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Novel selective biocatalytic deacetylation studies on dihydropyrimidinones

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Abstract

Five racemic ethyl 4-(3/4-acetoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylates have been synthesized by acetylation of corresponding hydroxy analogues, which in turn have been synthesized under microwave condition by multi-component Biginelli cyclocondensation of ethyl acetoacetate, urea and the corresponding hydroxybenzaldehydes in the presence of ferric chloride. These dihydropyrimidinones have been subjected to biocatalytic resolution using acetoxyl group on the phenyl ring as remote handle; Novozyme®-435, an immobilized lipase from *Candida antarctica* in THF:DIPE has been found to catalyze the deacetylation reactions in an enantioselective fashion.

Keywords: 4-Phenyl-3,4-dihydropyrimidinones; Biocatalysis; Novozyme-435; Enantioselective deacetylation

1. Introduction

The increase in different cardiovascular diseases has warranted the development of new and efficient cardioprotecting agents. Compounds belonging to the class of 4-aryl-1,4-dihydropyridines, such as nifedipine, nitrendipine, nimodipine, etc. are the most studied calcium channel modulators and have become almost indispensable for the treatment of cardiovascular diseases such as hypertension and angina since their introduction into clinical medicine in seventies [1–3]. Although compounds of this class have symmetrical scaffold, lot of desymmetrisation studies have been carried out to generate chiral dihydropyridines keeping in mind that chirality plays an important role in biological activities [4–6]. 4-Aryl-3,4-dihydropyrimidinones **A**, which have similar structure as 4-aryl-1,4-dihydropyridines **C** also exhibited calcium channel modulating activity, but in contrast to the latter compounds they are inherently asymmetric (Fig. 1). The calcium channel modulation activity of 4-aryl-3,4-dihydropyrimidinones is dependent on the absolute configuration at the stereogenic center C-4. The orientation (*R*-/*S*-configuration) of C-4 aryl group in 4-aryl-3,4-dihydropyrimidinones acts as a molecular switch between calcium channel blocking (antagonist) and activating activities (agonist) [7]. For example, it is only the (*R*)-enantiomer SQ 32926 **A** that carries the therapeutically desired calcium channel blocking activity and not the (*S*)-enantiomer SQ 32926 **B** (Fig. 1) [8]. Besides the difference in potency, two enantiomers of a drug candidate often exhibit differences in their pharmacological profile [9–13].

Although, several procedures are reported for the synthesis of 4-aryl-3,4-dihydropyrimidinones [14–19], the recently developed microwave assisted synthesis offers significant advantages over conventional methods with respect to yield, simplicity, eco-friendliness and time taken for the reaction [20,21]. All these classical chemical and microwave procedures of synthesis of 4-aryl-3,4-dihydropyrimidinones lead to the formation of racemic mixtures of the compounds, which necessitates the development of efficient methodologies for the resolution of (±)-4-aryl-3,4-dihydropyrimidinones. Literature reveals that optically pure dihydropyrimidinones have been obtained by resolution of the corresponding racemic C-5 carboxylic acids by salt formation with *R*/*S*-amines [22], by separation of diastereomeric derivatives bearing chiral auxiliaries at N-3 [23] or by HPLC using chiral stationary phases [24].

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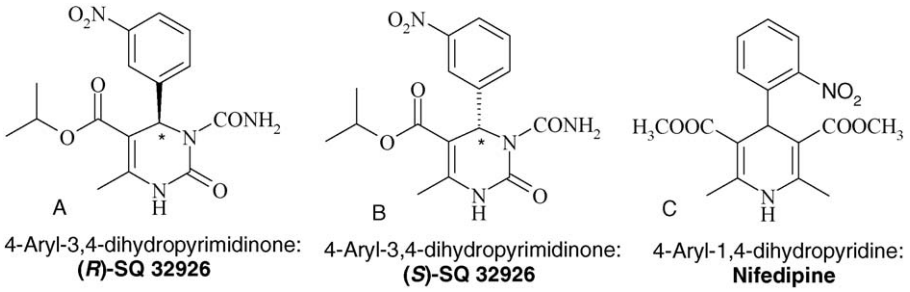
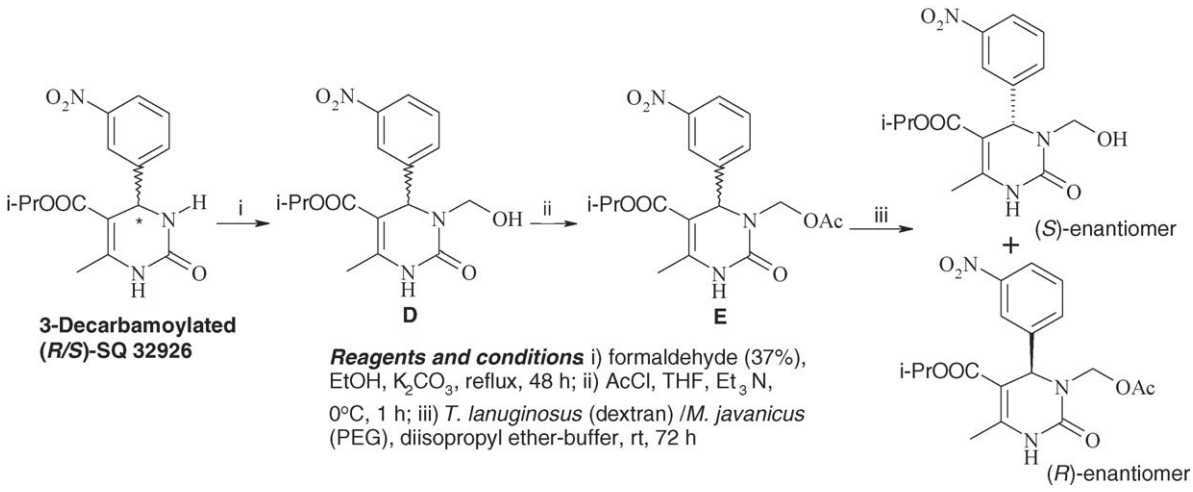


