 85-87°C, yield 43%. $[\alpha]_D^{25.5}$ = -52.59 (*c* 1.41 in CH_3OH, ee=95.5%). ^1H NMR (400 MHz, $CDCl_3$, δ): 7.96-7.98 (m, 2H, C_6H_4), 7.25-7.27 (m, 2H, C_6H_4), 4.64 and 4.68 (dd, 1H, CH_aH_bN, JH_bCH=3.6 Hz), 4.56 and 4.59 (dd, 1H, CH_aH_bN, JH_aCH=7.6 Hz, JH_aH_b=13,6 Hz), 4.42-4.46 (m, 1H, CH), 3.01 (s, 1H, OH), 2.40 (s, 3H, $CH_3C_6H_4$), 1.33 (d, 3H, CH_3, JCH_3CH=6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 165.16, 140.61, 129.55, 126.70, 124.14, 66.19, 59.73, 21.45, 20.20.

(–)-(*R*)-1-[5-(4-chlorophenyl)-2*H*-tetrazol-2-yl)propan-2-ol ((–)-(*R*)-4c). Colorless crystals, mp 100-102°C, yield 39%. $[\alpha]_D^{25.5}$ = -53.5 (*c* 1.37 in CH_3OH, ee=98%). ^1H NMR (400 MHz, $CDCl_3$, δ): 8.01-8.04 (m, 2H, C_6H_4), 7.42-7.45 (m, 2H, C_6H_4), 4.66 and 4.69 (dd, 1H, CH_aH_bN, JH_bCH=3.6 Hz), 4.58 and 4.61 (dd, 1H, CH_aH_bN, JH_aCH=7.6 Hz, JH_aH_b=13,6 Hz), 4.42-4.49 (m, 1H, CH), 2.79 (s, 1H, OH), 1.35 (d, 3H, CH_3, JCH_3CH=6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 164.23, 136.50, 129.18, 128.06, 125.47, 66.21, 59.87, 20.29.

(–)-(*R*)-1-[5-(2-chorophenyl)-2*H*-tetrazol-2-yl)propan-2-ol ((–)-(*R*)-4d). Oil, yield 43%. $[\alpha]_D^{25.6}$ = -52.42 (*c* 1.49 in CH_3OH, ee=98%). ^1H NMR (400 MHz, $CDCl_3$, δ): 7,96-7.98 (m, 1H, C_6H_4), 7.52-7.54 (m, 1H, C_6H_4), 7.39-7.42 (m, 2H, C_6H_4), 4.74 and 4.78 (dd, 1H, CH_aH_bN, JH_bCH=3.2 Hz), 4.63 and 4.66 (dd, 1H, CH_aH_bN, JH_aCH=8 Hz, JH_aH_b=14 Hz), 4.45-4.48 (m, 1H, CH), 2.67 (s, 1H, OH), 1.35 (d, 3H, CH_3, JCH_3CH=6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 163.30, 133.02, 131.28, 131.23, 130.86, 126.96, 126.09, 66.21, 59.80, 20.19.

(+)-(*S*)-4-(5-phenyl-2*H*-tetrazol-2-yl)butan-2-yl acetate ((+)-(*S*)-1a). Oil, yield 40%. $[\alpha]_D^{22}$ = +11.94 (*c* 1.55 in CH_3OH, ee=99%). ^1H NMR (400 MHz, $CDCl_3$, δ): 8.11-8.14 (m, 2H, C_6H_5), 7.45-7.50 (m, 3H, C_6H_5), 4.96-5.00 (m, 1H, CH), 4.70-4.73 (m, 2H, CH_2N), 2.29-2.35 (m, 2H, CH_2), 2.01 (s, 3H, CH_3CO), 1.29 (d, 3H, CH_3, JCH_3CH=6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.36, 165.11, 130.26, 128.84, 127.30, 126.75, 67.94, 49.66, 35.13, 21.11, 19.85.

(+)-(*S*)-4-[5-(4-methylphenyl)-2*H*-tetrazol-2-yl]butan-2-yl acetate ((+)-(*S*)-1b). Oil, yield 42%. $[\alpha]_D^{23}$ = +18.35 (*c* 1.39 in CH_3OH, ee=97%). ^1H NMR (400 MHz, $CDCl_3$, δ): 8.00-8.02 (m, 2H, C_6H_4), 7.26-7.29 (m, 2H, C_6H_4), 4.94-5.02 (m, 1H, CH), 4.69-4.72 (m, 2H, CH_2N), 2.40 (s, 3H, CH_3), 2.23-2.37 (m, 2H, CH_2), 2.01 (s, 3H, CH_3CO), 1.29 (d, 3H, CH_3, JCH_3CH=6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.37, 165,10, 140.43, 129.54, 126.67, 124.49, 67.99, 49.62, 35.13, 21.45, 21.12, 19.85.

