Indian Journal of Chemistry
Vol. 46B, September 2007, pp. 1501-1510

Biocatalytic acylation studies on novel 3-aryl-3-hydroxymethyl-2, 3-dihydro-4*H*-1-benzopyran-4-ones

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Received 3 August 2006; accepted (revised) 26 June 2007

(±)-3-Aryl-3-hydroxymethyl-2,3-dihydro-4*H*-1-benzopyran-4-ones have been synthesized in four steps starting with the coupling of resorcinol with corresponding phenylacetic acid leading to the formation of 2,4-dihydroxyphenyl aryl ketones, which upon monomethylation/benzylation and hydroxymethylation afford (±)-hydroxymethylisoflavanones in 65-70% yields. These isoflavanones have been subjected to lipase-catalyzed acylation reactions under different conditions (for optimization) of varying solvents, enzymes and acylating agents. *Candida antarctica* lipase B in tetrahydrofuran using heptanoic anhydride at 90 ^{o}C is found to be the best reaction protocol for the biocatalytic reaction.

Keywords: Isoflavanones, biocatalytic resolution, lipase, CAL-B, acylating agents, heptanoic anhydride

The tremendous potential of enzymes as practical catalysts is well recognized[1-4]. There are some marvellous advantages that enzymes offer which are difficult to obtain by conventional catalysis, mainly it is the selectivity and specificity that enzymes show in their reactions. No matter how simple or trivial the enzyme-catalysed chemical reaction is, this may be on three levels: chemoselectivity, regioselectivity and stereo-selectivity/specificity with the result that enzymes are being increasingly exploited for asymmetric synthetic transformations. This is also fuelled by the increased requirement of chiral switching technologies to fulfill the growing demand for enantiopure pharmaceuticals. Among the biocatalysts in organic reactions, lipases are most frequently used in the synthesis of many biologically active compounds and natural products[5,6]. This is due to their substrate specificity, which ranges from being very narrow to broad and also because they operate at RT and without any added cofactors, under neutral aqueous conditions, and without substrate functional-group protection. In recent years, lipases from porcine pancreas, and *Candida*, *Aspergillus* and *Pseudomonas* species have been successfully employed for carrying out regio- and stereoselective acylations/deacylations on different classes of compounds, *viz*. polyphenolics[7-9], benzopyranones[10], carbohydrates[11-13], chromanones[14], polyols[15,16], *etc*.

Isoflavonoids represent a relatively large group of naturally occurring secondary metabolites, displaying a wide array of physiological activity, *e.g.* antifungal[17], antibacterial[18] and as phytoalexins[19]. Polyhydroxylated ketones are precursors for the synthesis of a variety of useful target molecules and are used for the synthesis of chalcones, flavones, flavanones, coumarins, chromanones, chromanols, *etc*. Futhermore, hydroxymethylchromanones have been used as precursors for non-steroidal anti-inflammatory agents[20]. Additionally, these compounds have been used as components of anovulatory (dysfunctional uterine bleeding) pharmaceuticals[21]. Reports on the biological activity of chromanone derivatives and the realization that the two enantiomers of a chiral compound differ pharmacologically prompted the synthesis of a series of chromanone derivatives in enantiomerically enriched forms employing chemoenzymatic routes.

Results and Discussion

Four racemic 3-aryl-3-hydroxymethyl-2,3-dihydro-4*H*-1-benzopyran-4-ones have been synthesized in three steps starting from the nuclear acylation of

resorcinol **1** with the appropriately substituted phenylacetic acid **2a-c** to afford 2,4-dihydroxyphenyl aryl ketones **3a-c**[22-24] in 75-80% yield. The partial methylation/benzylation of ketones **3a-c** led to the formation of 2-hydroxy-4-methoxy/benzyloxyphenyl aryl ketones **4a-d**[22,24-26] in 82-92% yields, which on reaction with alkaline formaldehyde afforded the (±)-hydroxymethylisoflavanones **5a-d** in 65-70% yields (**Scheme I**).

