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Lipase-catalyzed alcoholytic resolution of (*R*,*S*)-flurbiprofenyl azolides for preparation of (*R*)-NO-flurbiprofen ester prodrugs

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ABSTRACT

A lipase-catalyzed alcoholysis of (*R*,*S*)-flurbiprofenyl azolide in anhydrous methyl *tert*-butyl ether (MTBE) has been developed for the preparation of (*R*)-flurbiprofenyl ester, (*S*)-flurbiprofenyl azolide and hence (*S*)-flurbiprofen. On the basis of enzyme enantioselectivity and activity, the best reaction condition of using (*R*,*S*)-flurbiprofenyl 4-bromopyrazolide and 2,3-dibromo-1-propanol as the substrates for *Candida antartica* lipase B (CALB) at 45 °C was selected, and led to excellent enantioselectivity (V_R/V_S = 200.3) with two order-of-magnitudes higher specific initial activity for the fast-reacting enantiomer in comparison with those for other lipases. A thermodynamic analysis indicated that both $-\Delta\Delta H$ and $-\Delta\Delta S$ gave equal contributions to $-\Delta\Delta G$ = 14.03 kJ/mol, and hence the excellent enantioselectivity, at the best reaction condition. The kinetic constants estimated from a thorough kinetic analysis were successfully employed for modeling the time-course conversions of both enantiomers. The optically pure (*R*)-flurbiprofenyl 2,3-dibromo-1-propyl ester obtained via reactive extraction after the alcoholysis was then employed for the synthesis of optically pure (*R*)-flurbiprofenyl 2,3-bisnitrooxypropyl ester prodrug.

1. Introduction

As an important class of non-steroidal anti-inflammatory drugs (NSAIDs), 2-arylpropionic acids (profens) have their pharmacological activity mainly on the (*S*)-enantiomer. Considerable efforts have been made for obtaining (*S*)-profens via lipase-catalyzed kinetic resolution [1–4] or dynamic kinetic resolution [5–10]. However, drawbacks of relatively low enzyme activity (or enantioselectivity) and stability, and substrate solubility in aqueous or organic solvents were reported, implying that more efficient resolution platforms should be explored in order to meet the industrial requirements.

Previous pharmacological studies of profens have shown that gastrointestinal side effects such as ulceration and hemorrhage constitute the most frequent adverse reactions. Therefore, the development of safer profens continues to be a very active research topic, such as syntheses of metal-complex profen derivatives as well as (*S*)-profen amide and ester prodrugs [11–13]. The latter include cyclo-oxygenase (Cox) inhibitor nitric oxide donors (CINODs) such as NO-(*S*)-naproxen and NO-(*S*)-ketoprofen, a new class of anti-inflammatory and analgesic drugs generated by adding a nitric oxide generating moiety to the parent NSAID via an ester linkage. The NO released by the metabolism of nitrate may provide protective effects in the gastrointestinal tract, aside from mediating the blood flow, neurotransmission, immune reactions, and muscle contraction [14–17].

Recent studies have revealed that some NSAIDs having anticancer effects on reducing cell growth and metastasis, and inducing apoptosis, require levels of dosage much higher than those required for Cox-inhibition. Therefore, NO-donating (*R*,*S*)-profens or (*R*)-profens such as (*R*)-etodolac and (*R*)-flurbiprofen lacking Cox inhibitory activity and in vivo metabolic interconversion to their (*S*)-antipodes were tested, demonstrating chemopreventive properties against a variety of cancers in several cell lines and animal tumor models [18,19]. Moreover, certain NSAIDs such as (*R*,*S*)-flurbiprofen were shown to decrease the production of amyloid-β(1–42) peptide ($A\beta_{42}$) associated with the Alzheimer's disease (AD), with the mechanism of action of $A\beta_{42}$-lowering activity not related to Cox-inhibition or other non-Cox targets of NSAIDs [20,21]. In order to reduce the gastrointestinal toxicity, (*R*)-profens such as (*R*)-flurbiprofen, NO-donating profens such as NO-(*R*,*S*)-flurbiprofen (HCT-1026), or other (*R*,*S*)-profen ester prodrugs were also tested, showing encouraging results on cognitive and functional outcomes among mildly affected patients in an earlier but not clinically established phase [22–27]. All the results indicate that it is highly desirable to effectively supply the market with not only enantiopure (*S*)-flurbiprofen, but also (*R*)-flurbiprofen, and their ester or amide prodrugs.