(+)-(*S*)-4-[5-(4-chlorophenyl)-2*H*-tetrazol-2-yl]butan-2-yl acetate ((+)-(*S*)-1c). Colorless crystals, mp 46-48°C, yield 48%. $[\alpha]_D^{23}$ = +13.32 (*c* 1.54 in CH_3OH, ee=99%). ^1H NMR (400 MHz, $CDCl_3$, δ): 8.05-8.07 (m, 2H, C_6H_4), 7.44-7.46 (m, 2H, C_6H_4), 4.93-5.01 (m, 1H, CH), 4.69-4.73 (m, 2H, CH_2N), 2.29-2.34 (m, 2H, CH_2), 2.01 (s, 3H, CH_3CO), 1.29 (d, 3H, CH_3, JCH_3CH=6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.34, 164.25, 136.30, 129.16, 128.05, 125.80, 67.87, 49.74, 35.11, 21.11, 19.86.

(+)-(*S*)-4-[5-(2-chlorophenyl)-2*H*-tetrazol-2-yl]butan-2-yl acetate ((+)-(*S*)-1d). Colorless crystals, mp 42-44°C, yield 39%.$[\alpha]_D^{23}$ = +23.26 (*c* 1.72 in CH_3OH, ee=99%). ^1H NMR (400 MHz, $CDCl_3$, δ): 7.91-7.94 (m, 1H, C_6H_4), 7.51-7.53 (m, 1H, C_6H_4), 7.35-7.42 (m, 2H, C_6H_4), 4.96-5.01 (m, 1H, CH), 4.74-4.78 (m, 2H, CH_2N), 2.31-2.38 (m, 2H, CH_2), 2.01 (s, 3H, CH_3CO), 1.29 (d, 3H, CH_3, JCH_3CH=6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.34, 163.30, 132.97, 131.28, 131.04, 130.77, 126.88, 126.40, 67.92, 49.81, 35.10, 21.12, 19.83.

(–)-(*S*)-1-(5-phenyl-2*H*-tetrazol-2-yl)propan-2-yl acetate ((–)-(*S*)-3a). Oil, yield 46%. $[\alpha]_D^{23.4}$ = -12.81 (*c* 1.64 in CH_3OH, ee=nd). ^1H NMR (400 MHz, $CDCl_3$, δ): 8.13-8.15 (m, 2H, C_6H_5), 7.46-7.50 (m, 3H, C_6H_5), 5.42-5.47 (m, 1H, CH), 4.77-4.79 (m, 2H, CH_2N), 2.01 (s, 3H, CH_3CO), 1.36 (d, 3H, CH_3, JCH_3CH=6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.03, 165.21, 130.35, 128.86, 127.19, 126.79, 67.84, 56.27, 20.89, 17.43.

(–)-(*S*)-1-[5-(4-methylphenyl)-2*H*-tetrazol-2-yl)propan-2-yl acetate ((–)-(*S*)-3b). Oil, yield 43%. $[\alpha]_D^{25.5}$ = -9.81 (*c* 1.68 in CH_3OH, ee=95.5%). ^1H NMR (400 MHz, $CDCl_3$, δ): 8.00-8.03 (m, 2H, C_6H_4), 7.26-7.29 (m, 2H, C_6H_4), 5.41-5.46 (m, 1H, CH), 4.77 and 4.80 (dd, 1H, CH_aH_bN, JH_bCH=1.6 Hz), 4.73 and 4.76 (dd, 1H, CH_aH_bN, JH_aCH=2.4 Hz, JH_aH_b=14 Hz), 2.40 (s, 3H, CH_3), 2.00 (s, 3H, CH_3CO), 1.35 (d, 3H, CH_3, JCH_3CH=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.03, 164.20, 140.52, 129.54, 126.71, 124.39, 67.86, 56.21, 21.45, 20.89, 17.43.