The racemic 3-hydroxymethylisoflavanones **5a-d** were subjected to enantioselective acetylation with vinyl acetate and *Candida antarctica* lipase (CAL B) in toluene (**Scheme II**). In all these cases, a weighed amount of the racemic substrate was incubated with vinyl acetate and enzyme in toluene at 38-40°C. The progress of the reaction was monitored by TLC/HPLC and the reaction work-up was carried out after about 50% conversion of the starting material into the product by filtering off the enzyme. The components of the reaction mixture, *e.g.*, the acetoxymethylisoflavanones **6a-d** and the unreacted hydroxymethylisoflavanones **5a-d** were separated by column chromatography and were found to be optically active (**Table I**). This indicates that CAL-catalyzed acetylation of 3-hydroxymethylisoflavanones **5a-d** is enantioselective. On the basis of the yields and time period required to transform the starting racemic substrates into the less polar products, **5c** was selected for further study.

Different lipases, *i.e. Candida antarctica* lipase (CAL B), *Candida rugosa* lipase (CRL), porcine pancreatic lipase (PPL), Amano PS lipase and Amano PY lipase were screened for enantioselective acetylation of (±)-3-(4'-chlorophenyl)-3-hydroxymethyl-7-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one **5c** in toluene, diisopropyl ether and tetrahydrofuran using vinyl acetate as the acylating agent at 38-40°C. During the screening of different lipases, reactions with CAL were facile in toluene and diisopropyl ether as compared to tetrahydrofuran. Further, the rate of transformation of starting racemic 3-hydroxymethylisoflavanone **5c** into the product was slow in the cases of reactions catalyzed by Amano PS, Amano PY and CRL, and PPL did not accept it as substrate (**Table II, Figure 1, Figure 2** and **Figure 3**).

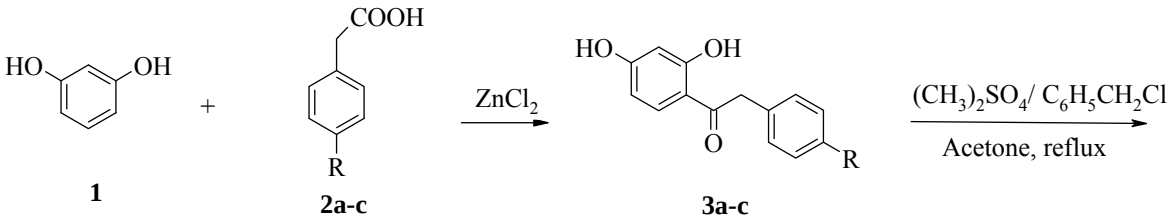
In order to find out the tolerance of CAL for different acylating agents of varying chain lengths, reactions with vinyl acetate/acetic anhydride, 2,2,2-trifluoroethyl butyrate and heptanoic anhydride were carried out for the acylation of (±)-3-(4'-chlorophenyl)-3-hydroxymethyl-7-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one **5c** in toluene and diisopropyl ether at 38-40°C (**Scheme III**). Heptanoic anhydride was found to be the most efficient acylating agent to transform the starting racemic substrate **5c** into the less polar products **7b** (seen on TLC) upon incubation with *Candida antarctica* lipase (CAL B) in toluene and diisopropyl ether. The rate of transformation became slow as the alkyl chain length in acylating agent kept on decreasing (**Table II** and **Figure 4**).

For temperature study, racemic substrate **5c** was incubated with heptanoic anhydride and CAL at 40°C, 60°, 90°, 130° and 170°C. Diphenyl ether was used as solvent for the screening of enzymatic resolution at different temperatures. In studies involving optimization of reaction conditions for resolution with CAL, it was found that time period required for transformation of starting racemic substrate **5c** into the less polar product **7b** (seen on TLC) decreases as temperature increases. The fastest reaction was at 90°C, 50% conversion of **5c** into its heptanoate taking place in 5 hr. However, there was no enzymatic resolution at temperatures higher than 90°C (**Table II** and **Figure 5**).

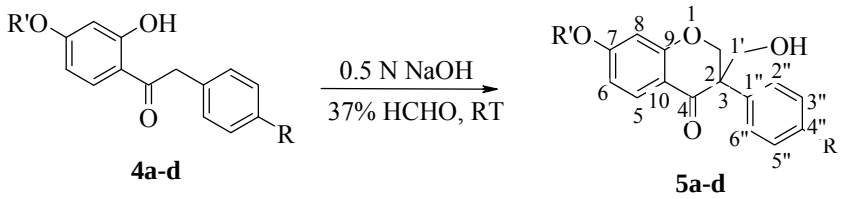
All the (±)-, (+)- and (-)-hydroxymethylisoflavanones **5a-d** and (±)-/(+)–acyloxymethyl-isoflavanones **6a-d** and **7a-b** were identified on the basis of their spectral data (*cf.* Experimental Section) and are being reported for the first time ever.