Fig. 1. Bioactive 4-aryldihydropyrimidinone and 4-aryldihydropyridine.



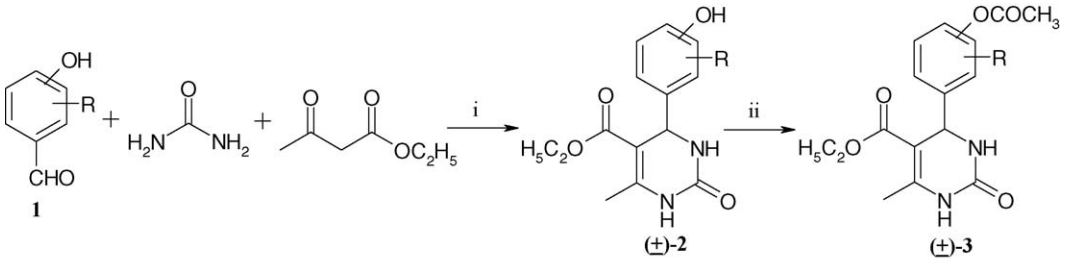
Scheme 1.

One of the recent additions in the armament of synthetic organic chemists for chiral resolution of organic compounds is the application of biocatalysts, in particular lipases. This is mainly because of their low cost and wide tolerance towards a variety of reaction conditions and substrates [25–27]. Earlier we have successfully demonstrated stereoselective acylation/deacylation, amidation and epoxidation reactions on amines, different polyphenolic compounds, alcohols, dienes, sugars and isoxazolidine derivatives using porcine pancreatic, *Candida antarctica*, *Candida rugosa* or *Pseudomonas* species lipases [28–38]. Recently, Schnell et al. [39] have resolved 3-decarbamoylated (*R*/*S*)-SQ32926 using lipases from *Mucor javanicus* (Amano M) and *Thermomyces lanuginosus* (Amano CE) in mixed aqueous organic solvents in the presence of enhancer, polyethylene glycol methyl ether (PEG, M. Wt. 5000) and dextran (M. Wt. 260,000), respectively (Scheme 1). The resolution of *N*-3-decarbamoylated SQ32926 has been affected using *N*-3-acetoxymethyl group as a handle for lipase-catalyzed deacetylation of *N*-3-acetoxymethylated 4-aryl-3,4-dihydropyrimidinone **E**, which was synthesized by the reaction of corresponding dihydropyrimidinone with formaldehyde, followed by acetylation of the resulted *N*-hydroxymethyldihydropyrimidinone **D** (Scheme 1). In the current study, we report the synthesis of a series of racemic ethyl 4-acetoxyphenyl-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylates and their lipase-catalyzed resolution using acetoxyl group as a remote handle.

2. Results and discussion

Racemic ethyl (±)-4-(3/4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylates **2a**–**2e** were prepared in 80–90% yields by multi-component Biginelli cyclocondensation of ethyl acetoacetate, urea and the corresponding aromatic hydroxyaldehydes **1a**–**1e** in the presence of ferric chloride under microwave conditions, following the modified literature procedure [40]. Compounds **2a**–**2e** were acetylated to afford the ethyl (±)-4-(3/4-acetoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylates **3a**–**3e** in quantitative yields using acetic anhydride-pyridine and a catalytic amount of *N*,*N*-dimethylaminopyridine (DMAP) (Scheme 2). All the 4-hydroxyaryl- and 4-acetoxyaryl-3,4-dihydropyrimidin-2-ones **2a**–**2e** and **3a**–**3e**, respectively, were unambiguously characterized on the basis of their spectral data (IR, ^{1}H and ^{13}C NMR and mass spectra). The structures of known compounds **2a**–**2d** were further confirmed by comparison of their melting points and/or spectral data with those reported in the literature [40–42].