(–)-(*S*)-1-[5-(4-chlorophenyl)-2*H*-tetrazol-2-yl)propan-2-yl acetate ((–)-(*S*)-3c). Colorless crystals, mp 66-68°C, yield 47%. $[\alpha]_D^{25.7}$ = -7.07 (*c* 1.63 in CH_3OH, ee=98%). ^1H NMR (400 MHz, $CDCl_3$, δ): 8.05-8.08 (m, 2H, C_6H_4), 7.43-7.47 (m, 2H, C_6H_4), 5.40-5.46 (m, 1H, CH), 4.72-4.81 (m, 2H, CH_2N), 2.00 (s, 3H, CH_3CO), 1.35 (d, 3H, CH_3, JCH_3CH=6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.00, 164.36, 136.38, 129.16, 128.09, 125.70, 67.79, 56.37, 20.88, 17.45.

(–)-(*S*)-1-[5-(2-chorophenyl)-2*H*-tetrazol-2-yl)propan-2-yl acetate ((–)-(*S*)-3d). Oil, yield 47%. $[\alpha]_D^{25.5}$ = -11.72 (*c* 1.66 in CH_3OH, ee=99%). ^1H NMR (400 MHz, $CDCl_3$, δ): 7.92-7.96 (m, 1H, C_6H_4), 7.50-7.55 (m, 1H, C_6H_4), 7.35-7.42 (m, 3H, C_6H_5), 5.41-5.49 (m, 1H, CH), 4.84 and 4.87 (dd, 1H, CHa*Hb*N, J_{HbCH}=4.4 Hz), 4.78 and 4.81 (dd, 1H, CH_aH_bN, J_{HaCH}=6.8 Hz, J_{HaHb}=14 Hz), 2.01 (s, 3H, CH_3CO), 1.37 (d, 3H, CH_3, JCH_3CH=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.01, 163.39, 133.04, 131.29, 131.10, 130.78, 126.88, 126.32, 67.86, 56.40, 20.88, 17.43.

Antifungal Activity Assay

The antifungal activity of compounds (±)-**1a-d**, (±)-**2a-d**, (–)-(*R*)-**2a-d**, (+)-(*S*)-**2a-d**, (±)-**3a–d**, (–)-(*S*)-**3d**, (±)-**4a-d**, (–)-(*R*)-**4a-d,** and (+)-(*S*)-**4a-d** against mold strains *Fusarium oxysporum* MF 5, *Fusarium sambucinum* MF 1, *Aspergillus niger* ATCC 16404, *Colletotrichum coccodes* MC 1 was determined by measuring the inhibition of radial growth on an agar medium in a Petri dish.[35,36] The compound was dissolved in dimethyl sulfoxide (DMSO) and added to a potato dextrose agar (PDA, Merck) medium to give a final concentration of 128 μg ml^{-1} (w/v) of tested compound and less than 0.2 % (v/v) of DMSO. The 6-mm diameter mycelial disk was cut out of the actively growing fungal mycelium on PDA and placed at the center of each Petri dish. The final DMSO concentration was identical in both control (without tested compound) and treated cultures. The radial fungal growth was measured after several days of incubation at 25 °C. The inhibitory activity of each compound was calculated as a percentage of the positive control growth using the following equation: Inhibition = ((control – treatment)/control) × 100%.[37] Each experiment was repeated twice and the data presented here are the average of two experiments. As a control to the antifungal activity assay the plates with amphotericin B (BioShop) at a final concentration of 21 μg ml^{-1} (w/v) were used.

RESULTS AND DISCUSSION

The racemic mixtures of 4-(5-aryl-2*H*-tetrazol-2-yl)butan-2-yl acetate (±)-**1a-d** and 1-(5-aryl-2*H*-tetrazol-2-yl)propan-2-yl acetate (±)-**3a-d** were prepared from the appropriate5-substituted tetrazoles according to the procedure reported earlier.[33] The synthesis involved three steps: preparation of appropriate ketones, reduction of carbonyl compounds to corresponding alcohols, and acetylation of hydroxy group with acetic anhydride in anhydrous pyridine solution.

Kinetic resolution of the racemic mixtures of acetates (±)-**1a-d** and (±)-**3a-d** was performed by lipase-catalyzed hydrolysis. The reactions were carried out at room temperature in a two-phase *tert*-butyl methyl ether (TBME)-phosphate buffer of pH=7.2 system (Scheme 1).