The biocatalyst-catalyzed esterification of (*R*,*S*)-flurbiprofen or hydrolysis of (*R*,*S*)-flurbiprofenyl (thio)esters have been reported in the literature [1,28,29,3,30–32], in which low enantioselectivity or

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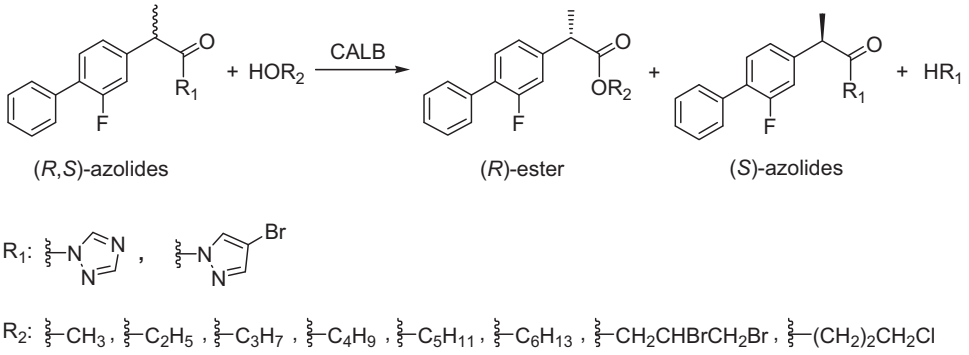


Scheme 1. CALB-catalyzed alcoholysis of (*R,S*)-flurbiprofenyl azolides.

activity were generally found. Therefore, it is aimed to employ (*R,S*)-flurbiprofenyl azolides as the extreme substrate for developing an efficient alcoholytic resolution process via lipases for preparing enantiopure (*R*)-flurbiprofenyl ester and (*S*)-flurbiprofenyl azolide in anhydrous MTBE (Scheme 1) [33–35]. After the reactive extraction via an alkaline solution, (*R*)-flurbiprofenyl 2,3-dibromo-1-propyl ester remained in the organic phase is separated from the resultant (*S*)-flurbiprofen dissolved in the aqueous phase, and employs for the synthesis of NO-containing ester prodrug (Scheme 2).

2. Materials and methods

2.1. Materials

Novozym 435 as a *Candida antartica* lipase B (CALB) immobilized on polyacrylic resin, 10 PLU/mg, lipase MY as a crude *Candida rugosa* lipase (CRL), 30 U/mg, and *Carica papaya* lipase (CPL) partially purified from a crude papain were provided by Novo Nordisk (Bagsvaerd, Denmark), Meito Sangyo (Tokyo, Japan), and Challenge Bioproducts (Yun-Lin, Taiwan), respectively. Other chemicals of analytical grade were commercially available: 1-chloro-3-propanol and 4-bromopyrazole from Acros (Geel, Belgium); *N,N*-carbonyldi-1,2,4-triazole (CDT) and chloroform from Aldrich (Milwaukee, WI); (*R,S*)-flurbiprofen (i.e. (*R,S*)-2-fluoro-α-methyl-4-biphenylacetic acid) from TCI (Tokyo, Japan); acetophenone and silver nitrate from Sigma (St. Louis, MO); 1,4-diazabicyclo[2,2,2]octane (DABCO) from Fluka (Buchs, Switzerland); acetonitrile, benzene, cyclohexane (CYC), butanol, ethanol, hexanol, isopropanol (IPA), hexane (HEX), methanol, methyl *tert*-butyl ether (MTBE), pentanol, propanol, and triethylamine from Tedia (Fairfield, OH). Anhydrous solvent was prepared by adding calcium hydride from Riedel-de Haen (Seelze, Germany) to the organic solvent for 24 h.

2.2. Analysis

The alcoholysis of (*R,S*)-flurbiprofenyl azolides was monitored by HPLC using a chiral OD-H, OJ, or OJ-H column from Daicel (Tokyo, Japan) that was capable of separating the internal standard of 4-nitrophenol or acetopheone, (*S*)- and (*R*)-azolide. The mobile phase was a mixture of HEX/IPA/glacial acetic acid at a flow rate of 2.0 mL/min. UV detection at 270 nm was employed for quantification at the room temperature.