Different lipases, i.e. porcine pancreatic lipase (PPL), *C. rugosa* lipase (CRL), Novozyme®-435 (immobilized CAL-B) and *Bacillus* sp. lipase were screened for enantioselective deacetylation of ethyl (±)-4-(3/4-acetoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylates **3a**–**3e**. None of the five (±)-4-(3/4-acetoxyphenyl)-3,4-dihydropyrimidin-2-ones **3a**–**3e** under study were accepted as substrates by PPL and *Bacillus* sp. lipase. Although, CRL in tetrahydrofu-



	1 and (±)-2		(±)-3	
	OH	R	$OCOCH_3$	R
a	C-3	H	C-3	H
b	C-3	C-4 OCH_3	C-3	C-4 OCH_3
c	C-4	H	C-4	H
d	C-4	C-3 OCH_3	C-4	C-3 OCH_3
e	C-4	C-3 OC_2H_5	C-4	C-3 OC_2H_5

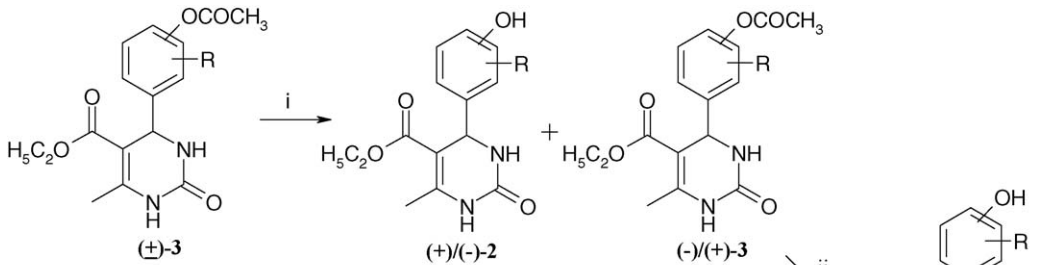
Reagents and Conditions : i) microwave 1-2 min, $FeCl_3$, SiO_2; ii) acetic anhydride, DMAP, 60 min, 25-30°C

Scheme 2.

ran:diisopropyl ether (THF:DIPE) catalyzed the deacetylation of (±)-3,4-dihydropyrimidin-2-ones **3a**–**3e**, the reaction was too slow to be of any practical utility. On the basis of the screening tests, Novozyme®-435 in THF:DIPE (2:1) was selected for further deacetylation studies on 3,4-dihydropyrimidin-2-ones **3a**–**3e**. The mixture of solvents, i.e. THF:DIPE (2:1) was selected on the basis of many test reactions in different mixtures of solvents and on the basis of our experience of better efficiency of CAL in relatively non-polar solvents (Scheme 3).

In a typical reaction, the racemic 3,4-dihydropyrimidin-2-one (**3a**–**3e**, 2 mmol) was treated in an incubator shaker with Novozyme®-435 (∼300 mg) in THF:DIPE (2:1, 20–25 ml) at 50–55 °C in the presence of butan-1-ol. The reaction was monitored by TLC and stopped by filtering off the enzyme after about 50% conversion of the starting acetate to a slow moving product on TLC. The solvent was removed from the reaction mixture and residue was subjected to column chromatography to afford the enzymatically deacetylated 4-hydroxyphenyl-3,4-dihydropyrimidin-2-ones **2a**–**2e** and unreacted 4-acetoxyphenyl-3,4-dihydropyrimidin-2-ones **3a**–**3e** in pure forms in 39–48 and 35–45% yields, respectively (Table 1).

The results of lipase-catalyzed deacetylation as summarized in Table 1 indicated that the nature and position of substituents on the phenyl ring of 4-acetoxyaryldihydropyrimidinones **3a**–**3e** affect the rate of deacetylation of the compound. Thus, the rate of deacetylation of 4-(3-acetoxyphenyl)-dihydropyrimidinone **3a** is 3.5 times faster than the rate of deacetylation of 4-(3-acetoxy-4-methoxyphenyl)-dihydropyrimidinone **3b**, which differs only in the presence of an extra methoxyl group at C-4 position of the C-4 phenyl moiety. Similarly, the rate of deacetylation of 4-(4-acetoxyphenyl) dihydropyrimidinone **3c** is about two times faster than the rate of deacetylation of 4-(4-acetoxy-3-methoxyphenyl)-dihydropyrimidinone **3d**, which in turn is 1.2 times faster than the rate of deacetylation of 4-(4-acetoxy-3-ethoxyphenyl)-dihydropyrimidinone **3e**. The rate of lipase-catalyzed deacetylation reactions are so sensitive to



	(+)/(-)-2 & (-)/(+)-2		(±)-3 & (-)/(+)-3	
	OH	R	$OCOCH_3$	R
a	C-3	H	C-3	H
b	C-3	C-4 OCH_3	C-3	C-4 OCH_3
c	C-4	H	C-4	H
d	C-4	C-3 OCH_3	C-4	C-3 OCH_3
e	C-4	C-3 OC_2H_5	C-4	C-3 OC_2H_5

Reagents and Conditions:
i) Novozyme®- 435, THF-DIPE (2:1), butan-1-ol, 10-35 h, 50-55 °C;
ii) Sat. methanolic-ammonia, 30-45 min, 25-30 °C

Scheme 3.