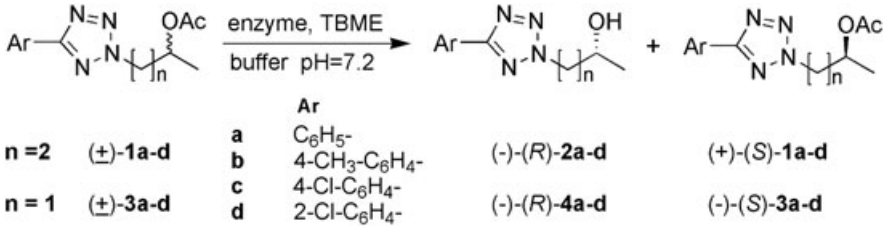
The control experiment revealed that the reaction did not proceed in the absence of the enzyme. Three commercially available lipases: Novozyme SP 435 from *Candida antarctica*, Amano AK from *Pseudomonas fluorescens*, and Amano PS from *Burkholderia cepacia* (earlier *Pseudomonas cepacia*) were used as catalysts and the influence of lipase type on the enantioselectivity of the reaction was investigated. The progress of the reactions was monitored on TLC plates using toluene:ethyl acetate (5:1 v/v) as the eluent and by GC. After an appropriate time reactions were stopped by filtering off the enzyme. Enantiomerically enriched products were separated by silica gel column chromatography. Enantiomeric excesses (ee) of alcohols (–)-**2a-d** and (–)-**4b-d**, and esters (–)-**3c-d** were determined by HPLC analysis using a Chiralcel OD-H column. This column was inappropriate to the detection of ee's of the remaining esters (+)-**1a-d** and (–)-**3b**; therefore they were hydrolyzed to the corresponding alcohols (+)-**2a-d** and (–)-**4b**. The reaction was performed in MeOH with 1M NaOH solution at room temperature. It seems reasonable to assume that the ee of acetates (+)-**1a-d** and (–)-**3c-d** were the same or even higher than alcohols prepared from them. This column was also unsuitable for detection of the optical purity of alcohol (–)-**4a** and ester (–)-**3a**; therefore the results of the enzymatic hydrolysis of ester (±)-**3a** are unknown.

Taking into consideration the results of the enzymatic hydrolysis of esters (±)-**1a-d**, the most effective catalyst was Novozyme SP 435, which catalyze reactions in good stereoselectivity and in a relatively short time, giving excellent conversion rates. Indeed, the resolution was complete and optically pure products were obtained after 20–24 h. Furthermore, the data presented in Table 1 indicate that chloro and methyl substituents improve the enantioselectivity of the reaction without significant change of their times. For comparison, when these reactions were performed in the presence of other lipases (Amano PS and Amano AK), the decrease in conversion, selectivity, and extending the reaction times were observed. Even, after 2 w, the resolutions hardly reached conversions close to 13.5–43%. The reactions with Amano PS as catalyst were the most sluggish and almost not selective.

A similar observation was done for kinetic resolution of esters (±)-**3a-d**. The results of these studies are summarized in Table 2. As can be seen, immobilized lipase (Novozyme SP 435) was a more effective catalyst than the two other native enzymes (Amano AK and Amano PS). The reactions catalyzed by Novozyme SP 435 required shorter times (21–25 h) to reach 50% conversion, and achieved the highest enantioselectivity values (E > 100).

Comparison of the results in Tables 1 and 2 clearly shows that the distance between the acetoxy group and the tetrazole ring does not significantly affect the selectivity of enzymes. Additionally, in all cases (*R*)-stereopreference was observed. The absolute configurations of the products, that is, alcohols (–)-**2a-d**, (–)-**4a-d** and esters (+)-**1a-d**, (–)-**3a-d,** were determined by comparing their specific rotation values with those described earlier.[33] According to them, the alcohols and unchanged esters have (*R*) and (*S*) configuration, respectively.

In comparison to our previous article[33] we found that reversing direction of the reaction, and the use of hydrolysis reaction instead of acetylation, significantly affects the



Scheme 1. Lipase-catalyzed hydrolysis of acetate (±)-**1a-d** and (±)-**3a-d**.