2.3. Substrate preparations

To 2 mL benzene was added 1 mmol (*R,S*)-flurbiprofen and 1.5 mmol CDT and stirred at 55 °C for 30 min. The resultant mixture was filtered and then kept at 4 °C for obtaining the desired (*R,S*)-flurbiprofenyl 1,2,4-triazolide in white crystal. For the synthesis of (*R,S*)-flurbiprofenyl 4-bromopyrazolide, a mixture containing 5 mL benzene and 13 mmol thionyl chloride was added dropwise to 25 mL benzene containing 13 mmol (*S*)- or (*R,S*)-flurbiprofen, 10 mmol 4-bromopyrazole, and 40 mmol triethylamine at 0 °C with stirring, and then at the room temperature for 2 h. The resultant mixture was quenched in succession with 0.1 M HCl solution (3× 10 mL), 0.1 M NaOH solution (3× 10 mL), and 0.1 M NaCl solution (3× 10 mL). The organic phase was separated, dried over anhydrous $MgSO_4$ for 12 h, filtered and concentrated under reduced pressure for giving the desired powdered product. All the synthesized substrates and products were confirmed from HPLC analysis and ^{1}H NMR spectra recorded at 400 MHz on Brucker AC-500 spectrometer in DMSO-d_6 solution with TMS as an internal standard as follows.

(R,S)-Flurbiprofenyl 1,2,4-triazolide: ^{1}H NMR (DMSO-d_6/TMS) δ: 1.58 (3H, d), 4.99–5.05 (1H, q), 7.28–7.52 (8H in benzene ring, m), 8.31 (1H, s), 9.38 (1H, s). The abbreviations d, q, m and s were the peak multiplicities of doublet, quartet, multiplet and single, respectively. HPLC analysis: OJ-H column with mobile phase HEX/IPA/glacial acetic acid = 94.5/5.0/0.5 (v/v/v); retention time: 2-nitrophenol/(*S*)-azolide/(*R*)-azolide = 12.1/17.1/22.3, min.

(R,S)-Flurbiprofenyl 4-bromopyrazolide: ^{1}H NMR (DMSO-d_6/TMS) δ: 1.55 (3H, d), 5.11–5.16 (1H, q), 7.22–7.55 (8H in benzene ring, m), 8.05 (1H, s), 8.72 (1H, s). HPLC analysis: OJ column with mobile phase HEX/IPA = 98/2 (v/v); retention time: acetophenone/(*S*)-azolide/(*R*)-azolide = 4.2/7.0/10.4, min.

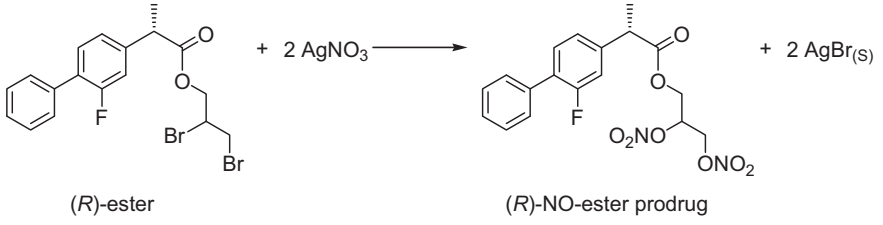
(R)-Flurbiprofenyl 2,3-dibromo-1-propyl ester: ^{1}H NMR (DMSO-d_6/TMS) δ: 1.10–1.23 (3H, d), 3.68–3.76 (1H in 2-C and 2H in 3′-C, m), 4.16–4.53 (2H in 1′-C and 1H in 2′-C, m), 7.22–7.55 (8H in benzene ring, m). HPLC analysis: OJ-H column with mobile phase HEX/IPA/glacial acetic acid = 98.5/1.0/0.5 (v/v/v); retention time: acetophenone/(*R*)-ester = 4.6/12.4, min.

(R)-Flurbiprofenyl 2,3-bisnitrooxypropyl ester: ^{1}H NMR (DMSO-d_6/TMS) δ: 1.17–1.19 (3H, d), 3.23 (2H, d), 4.00–4.17 (2H in 1′-C and 1H in 2′-C, m), 7.39–7.55 (8H in benzene ring, m). HPLC analysis: OJ-H column with mobile phase HEX/IPA/glacial acetic acid = 98.5/1.0/0.5 (v/v/v); retention time: acetophenone/(*R*)-ester = 4.6/9.8, min.