Table 1
Enantioselective deacetylation of ethyl (±)-4-(3/4-acetoxyaryl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylates **3a–3e** mediated by Novozyme®-435 in THF:DIPE (2:1) at 50–55 °C in the presence of butan-1-ol[a,b]

Substrate	Time (h)	Products[c]	Yield[d]
Ethyl (±)-4-(3-acetoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3a**)	10	Ethyl (+)-4-(3-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**2a**)	42
		Ethyl (−)-4-(3-acetoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3a**)	45
Ethyl (±)-4-(3-acetoxy-4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3b**)	35	Ethyl (−)-4-(3-hydroxy-4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**2b**)	43
		Ethyl (+)-4-(3-acetoxy-4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3b**)	40
Ethyl (±)-4-(4-acetoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3c**)	8	Ethyl (−)-4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**2c**)	48
		Ethyl (+)-4-(4-acetoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3c**)	42
Ethyl (±)-4-(4-acetoxy-3-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3d**)	15	Ethyl (−)-4-(4-hydroxy-3-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**2d**)	39
		Ethyl (+)-4-(4-acetoxy-3-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3d**)	45
Ethyl (±)-4-(4-acetoxy-3-ethoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3e**)	18	Ethyl (−)-4-(4-hydroxy-3-ethoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**2e**)	41
		Ethyl (+)-4-(4-acetoxy-3-ethoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3e**)	35

[a] All these reactions, when performed under identical conditions but without adding lipase did not yield any product.
[b] All deacetylation reactions were stopped by filtering off the enzyme after about 50% conversion of the starting racemic acetoxy dihydropyrimidin-2-ones to the product, i.e. corresponding hydroxy compound.
[c] Deacetylated dihydropyrimidin-2-ones and recovered, unreacted acetoxy dihydropyrimidin-2-ones.
[d] Yields were calculated by assuming corresponding single enantiomer as 100% in the starting ethyl (±)-4-(3/4-acetoxyaryl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylates **3a–3e**.

the substituents in the C-4 phenyl moiety of dihydropyrimidinones that there is 1.25 times difference even in the rate of deacetylation of 4-(3-acetoxyphenyl)-dihydropyrimidinone **3a** and 4-(4-acetoxyphenyl)-dihydropyrimidinone **3c** and 2.33 times difference in the rate of deacetylation of 4-(3-acetoxy-4-methoxyphenyl)-dihydropyrimidinone **3b** and 4-(4-acetoxy-3-methoxyphenyl)-dihydropyrimidinone **3d**; which differ only in the position of acetoxy or acetoxy and methoxy substituent in the phenyl ring. The differences in the rates of deacetylation of different 4-aryldihydropyrimidinone **3a–3e** may be because of the fact that occupancy of aryl group having acetoxyl function in the binding site of Novozyme®-435 changes with the presence of different substituents in the ring and also because of the presence of the same substituent at different positions of the aromatic ring. These differences thereby change the accessibility of acetoxyl group on the phenyl ring of dihydropyrimidinone to the same residue of the catalytic triad present in the catalytic site, which is responsible for catalysis of acetyl/acyl transfer reactions. Further, the enzymatically deacetylated compounds **2a–2e** and recovered, unreacted acetates **3a–3e** had significant optical rotation values (Table 2), thus indicating that Novozyme®-435 catalyzes the deacetylation of (±)-4-(3/4-acetoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-ones **3a–3e** in an enantioselective fashion.

In order to determine enantiomeric excess (ee) values of biocatalytically deacetylated (+)/(−)-4-hydroxyphenyl-3,4-dihydropyrimidin-2-ones **2a–2e** and recovered, unreacted (−)/(+)-4-acetoxyphenyl-3,4-dihydropyrimidin-2-ones **3a–3e**, the ^{1}H NMR spectra of (±)-**2a–2e** and (±)-**3a–3e** were recorded in the presence of different concentrations of chiral-shift reagent, (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)-ethanol [(+)-TFAE], but the separation of the signals in the ^{1}H NMR spectra of these compounds was not observed. Further, ee determination of enzymatically deacetylated (+)/(−)-4-hydroxyphenyl-3,4-dihydropyrimidin-2-ones **2a–2e** was attempted by synthesis of *O*-acetylmandelic acid ester of (+)/(−)- and (±)-**2a–2e** with *S*-(+)-*O*-acetylmandelic acid in dichloromethane according to the procedure of Whitesell and Reynolds [43]. The ^{1}H NMR spectral analysis of the diastereomeric mandelates obtained from esterification of (±)-**2a–2e** were analyzed to find out the splitting in chemical shift values of diastereomeric protons. Unfortunately, no separation of the signals in the ^{1}H NMR spectra of diastereomeric mandelates was observed, thus enantiomeric excess values of deacetylated, optically enriched (+)/(−)-4-hydroxyphenyl-3,4-dihydropyrimidin-2-ones **2a–2e** or those of the recovered, unreacted (−)/(+)-4-acetoxyphenyl-3,4-dihydropyrimidin-2-ones **3a–3e** could not be determined by NMR techniques.