TABLE 1. Results of lipase-catalyzed hydrolysis of esters (±)-1a-d

Entry	Ar	Enzyme	Time (h)	c[a] (%)	$ee_{alk.}$[b] (%)	$ee_{est.}$[b] (%)	E[a]
1	C_6H_5-	Novozyme SP 435	24	50	97	99	>100
	4-CH_3-C_6H_4-		20	49	99	97	>100
	4-Cl-C_6H_4-		23	50	98	99	>100
	2-Cl-C_6H_4-		23	50	99	99	>100
2	C_6H_5-	Amano AK	336	43	99	76	>100
	4-CH_3-C_6H_4-		336	41	97	69	>100
	4-Cl-C_6H_4-		336	40	93	62	56
	2-Cl-C_6H_4-		336	37	95	57	69
3	C_6H_5-	Amano PS	312	27	85	32	17
	4-CH_3-C_6H_4-		312	13	77	12	9
	4-Cl-C_6H_4-		312	17	67	14	6
	2-Cl-C_6H_4-		312	32	71	33	8

[a]Conversion (c) and E values were calculated from the enantiomeric excess of substrate (+)-**1a-d** ($ee_{est.}$) and product **(-)-2a-d** ($ee_{alk.}$) using the formula: $c=ee_{sub}/(ee_{sub}+ee_{prod})$, $E=Ln[(1-ee_{sub})(ee_{prod}/ee_{sub}+ee_{prod})]/Ln[(1+ee_{sub})(ee_{sub}+ee_{prod})]$.
[b]Determined by HPLC analysis using a Chiracel OD-H column.

TABLE 2. Results of lipase-catalyzed hydrolysis of esters (±)-3a-d

Entry	Ar	Enzyme	Time (h)	c[a](%)	$ee_{alk.}$[b](%)	$ee_{est.}$[b](%)	E[a]
1	C_6H_5-	Novozyme SP 435	21	nd	nd	nd	nd
	4-CH_3-C_6H_4-		25	50	95	95	>100
	4-Cl-C_6H_4-		23	50	98	98	>100
	2-Cl-C_6H_4-		23	50	98	99	>100
2	C_6H_5-	Amano AK	336	nd	nd	nd	nd
	4-CH_3-C_6H_4-		336	51	92	95	89
	4-Cl-C_6H_4-		95	33	97	48	>100
	2-Cl-C_6H_4-		336	49	88	83	41
3	C_6H_5-	Amano PS	432	nd	nd	nd	nd
	4-CH_3-C_6H_4-		432	43	87	67	29
	4-Cl-C_6H_4-		95	26	93	32	38
	2-Cl-C_6H_4-		336	61	34	54	3

[a]Conversion (c) and E values were calculated from the enantiomeric excess of substrate (-)-**3b-d** ($ee_{est.}$) and product (-)-**4b-d** ($ee_{alk.}$) using the formula: $c=ee_{sub}/(ee_{sub}+ee_{prod})$, $E=Ln[(1-ee_{sub})(ee_{prod}/ee_{sub}+ee_{prod})]/Ln[(1+ee_{sub})(ee_{sub}+ee_{prod})]$.
[b]Determined by HPLC analysis using a Chiracel OD-H column.
nd, no data.

selectivity of enzymes. In hydrolysis the most stereoselective catalyst is Novozyme SP 435, while in the acetylation lipase Amano AK. Furthermore, taking into account the time of reactions, the lipase-mediated hydrolysis approach appeared to be better than the transesterification reaction. The enzymatic hydrolysis runs faster, and the pure enantiomers of alcohols **2a-d** and **4a-d** can be obtained after 20–25 h of the reaction.

Synthesized tetrazole derivatives were tested for antimicrobial activity. An agar-diffusion method was used for determination of the antibacterial activity against Gram-negative bacteria *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* as well as Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*. Unfortunately, all the tested compounds did not reveal appreciable antibacterial activity, since the MIC (minimal inhibitory concentration) values were 512 μg. These compounds were also evaluated for antifungal activity against the yeast *Candida albicans* using a broth microdilution susceptibility procedure[38] and against mold strains *Fusarium sambucinum*, *Fusarium oxysporum*, *Colletotrichum coccodes*, *Aspergillus niger* as described in the Materials and Methods. In the case of *C. albicans*, only compounds (±)-**2c**, (–)-(*R*)-**2c**, and (+)-(*S*)-**2c** showed very weak antifungal activity (MIC value of 128 μg ml^{-1}). Some of the synthesized compounds showed promising antifungal activity against the tested mold strains. The median inhibitory concentration (IC_{50}) was considered as the tetrazole derivative concentration that resulted in 50% inhibition of the fungal growth when compared to the control group. The results are given in Table 3. As can be seen, only compounds (±)-**1c**, (±)-**2c**, (*R*)-(–)-**2c**, (*S*)-(+)-**2c**, and (±)-**3c** showed promising antifungal activity against all tested mold strains when applied to the culture medium at concentration 128 μg ml^{-1} to reach IC_{50}. We also observed that even a 64 μg ml^{-1} concentration of compound (±)-**2c** was effective for 50% growth inhibition of *A. niger* ATCC16404. The compounds (±)-**1a-b**, **2a**, (–)-(*R*)-**2b**, (+)-(*S*)-**2d**, (±)-**3c**, (+)-(*S*)-**3d**, (±)-**4a-d**, **4c**, and (+)-(*S*)-**4d** effectively inhibited the growth of at least one of the tested mold strains. Comparison of all results clearly shows that esters and alcohols substituted by chlorine at *para* position of the aromatic ring possess the highest antifungal activity. Moreover, tetrazole derivatives with a longer alkyl chain (**1a-d** and **2-a-d**) show better antifungal activity. It is also worth mentioning that in most cases the antifungal activity of both enantiomers is similar.