(R,S)-Flurbiprofen: HPLC analysis: OD-H column with mobile phase HEX/IPA = 98/2 (v/v); retention time: acetophenone/(*R*)-flurbiprofenyl 2,3-dibromo-1-propyl ester/(*R*)-flurbiprofen/(*S*)-flurbiprofen = 2.8/5.4/10.5/11.4, min.

2.4. Effects of lipase sources, alcohol, temperature, and leaving azole on alcoholytic resolution

To 10 mL anhydrous MTBE containing 3 mM (*R,S*)-flurbiprofenyl 1,2,4-triazolide and 100 mM methanol in 25 mL Schott bottle at 45 °C was added a specific amount of crude CPL, CRL or CALB as shown in Table 1 and figure legends. The resultant solution was stirred with a magnetic stirrer at 400 rpm, and samples were removed at different time intervals for HPLC analysis, from which the time-course conversions X_R and X_S, specific initial rates $V_R/(E_t)$ and $V_S/(E_t)$ based on several conversion determinations, racemate conversion X_t, and enantiomeric excess for the substrate ee_s were determined. Similar experiments were performed except that the temperature was changed to 25, 35 or 55 °C.



Scheme 2. Synthesis of (*R*)-NO-flurbiprofenyl 2,3-bisnitrooxypropyl ester prodrug.

Table 1
Effects of lipase sources, leaving azole, alcohol as acyl acceptor, and temperature on $V_R/(E_t)$, $V_S/(E_t)$, V_R/V_S, X_t and ee_s for lipase-catalyzed alcoholysis of (*R*,*S*)-flurbirpofenyl azolides.

Alcohol	Lipase	Temp (°C)	$V_R/(E_t)$ (mmol/h g)	$V_S/(E_t)$ (mmol/h g)	V_R/V_S	(E_t) (mg/mL)	Time (h)	X_t (%)	ee_s (%)
For 1,2,4-triazolides									
Methanol	CPL	45	4.12E−2	4.53E−2	1.1	30	1.25	29.0	0.8
Methanol	CRL	45	1.05E−2	2.15E−2	2.0	30	5.0	41.6	17.9
Methanol	CALB	45	1.08E−1	1.31E−2	82.4	6	3.0	56.1	100.0
Ethanol	CALB	45	6.20E−1	6.72E−3	92.3	6	5.0	51.6	100.0
Propanol	CALB	45	7.60E−1	8.10E−3	93.8	2	1.0	51.7	100.0
Butanol	CALB	45	6.28E−1	6.68E−3	94.0	2	3.7	50.2	100.0
Pentanol	CALB	45	9.17E−1	9.83E−3	93.3	2	9.0	57.4	100.0
Hexanol	CALB	45	7.53E−1	7.38E−3	102.0	6	1.0	50.7	100.0
1-Chloro-3-propanol	CALB	45	8.10E−1	1.95E−2	105.6	10	1.0	54.4	100.0
2,3-Dibromo-1-propanol	CALB	45	2.06	7.65E−3	105.9	6	1.0	51.2	100.0
Methanol	CALB	55	1.79	2.38E−2	75.2	6	2.0	58.1	100.0
Methanol	CALB	35	7.66E−1	8.28E−3	92.5	12	3.0	58.0	100.0
Methanol	CALB	25	3.11E−1	3.04E−3	102.3	12	1.0	51.3	100.0
For 4-bromopyrazolides									
Hexanol	CALB	45	4.38E−1	3.25E−3	134.8	6	9.0	55.2	100.0
1-Chloro-3-propanol	CALB	45	1.62E−1	1.01E−3	160.4	20	1.7	50.6	100.0
2,3-Dibromo-1-propanol	CALB	45	5.95E−1	2.97E−3	200.3	6	10.2	56.2	100.0
2,3-Dibromo-1-propanol	CPL	45	1.95E−4	1.04E−3	5.3	30	21.5	23.1	19.1
2,3-Dibromo-1-propanol	CRL	45	4.05E−4	3.91E−3	9.7	30	5.0	21.4	19.9
2,3-Dibromo-1-propanol	CALB	35	2.24E−1	1.02E−3	220.3	6	23.0	57.6	100.0
2,3-Dibromo-1-propanol	CALB	25	1.31E−1	5.40E−4	242.5	6	23.0	53.5	100.0

Conditions: 3 mM racemate and 100 mM alcohol in 10 mL anhydrous MTBE for CALB or CYC for CPL and CRL at 400 rpm. Symbol of E−1 as 10^{-1}.