However to know the extent of optical enrichment of enzymatically deacetylated (+)/(−)-4-hydroxyphenyl-3,4-dihydropyrimidin-2-ones **2a–2e**, chemical deacetylation of recovered, unreacted acetates (−)/(+)-**3a–3e** was affected with saturated methanolic ammonia and optical rotation values of the enzymatically deacetylated (+)/(−)-**2a–2e** and chemically deacetylated (−)/(+)-**2a–2e** were compared. These were quite comparable and were of opposite signs in all the five cases (Table 2). This indicates that the order of enantioselectivity dur-

Table 2
Optical rotation values ($[\alpha]_D^{25}$) of (+)/(−)-4-hydroxyaryl-3,4-dihydropyrimidin-2-ones **2a–2e** obtained by Novozyme®-435 catalyzed deacetylation of (±)-**3a–3e**; recovered, unreacted acetates (−)/(+)-4-acetoxyaryl-3,4-dihydropyrimidin-2-ones **3a–3e** and (−)/(+)-4-hydroxyaryl-3,4-dihydropyrimidin-2-ones **2a–2e** obtained by chemical deacetylation of (−)/(+)-**3a–3e**

Hydroxy dihydropyrimidinones (+)/(−)-**2a–2e** obtained by enzymatic deacetylation of (±)-**3a–3e**	Recovered, unreacted acetoxy dihydropyrimidinones (−)/(+)-**3a–3e**	Dihydropyrimidinones (−)/(+)-**2a–2e** obtained by chemical deacetylation of (−)/(+)-**3a–3e**
(+)-**2a**: +21.0	(−)-**3a**: −18.0	(−)-**2a**: −18.0
(−)-**2b**: −33.5	(+)-**3b**: +12.0	(+)-**2b**: +30.0
(−)-**2c**: −38.0	(+)-**3c**: +32.0	(+)-**2c**: +30.0
(−)-**2d**: −3.0	(+)-**3d**: +8.9	(+)-**2d**: +3.1
(−)-**2e**: −11.0	(+)-**3e**: +14.2	(+)-**2e**: +8.8

ing lipase-catalyzed deacetylation of (±)-**3a–3e** is moderate to high. The structures of enzymatically deacetylated (+)/(−)-4-hydroxyphenyl-3,4-dihydropyrimidin-2-ones **2a–2e,** and those of the recovered, unreacted (−)/(+)-4-acetoxyphenyl-3,4-dihydropyrimidin-2-ones **3a–3e** and (−)/(+)-4-hydroxyphenyl-3,4-dihydropyrimidin-2-ones **2a–2e** obtained from chemical deacetylation of (−)/(+)-**3a–3e** were unambiguously established on the basis of their spectral data (IR, ^{1}H, ^{13}C NMR and mass spectra) analysis. The spectral data of compounds (+)/(−)-**2a–2e**, (−)/(+)-**3a–3e** and (−)/(+)-**2a–2e** were found identical with the data recorded for the corresponding racemic compounds. All these reactions, when performed under identical conditions but without adding lipase did not yield any product.

3. Conclusion

We have demonstrated the application of Novozyme-435, a 'Greener' catalyst, for the preparation of optically enriched ethyl 4-(3/4-hydroxyaryl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylates **2a–2e** and ethyl 4-(3/4-acetoxyaryl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylates **3a–3e**, which belong to a class of compounds exhibiting interesting biological activities, in particular calcium channel modulating activity. This is the first report of lipase-catalyzed resolution of 4-aryl-6-methyl-3,4-dihydropyrimidin-2-ones involving hydroxyl group in C-4 aryl moiety of the molecule as remote handle. The lipase-catalyzed methodology developed by us for the resolution of 4-aryldihydropyrimidinones is more efficient than the earlier reported procedure developed by Schnell et al. [39] because it does not require any derivatization of the substrate. As it is difficult to synthesize compounds of this class in enantiomerically pure forms, the enzymatic methodology reported herein may find utility in the synthesis of optically enriched/pure compounds of this medicinally important class.

4. Experimental

Reactions were conducted under an atmosphere of nitrogen, when anhydrous solvents were used. Microwave reactions were performed in a domestic microwave oven of 850 W 1.2 Cft (33 L, infodisplay, Sharp Carosel). Column chromatography was carried out using silica gel (100–200 mesh). Melting points were determined using H_2SO_4 bath and are uncorrected. Analytical TLCs were performed on pre-coated Merck silica gel $60F_{254}$ plates; the spots were detected either using UV light or by charring with 4% alcoholic H_2SO_4. The IR spectra were recorded on a Perkin-Elmer model 2000 FT-IR spectrophotometer. The optical rotations were measured on Bellingham-Stanley AD 220 polarimeter. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance AC-300 spectrometer at 300 and at 75.5 MHz, respectively. The chemical shift values are reported as δ ppm relative to TMS used as internal standard and the coupling constants (J) are measured in Hz. The FAB-HRMS spectra of all the compounds were recorded on a JEOL JMS-AX505W high-resolution mass spectrometer in positive ion mode using the matrix HEDS (bishydroxyethylsulphide) doped with sodium acetate. Novozyme-435 was a gift from Novozymes A/S (Copenhagen, Denmark) and *Bacillus* sp. Lipase was obtained from the laboratory of Professor R.K. Saxena, Department of Microbiology, University of Delhi South Campus, whereas *C. rugosa* lipase (CRL) and porcine pancreatic lipase (PPL) were purchased from Sigma Chemical Co. (USA). These lipases were used after storing in vacuo over P_2O_5 for 24 h. The chiral-shift reagents (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol [(+)-TFAE] and chiral derivatising agent (*S*)-(+)-*O*-acetylmandelic acid were purchased from Aldrich Chemical Co. (USA). The organic solvents THF and dioxane were used after drying and distillation over sodium pieces.