TABLE 3. Antifungal activity (% of growth inhibition) of the tetrazole derivatives at concentration of 128 μg ml^{-1}

Entry	Compound	*F. sambucinum* MF 1	*F. oxysporum* MF 5	*C. coccodes* MC 1	*A. niger* ATCC 16404
1	(±)-**1a**	28.5	51.9	43.3	37.3
2	(±)-**1b**	51.1	51.1	47.6	59.9
3	(±)-**1c**	54.3	60.9	57.8	67.3
4	(±)-**1d**	33.3	43,6	43.1	33.3
5	(±)-**2a**	5	20.2	53.1	28.4
	(-)-(*R*)-**2a**	3.7	24.5	53.9	32.4
	(+)-(*S*)-**2a**	7.8	25.7	55.6	33.2
6	(±)-**2b**	44.2	41.3	36.9	47
	(-)-(*R*)-**2b**	42.9	35	43.8	54.3
	(+)-(*S*)-**2b**	41.8	26.1	44.4	36.2
7	(±)-**2c**	60.5	55.2	72	83
	(-)-(*R*)-**2c**	60.2	54.7	66.7	85
	(+)-(*S*)-**2c**	61.6	55.8	67.5	80.6
8	(±)-**2d**	16.7	18.4	46.6	27.5
	(-)-(*R*)-**2d**	7.6	20.9	47.5	30.5
	(+)-(*S*)-**2d**	28.6	23.1	52	31.6
9	(±)-**3a**	9.3	38.7	41.1	18.3
10	(±)-**3b**	38.3	45.1	45.1	39.7
11	(±)-**3c**	49.6	58.1	60.8	61.5
12	(±)-**3d**	8.6	45.1	43.6	28.6
	(+)-(*S*)-**3d**	26.9	48.6	55.6	43.2
13	(±)-**4a**	6.8	7.9	53.7	4.7
	(-)-(*R*)-**4a**	nd	nd	nd	nd
	(+)-(*S*)-**4a**	nd	nd	nd	nd
14	(±)-**4b**	14	35.1	61.8	39.6
	(-)-(*R*)-**4b**	16.7	28.4	40	11.1
	(+)-(*S*)-**4b**	16.7	22.4	38	25
15	(±)-**4c**	47.1	49.7	59.7	72.2
	(-)-(*R*)-**4c**	42.9	48.2	63.6	66.8
	(+)-(*S*)-**4c**	41	46.7	56.8	65.5
16	(±)-**4d**	3.1	12.8	55.2	17.9
	(-)-(*R*)-**4d**	7.2	14.3	39.9	11.3
	(+)-(*S*)-**4d**	8.3	11.4	53.9	17.3
17	control*	83.1	64.7	88.9	88.2

nd, no data.
*Amphotericin B-standard antifungal agent at a concentration of 21 μg ml^{-1}.

CONCLUSION

In summary, the enzymatic hydrolysis of racemic esters containing a tetrazole ring is a very efficient and convenient method for obtaining enantiomerically enriched secondary alcohols and their acetates. The best results from the point of view of enantioselectivity and enantiopurity of the formed products were achieved by hydrolysis in the presence of Novozyme SP 435. Some tetrazole derivatives possess interesting antifungal activity against *F. sambucinum*, *F. oxysporum*, *C. coccodes*, and *A. niger*. An especially active compound proved to be 4-[5-(4-chlorophenyl)-2*H*-tetrazol-2yl]butan-2-ol.

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