In order to study effects of the acyl acceptor on the lipase performance, more experiments using other alcohols were carried out at 45 °C. Similarly in order to investigate the substrate structure on CALB activity and enantioselectivity, (*R*,*S*)-flurbiprofenyl 4-bromopyrazolide was also employed as the substrate. The kinetic analysis for CALB at 45 °C was performed, in which the concentrations of (*R*,*S*)-flurbiprofenyl 4-bromopyrazoolide and 2,3-dibromo-1-propanol were varied from 5.2 to 63.4 mM and 5 to 100 mM, respectively.

2.5. Reactive extraction for product separation and prodrug preparation

The alcoholysis was carried out at 45 °C in 10 mL anhydrous MTBE containing 30 mM (*R*,*S*)-flurbiprofenyl 4-bromopyrazolide, 100 mM 2,3-dibromo-1-propanol, and 6 mg/mL CALB. After depleting the fast-reacting enantiomer and removing the lipase via filtration, the resultant mixture was quenched in 0.1 M NaOH solution (10 mL) at 4 °C for 24 h, where the remaining (*S*)-flurbiprofenyl 4-bromopyrazolide might hydrolyze into (*S*)-flurbiprofen and 4-bromopyrazole dissolving in the aqueous phase. The organic phase was separated, dried over anhydrous $MgSO_4$ for 12 h, filtered and concentrated under reduced pressure to give the desired (*R*)-ester. The prodrug was then synthesized in dark in acetonitrile containing 45.5 mM of (*R*)-flurbiprofenyl 2,3-dibromo-1-propyl ester and 200 mM silver nitrate at the room temperature for 12 h. After filtering the precipitate, 0.1 mL chloroform was added to the resultant solution with stirring for 4 h. The organic phase was separated, dried over anhydrous $MgSO_4$ for 12 h, filtered and concentrated under reduced pressure to give the desired prodrug in yellow liquid.

3. Model development

An irreversible ping-pong Bi–Bi mechanism by considering a competitive alcohol inhibition (Supplementary Information) can be employed to derive rate equations for the alcoholysis of (*R*,*S*)-flurbiprofenyl azolide [34]:

$$V_R = \frac{-\mathrm{d}(S_R)}{\mathrm{dt}} = \frac{k_{2R}(S_R)(E_t)/K_{mR}}{G} \quad (1)$$

$$V_S = \frac{-\mathrm{d}(S_R)}{\mathrm{dt}} = \frac{k_{2S}(S_S)(E_t)/K_{mR}}{G} \quad (2)$$

$$G = 1 + \frac{(S_R)[1 + k_{2R}/k_{4R}]}{K_{mR}} + \frac{(S_S)[1 + k_{2S}/k_{4S}]}{K_{mS}} + \frac{(M)}{K_I} + \left[\frac{k_{2R}K_{m3R}(S_R)}{k_{4R}K_{mR}} + \frac{k_{2S}K_{m3S}(S_S)}{k_{4S}K_{mS}}\right]\left[\frac{1}{(M)}\right] \quad (3)$$

Details for estimating the kinetic constants and hence modeling the time-course variations of X_R and X_S are given in Supplementary Information.

4. Results and discussion

4.1. Effects of lipase sources, alcohol, temperature, and leaving azole

Table 1 demonstrates effects of lipase sources, alcohol as the acyl acceptor, temperature, and leaving azole on initial $V_R/(E_t)$ and $V_S/(E_t)$ and enzyme enantioselectivity in terms of V_R/V_S in anhydrous MTBE. In comparison with CPL and CRL performances for the alcoholysis of (*R*,*S*)-flurbiprofenyl 1,2,4-triazolide by methanol, CALB has the highest specific initial rate for the fast-reacting enantiomer and excellent enantioselectivity of $V_R/V_S = 82.4$ at 45 °C. A replacement of methanol with other alcohols containing a longer carbon-chain with or without having a chloro or dibromo substituent results in minor influences on CALB activity and enantioselectivity, leading to the rate-limiting acylation step for each enantiomer. Similar kinetic behaviors have been reported using (*R*,*S*)-naproxenyl 1,2,4-triazolide as the acyl donor [34]. A decrease of temperature also results in enhancements of enzyme enantioselectivity but not specific activity for each enantiomer (Table 1). Moreover, liner variations of $\ln(V_R/(E_t))$, $\ln(V_S/(E_t))$, and hence $\ln(V_R/V_S)$ with the inverse of absolute temperature were found (Supplementary Information), implying that CALB was thermally stable at 55 °C. Yet, the best temperature of 45 °C was selected by considering a compromise between the lipase activity and enantioselectivity.