4.1. General procedure for the preparation of ethyl (±)-4-(3/4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylates *2a–2e*

To a mixture of hydroxy aldehydes **1a–1e** (50 mmol), urea (150 mmol), ethyl acetoacetate (55 mmol) and ferric chloride hexahydrate (25 mmol), silica gel (100 g) was added to make a thick paste. The resulting paste was irradiated in a microwave for 1–2 min until TLC showed completion of reaction. The crude product was purified by column chromatography on silica gel using a gradient solvent system of chloroform–methanol to obtain the pure ethyl (±)-4-(3/4-hydroxyphenyl)-3,4-dihydropyrimidin-2-one-5-carboxylates **2a–2e** in 80–90% yields. All the 4-hydroxyaryl-3,4-dihydropyrimidin-2-ones were unambiguously characterized on the basis of their spectral data (IR, ^{1}H and ^{13}C NMR and mass spectra) analysis. The structures of known compounds

2a–**2d** were further confirmed by comparison of their melting points and/or spectral data with those reported in the literature [40–42].

4.1.1. Ethyl (±)-4-(4-hydroxy-3-ethoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**2e**)

It was obtained as a white crystalline solid (13.60 g) in 85% yield. M.p.: 185–186 °C; R_f: 0.38 (10% methanol in chloroform); IR (KBr): 3234 (NH), 3099 (NH), 1705 (ester CO), 1655 (amidic CO), 1518, 1476, 1382, 1324, 1286, 1254, 1229, 1100, 1045, 815, 751 and 720 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.14 (3H, t, J = 7.0 Hz, $COOCH_2CH_3$), 1.36 (3H, t, J = 6.9 Hz, OCH_2CH_3), 2.25 (3H, s, C-6 CH_3), 3.99 (4H, m, $COOCH_2CH_3$ and OCH_2CH_3), 5.08 (1H, d, J = 2.5 Hz, C-4H), 6.64 (1H, dd, J = 8.1 and 1.3 Hz, C-6′H), 6.71 (1H, d, J = 8.1 Hz, C-5′H), 6.79 (1H, br s, C-2′H), 7.49 (1H, br s, OH), and 8.58 and 8.99 (2H, 2s, 1H each, N-1H and N-3H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 15.12 and 15.75 ($COOCH_2CH_3$ and OCH_2CH_3), 18.81 (C-6 CH_3), 54.83 (C-4), 60.01 ($COOCH_2CH_3$), 65.07 (OCH_2CH_3), 100.89 (C-5), 113.33, 116.23 and 119.65 (C-2′, C-5′ and C-6′), 137.03 (C-6), 147.11, 147.28 and 148.54 (C-1′, C-3′ and C-4′), 153.44 (amidic CO) and 166.51 (ester CO); HRMS *m/z* calculated for $C_{16}H_{20}N_2O_5^+$ 320.1372, observed 320.1372.

4.2. General procedure for the preparation of ethyl (±)-4-(3/4-acetoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylates **3a**–**3e**

To a solution of ethyl (±)-4-(3/4-hydroxyphenyl)-3,4-dihydropyrimidin-2-one-5-carboxylate (**2a**–**2e**, 15 mmol) in acetic anhydride (75 mmol), a catalytic amount of 4-*N*,*N*-dimethylaminopyridine was added and the reaction mixture stirred at 25–30 °C for 1–2 h. On completion, as indicated by TLC examination, the reaction mixture was poured over ice-cold water and the white precipitate so obtained was filtered to afford the crude product. Pure ethyl 4-(3/4-acetoxyphenyl)-3,4-dihydropyrimidin-2-one-5-carboxylates was obtained in 88–94% yields by crystallization of the crude products from ethyl acetate–petroleum ether mixtures. All five novel 4-acetoxyaryl-3,4-dihydropyrimidin-2-ones **3a**–**3e** were unambiguously characterized on the basis of their spectral data (IR, ^{1}H and ^{13}C NMR and mass spectral) analysis.

4.2.1. Ethyl (±)-4-(3-acetoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3a**)

It was obtained as a white crystalline solid (4.34 g) in 91% yield. M.p.: 210–212 °C; R_f: 0.60 (10% methanol in chloroform); IR (KBr): 3352 (NH), 3223 (NH), 1764 (phenolic ester CO), 1696 (aliphatic ester CO), 1639 (amidic CO), 1456, 1372, 1324, 1295, 1226, 1093, 1017 and 799 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.17 (3H, t, J = 6.9 Hz, $COOCH_2CH_3$), 2.28 and 2.34 (6H, 2s, 3H each, C-6 CH_3 and $OCOCH_3$), 4.08 (2H, q, J = 6.9 Hz, $COOCH_2CH_3$), 5.42 and 5.48 (2H, 2s, 1H each, C-4H and N-1/3H), 7.02 (1H, m, C-5′H), 7.25 (3H, m, C-2′H, C-4′H and C-6′H) and 8.15 (1H, br s, N-3/1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.14 ($COOCH_2CH_3$), 18.73 (C-6 CH_3), 21.14 ($OCOCH_3$), 55.40 (C-4), 60.14 ($COOCH_2CH_3$), 101.08 (C-5), 119.82, 121.20, 123.99 and 129.71 (C-2′, C-4′, C-5′ and C-6′), 145.41 and 146.57 (C-6 and C-1′), 150.90 (C-3′), 153.15 (amidic CO), 165.50 (aliphatic ester CO) and 169.28 (phenolic ester CO); HRMS *m/z* calculated for $C_{16}H_{18}N_2O_5^+$ 318.1216, observed 318.1201.