The enantioselectivity can further increase from $V_R/V_S = 102.0$ to 134.8 for the alcoholysis of (*R*,*S*)-flurbiprofenyl 4-bromopyrazolide by hexanol, yet with a penalty of 1.72-folds lower of $V_R/(E_t)$. Similar enantioselectivity enhancements are shown using 1-chloro-3-propanol or 2,3-dibromo-1-propanol as the acyl acceptor for CALB, CPL or CRL. A comparison of $pK_a = 0.64$ for 4-bromopyrazolium with 2.19 for 1,2,4-triazolium [33] indicates that the leaving 4-bromopyrazole moiety should exert a stronger electron-withdrawing effect on the carbonyl carbon atom, making it more electrophilic and susceptible to the nucleophilic attack. Yet in comparison with 1,2,4-triazolide, the 3.46-folds lower of $V_R/(E_t)$ for 4-bromopyrazolide imply that some interactions between the 4-bromo substituent and amino acid residues of the active site may occur to weaken the electron-withdrawing capability of 4-bromo substituent. Based on the above results, 2,3-dibromo-

Table 2
Thermodynamic analysis for CALB-catalyzed alcoholysis in anhydrous MTBE.

Entry	ΔH_R (kJ/mol)	ΔH_S (kJ/mol)	$-\Delta\Delta H$ (kJ/mol)	$-\Delta\Delta S$ (J/mol K)	$-\Delta\Delta G$ (kJ/mol)
1[a]	45.60	54.03	8.43	−10.23	11.68
2[b]	59.63	67.17	7.54	−20.38	14.03

Reaction conditions as shown in Table 1; $-\Delta\Delta G$ calculated at 45 °C.
[a] For the alcoholysis of (*R*,*S*)-flurbirpofenyl 1,2,4-triazolide by methanol.
[b] For the alcoholysis of (*R*,*S*)-flurbirpofenyl 4-bromopyrazolide by 2,3-dibromo-1-propanol.

1-propanol was selected as the best acyl acceptor for preparing (*R*)-flurbiprofenyl ester and (*S*)-flurbiprofenyl 4-bromopyrazolide, and hence (*S*)-flurbiprofen via reactive extraction. Apparently, decreasing of temperature can improve the CALB enantioselectivity to V_R/V_S = 220.3 and 242.5 at 35 °C and 25 °C, respectively.

4.2. Thermodynamic analysis

The thermodynamic analysis has been proposed to investigate effects of solvent type and mixture, acyl donor and acceptor, lipase type and mutant on the temperature dependence of enantioselectivity in lipase-catalyzed kinetic resolutions. The logarithm of specific initial rate varied with the inverse of absolute temperature (Fig. S1) was employed for estimating ΔH_R and ΔH_S. Similarly, the differences in activation enthalpy ($-\Delta\Delta H$) and activation entropy ($-\Delta\Delta S$) for the transition states of both enantiomers were calculated and tabulated in Table 2.

In comparison with ΔH_R = 41.88 kJ/mol, ΔH_S = 59.15 kJ/mol, $-\Delta\Delta H$ = 16.97 kJ/mol, $-\Delta\Delta S$ = 15.04 J/mol K, and $-\Delta\Delta G$ = 12.18 kJ/mol for the alcoholysis of (*R*,*S*)-naproxenyl 1,2,4-triazolide by methanol at 45 °C [34], the replacement of acyl group to a flurbiprofenyl moiety has caused minor changes of the thermodynamic parameters. Yet, it is interesting to find the changing sign of $-\Delta\Delta S$, implying that the fluoro substituent might play an important role on decreasing the entropic contribution for the slow-reacting (*S*)-enantiomer at the transient state. This was also valid for the alcoholysis of (*R*,*S*)-flurbiprofenyl 4-bromopyrazolide by 2,3-dibromo-1-propanol (Table 2). Moreover, the excellent enantioselectivity of V_R/V_S = 200.3 at 45 °C indicated that both $-\Delta\Delta H$ and $-\Delta\Delta S$ gave equal contributions to $-\Delta\Delta G$ = 14.03 kJ/mol, and hence the enantiomer discrimination.

4.3. Kinetic analysis

The variations of initial $V_R/(E_t)$ and $V_S/(E_t)$ with enantiomer and methanol concentrations for CALB-catalyzed alcoholysis of (*R*,*S*)-flurbiprofenyl 4-bromopyrazolide by 2,3-dibromo-1-propanol at 45 °C are illustrated in Fig. 1(A and B). As described in Supplementary Information, the kinetic constants k_{2R} = 36.74 mmol/h g, k_{2S} = 0.20 mmol/h g, $K_{mS} = K_{mR}$ = 31.4 mM, K_I = 36.1 mM, $k_{4R}/K_{m3R} = k_{4S}/K_{m3S}$ = 0.79 L/h g, $k_{2R}/k_{4R} \ll 1$, $k_{2S}/k_{4S} \ll 1$, and hence k_{2R}/K_{mR} = 1.17 L/h g, $k_{2S}/K_{mS} = 6.41 \times 10^{-3}$ L/h g, and $E = k_{2R}K_{mS}/k_{2S}K_{mR}$ = 182 were estimated. They are very similar to those (k_{2R} = 181 mmol/h g, k_{2S} = 0.43 mmol/h g, K_{mR} = 86.8 mM, K_{mS} = 33.3 mM, K_I = 34.7 mM, k_{2R}/K_{mR} = 2.08 L/h g, $k_{2S}/K_{mS} = 1.29 \times 10^{-2}$ L/h g, and E = 161) estimated from the alcoholysis of (*R*,*S*)-naproxenyl 1,2,4-triaozlide by methanol [34]. By substituting the kinetic constants into Eqs. (1)–(3) coupled with the alcohol balance of $(M) = (M)_0 - 2(S_R)_0 + (S_R) + (S_S)$, the time-course variations of (S_R)

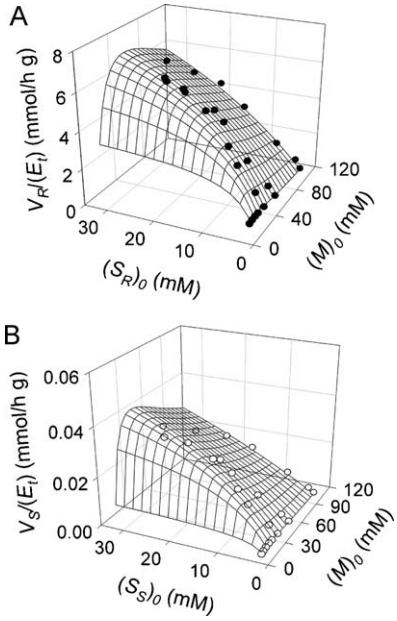


Fig. 1. Variations of initial (A) $V_R/(E_t)$ (●) and (B) $V_S/(E_t)$ (○) with methanol and (*R*)- or (*S*)-furbiprofenyl 4-bromopyrazolide concentrations in anhydrous MTBE consisting of 6 mg/mL CALB at 45 °C. (—) Best-fit results.

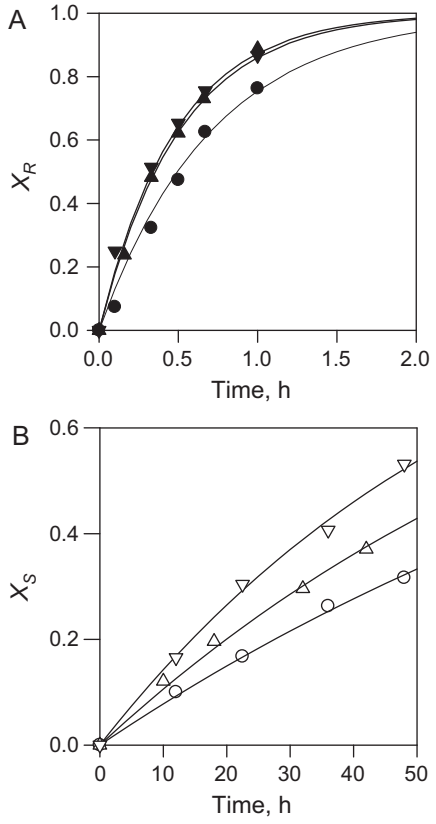


Fig. 2. Time-course conversions of (A) X_R, and (B) X_S using 6 mg/mL CALB at 45 °C; (●, ○) for 38.3 mM racemate and 100 mM methanol, (▲, △) for 28.5 mM racemate and 50 mM methanol, (▼, ▽) for 26.4 mM racemate and 30 mM methanol. (—) Theoretical predictions.

and (S_S), and hence X_R and X_S, were solved using a fourth-order Runge–Kutta method. Typical experimental data in agreements with the theoretical predictions are illustrated in Fig. 2.