4.2.2. Ethyl (±)-4-(3-acetoxy-4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3b**)

It was obtained as a white crystalline solid (4.80 g) in 92% yield. M.p.: 132–134 °C; R_f: 0.58 (10% methanol in chloroform); IR (KBr): 3248 (NH), 3117 (NH), 1762 (phenolic ester CO), 1703 (aliphatic ester CO), 1649 (amidic CO), 1514, 1465, 1369, 1293, 1269, 1224, 1205, 1091, 1025 and 796 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ 1.15 (3H, t, J = 6.9 Hz, $COOCH_2CH_3$), 2.23 and 2.32 (6H, 2s, 3H each, C-6 CH_3 and $OCOCH_3$), 3.78 (3H, s, OCH_3), 4.04 (2H, q, J = 6.9 Hz, $COOCH_2CH_3$), 5.26 (1H, s, C-4H) and 7.09 (3H, m, C-2′H, C-5′H and C-6′H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 14.89 ($COOCH_2CH_3$), 18.54 (C-6 CH_3), 20.82 ($OCOCH_3$), 55.96 and 56.87 (C-4 and OCH_3), 61.47 ($COOCH_2CH_3$), 102.42 (C-5), 114.00, 122.56 and 126.46 (C-2′, C-5′ and C-6′), 138.97 (C-6), 141.48 (C-1′), 149.23 (C-3′), 152.51 (C-4′), 155.27 (amidic CO), 167.85 (aliphatic ester CO) and 171.06 (phenolic ester CO); HRMS *m/z* calculated for $C_{17}H_{20}N_2O_6^+$ 348.1321, observed 348.1306.

4.2.3. Ethyl (±)-4-(4-acetoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3c**)

It was obtained as a white crystalline solid (4.48 g) in 94% yield. M.p.: 141–142 °C; R_f: 0.60 (10% methanol in chloroform); IR (KBr): 3249 (NH), 3117 (NH), 1759 (phenolic ester CO), 1704 (aliphatic ester CO), 1651 (amidic CO), 1508, 1459, 1367, 1286, 1225, 1094, 1017, 913, 853 and 785 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.17 (3H, t, J = 7.0 Hz, $COOCH_2CH_3$), 2.28 and 2.33 (6H, 2s, 3H each, C-6 CH_3 and $OCOCH_3$), 4.08 (2H, q, J = 7.0 Hz, $COOCH_2CH_3$), 5.40 (1H, d, J = 1.9 Hz, C-4H), 5.85 (1H, s, N-1H/N-3H), 7.02 (2H, d, J = 8.5 Hz, C-3′H and C-5′H), 7.32 (2H, d, J = 8.5 Hz, C-2′H and C-6′H) and 8.19 (1H, s, N-3H/N-1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.86 ($COOCH_2CH_3$), 18.39 (C-6 CH_3), 20.79 ($OCOCH_3$), 54.86 (C-4), 59.79 ($COOCH_2CH_3$), 101.03 (C-5), 121.46 (C-3′ and C-5′), 127.44 (C-2′ and C-6′), 140.99 (C-6), 146.10 (C-1′), 149.97 (C-4′), 153.01 (amidic CO), 165.24 (aliphatic ester CO) and 169.01 (phenolic ester CO); HRMS *m/z* calculated for $C_{16}H_{18}N_2O_5^+$ 318.1216, observed 318.1201.

4.2.4. Ethyl (±)-4-(4-acetoxy-3-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylate (**3d**)

It was obtained as a white crystalline solid (4.59 g) in 88% yield. M.p.: 176–178 °C; R_f: 0.58 (10% methanol in chloroform); IR (KBr): 3244 (NH), 3115 (NH), 1770 (phenolic ester CO), 1705 (aliphatic ester CO), 1655 (amidic CO), 1511, 1458, 1369, 1321, 1281, 1229, 1095, 1033, 902 and 783 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + few drops of CD_3OD): δ 1.19 (3H, t, J = 7.0 Hz, $COOCH_2CH_3$), 2.29 and 2.34 (6H, 2s, 3H each, C-6 CH_3 and $OCOCH_3$), 3.81 (3H, s, OCH_3), 4.10 (2H, q,

$J = 7.0$ Hz, $COOCH_2CH_3$), 5.37 (1H, s, C-4H) and 6.92 (3H, m, C-5′H, C-6′H and C-2′H); ^{13}C NMR (75.5 MHz, $CDCl_3$ + few drops of CD_3OD): δ 13.92 ($COOCH_2CH_3$), 18.20 (C-6 CH_3), 20.34 ($OCOCH_3$), 54.95 and 55.65 (C-4 and OCH_3), 60.02 ($COOCH_2CH_3$), 100.99 (C-5), 110.77, 118.50 and 122.62 (C-2′, C-5′ and C-6′), 139.07 (C-6), 142.59 (C-1′), 146.45 (C-3′), 150.91 (C-4′), 152.0 (amidic CO), 165.74 (aliphatic ester CO) and 169.19 (phenolic ester CO); HRMS *m/z* calculated for $C_{17}H_{20}N_2O_6^+$ 348.1321, observed 348.1289.