4.4. Prodrug synthesis

A typical example for the alcoholytic resolution has been described in Section 2.4, in which 60.6% molar yield of pure (*R*)-flurbiprofenyl 2,3-dibromo-1-propyl ester was obtained and confirmed from NMR and HPLC spectra after the reactive extraction via 0.1 M NaOH solution. The optically pure (*R*)-flurbiprofenyl 2,3-bisnitrooxypropyl ester prodrug was then synthesized in acetonitrile, recovered via extraction and solvent evaporation, confirmed from the NMR and HPLC spectra, and gave 79.9% molar yield.

5. Conclusions

A CALB-catalyzed alcoholysis of (*R*,*S*)-flurbiprofenyl azolide in anhydrous MTBE was developed for the preparation of (*R*)-flurbiprofenyl ester, (*S*)-flurbiprofenyl azolide, and hence (*S*)-flurbiprofen via reactive extraction. By varying the leaving azole moiety and alcohol as an acyl acceptor, (*R*,*S*)-flurbiprofenyl 4-bromopyrazolide and 2,3-dibromo-1-propanol were selected as the best substrates at 45 °C, and led to excellent enantioselectivity (V_R/V_S = 200.3) with high specific activity. A decrease of temperature might result in enhancements of the enzyme enantioselectivity but not specific activity.

A thermodynamic analysis indicated that changing of the leaving azole caused minor effects on varying $-\Delta\Delta H$ and $-\Delta\Delta S$ for the transition states of both enantiomers. Yet in comparison with the alcoholysis of (*R*,*S*)-naproxenyl 1,2,4-triaozlide by methanol, the changing sign of $-\Delta\Delta S$ was advantageous for giving the excellent enantioselectivity at 45 °C, where both $-\Delta\Delta H$ and $-\Delta\Delta S$ gave equal contributions to $-\Delta\Delta G$ = 14.03 kJ/mol. A thorough kinetic analysis for the alcoholysis at the best reaction condition was performed, with which the kinetic constants were estimated and successfully employed for modeling the time-course conversions for both enantiomers. The optically pure (*R*)-flurbiprofenyl 2,3-dibromo-1-propyl ester, obtained via reactive extraction after the alcoholytic resolution, was separated and employed for synthesizing the desired (*R*)-flurbiprofenyl 2,3-bisnitrooxypropyl ester prodrug.

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Appendix A. Nomenclature

ee_s	enantiomeric excesses for the substrate
E	enantiomeric ratio, defined as $k_{2R}K_{mS}/k_{2S}K_{mR}$
(E_t)	enzyme concentration (mg/mL)
G	parameter defined in Eq. (3)
k_{2i}, k_{4i}	kinetic constants, $i = R$ or S for (*R*)- or (*S*)-enantiomer (mmol/g h)
K_I	inhibition constant (mM)
K_{mi}, K_{m3i}	kinetic constants, $i = R$ or S for (*R*)- or (*S*)-enantiomer (mM)
(M)	alcohol concentration (mM)
$(M)_0$	initial alcohol concentration (mM)
(S_i)	substrate concentration, $i = R$ or S for (*R*)- or (*S*)-enantiomer (mM)
$(S_i)_0$	initial concentration, $i = R$ or S for (*R*)- or (*S*)-enantiomer (mM)
T	absolute temperature (K)
V_i	initial rate, $i = R$ or S for (*R*)- or (*S*)-enantiomer (mM/h)
X_i, X_t	enantiomer conversion defined as $[1-(S_i)/(S_i)_0]$, $i = R$ or S; racemate conversion defined as $(X_R + X_S)/2$
ΔH_i	enthalpy difference between transition and ground states, $i = R$ or S (kJ/mol)
ΔS_i	entropy difference between transition and ground states, $i = R$ or S (J/mol K)
$\Delta\Delta G$	defined as $(\Delta\Delta H - T\Delta\Delta S)$ (kJ/mol)
$\Delta\Delta H$	defined as $(\Delta H_R - \Delta H_S)$ (kJ/mol)
$\Delta\Delta S$	defined as $(\Delta S_R - \Delta S_S)$ (J/mol K)

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.procbio.2011.01.017.

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