4.2.5. Ethyl (±)-4-(4-acetoxy-3-ethoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2-one (3e)

It was obtained as a white crystalline solid (4.83 g) in 88% yield. M.p.: 168–171 °C; R_f: 0.59 (10% methanol in chloroform); IR (KBr): 3239 (NH), 3114 (NH), 1765 (phenolic ester CO), 1707 (aliphatic ester CO), 1652 (amidic CO), 1513, 1463, 1424, 1372, 1284, 1224, 1095, 1041, 904 and 790 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.18 (3H, t, $J = 7.0$ Hz, $COOCH_2CH_3$), 1.36 (3H, t, $J = 6.9$ Hz, OCH_2CH_3), 2.28 and 2.33 (6H, 2s, 3H each, C-6 CH_3 and $OCOCH_3$), 4.05 (4H, m, $COOCH_2CH_3$ and OCH_2CH_3), 5.39 (1H, s, C-4H), 5.69 (1H, s, N-1H/N-3H), 6.91 (3H, m, C-2′H, C-5′H and C-6′H) and 7.77 (1H, s, N-3H/N-1H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 14.36 ($COOCH_2CH_3$), 14.85 (OCH_2CH_3), 18.50 (C-6 CH_3), 20.75 ($OCOCH_3$), 55.35 (C-4), 56.10 (OCH_2CH_3), 60.52 ($COOCH_2CH_3$), 101.33 (C-5), 111.33, 118.99 and 122.55 (C-2′, C-5′ and C-6′), 139.53 (C-6), 143.25 (C-1′), 147.35 (C-3′), 151.39 (C-4′), 153.82 (amidic CO), 166.42 (aliphatic ester CO) and 169.85 (phenolic ester CO); HRMS *m/z* calculated for $C_{18}H_{22}N_2O_6^+$ 362.1478, observed 362.1477.

4.3. General procedure for enzymatic deacetylation of ethyl (±)-4-(3/4-acetoxyaryl)-6-methyl-3,4-dihydropyrimidin-2-one-5-carboxylates 3a–3e

To a solution of ethyl (±)-4-(3/4-acetoxyaryl)-3,4-dihydropyrimidin-2-one-5-carboxylates (**3a**–**3e**, 2 mmol) in anhydrous THF:DIPE (2:1, 20–25 ml), *n*-butanol (5 ml) was added, followed by Novozyme®-435 (300 mg). The suspension was stirred at 50–53 °C in an incubator shaker and the progress of the reaction was monitored periodically by analytical TLC. After about 50% conversion of the starting material into the product, reaction was quenched by filtering off the enzyme and the solvent evaporated to dryness in vacuo to afford a gummy residue, which was purified by column chromatography on silica gel using a gradient solvent system of chloroform–methanol to afford optically enriched (+)/(−)-ethyl 4-(3/4-hydroxyaryl)-3,4-dihydropyrimidin-2-one-5-carboxylates **2a**–**2e** and (−)/(+)-ethyl 4-(3/4-acetoxyaryl)-3,4-dihydropyrimidin-2-one-5-carboxylates **3a**–**3e** in 39–48 and 35–45% yields, respectively. The (+)/(−)-ethyl 4-(3/4-hydroxyaryl)-3,4-dihydropyrimidin-2-one-5-carboxylates **2a**–**2e** and (−)/(+)-ethyl 4-(3/4-acetoxyaryl)-3,4-dihydropyrimidin-2-one-5-carboxylates **3a**–**3e** were unambiguously identified by comparison of their spectral data with the corresponding racemic compounds, i.e. (±)-**2a**–**2e** and (±)-**3a**–**3e**, respectively, as reported above.

4.4. General procedure for chemical deacetylation of unreacted, recovered acetates (−)/(+)-3a–3e

To a solution of (−)/(+)-ethyl 4-(3/4-acetoxyaryl)-3,4-dihydropyrimidin-2-one-5-carboxylates **3a**–**3e** (100 mg) in methanol (5 ml) was and added saturated methanolic ammonia (10 ml) and the reaction mixture stirred for 30–45 min at 25–30 °C until TLC showed completion of reaction. The solvent was evaporated under reduced pressure to afford a gummy residue, which was co-evaporated with toluene. The resulting crude solid product was washed repeatedly with chloroform–petrol mixture (5 ml × 2.5 ml) to afford pure optically enriched (−)/(+)-ethyl 4-(3/4-hydroxyaryl)-3,4-dihydropyrimidin-2-one-5-carboxylates **2a**–**2e** in 85-90% yields. The (−)/(+)-ethyl 4-(3/4-hydroxyaryl)-3,4-dihydropyrimidin-2-one-5-carboxylates **2a**–**2e** were unambiguously identified on the basis of their spectroscopic data, which were found identical with the spectroscopic data of the corresponding enantiomers (+)/(−)-**2a**–**2e** and (±)-**2a**–**2e**.

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