Experimental Section

Progress of reaction was monitored by TLC on aluminium backed precoated Merck silica gel $60F_{254}$ plates, the spots were visualised either by UV light, charring with 10% ethanolic H_2SO_4 or by spraying with 5% alcoholic $FeCl_3$ solution. Silica gel (100-200 mesh) was used for column chromatography. Melting points were determined in a sulphuric acid bath and are uncorrected. Optical rotation values were measured with Perkin-Elmer 241 polarimeter. Fourier-transform infrared spectra (FT-IR) were recorded on a Perkin-Elmer model 2000 FT-IR spectrometer. Pellets were prepared by mixing KBr with samples for FT-IR study. UV-Vis absorption spectra were recorded on a Perkin-Elmer Lambda 9 spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on Bruker ARX-500 NMR spectrometer and Avance-300 spectrometer using TMS as internal standard. The chemical shift values are on δ scale in ppm and the coupling constant values (*J*) are in Hz. The HRMS were recorded on JOEL JMS-AX 505W



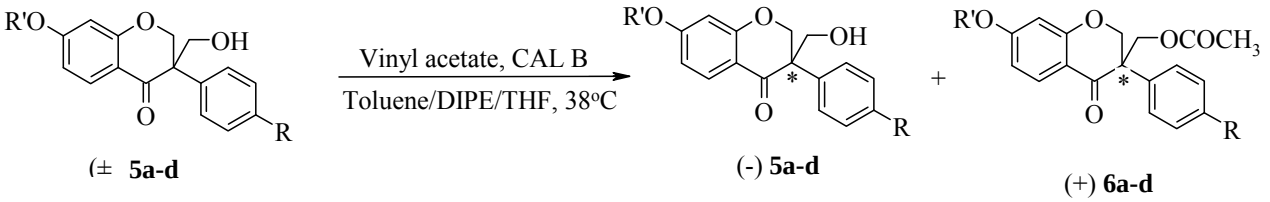
1 **2a-c** **3a-c**



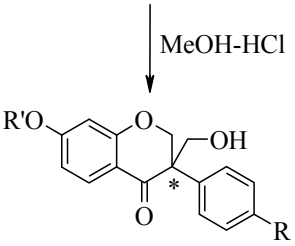
4a-d **5a-d**

2- 5	R	R'
a	H	CH_3
b	OCH_3	CH_3
c	Cl	CH_3
d	H	$CH_2C_6H_5$

Scheme I



(± **5a-d** (-) **5a-d** (+) **6a-d**



(+) **5a-d**

5, 6	R	R'
a	H	CH_3
b	OCH_3	CH_3
c	Cl	CH_3
d	H	CH_2 (1‴–6‴)

Scheme II

Table I—Enantioselective acylation of (±)-**5a-d** catalyzed by *Candida antarctica* lipase B in toluene using vinyl acetate at 38-40°C[a]

Substrate	Reaction time (hr)	Product mixture (% yield)[b]	$[\alpha]_D^{27}$	$[\alpha]_D^{27}$ of deacetylated **(+)-5a-d**
(±) 5a	15	**(+)-6a** (62) and **(-)-5a** (60)	**6a**: +2.0 **5a**: -4.0	**(+)-5a**: +3.2
(±) 5b	12	**(+)-6b** (71) and **(-)-5b** (60)	**6b**: +1.2 **5b**: -1.7	**(+)-5b**: +1.4
(±) 5c	9	**(+)-6c** (79) and **(-)-5c** (72)	**6c**: +1.5 **5c**: -3.2	**(+)-5c**: +2.3
(±) 5d	15	**(+)-6d** (67) and **(-)-5d** (55)	**6d**: +1.9 **5d**: -2.1	**(+)-5d**: +2.0
(±) 5c	11	**(+)-7a** (61) and **(-)-5c** (67)	**7a**: +1.3 **5c**: -2.2	**(+)-5c**: +1.1
(±) 5c	7	**(+)-7b** (66) and **(-)-5c** (69)	**7b**: +1.0 **5c**: -2.4	**(+)-5c**: +1.3

[a]All these reactions, when performed under identical conditions, but without adding *Canida antarctica* lipase, did not yield any product.
[b]Yields are calculated by assuming corresponding single enantiomer as 100% in the starting (±)-3-hydroxymethylbenzopyranones **5a-d**

Table II—Enantioselective acylation of (±)-3-(4'-chlorophenyl)-3-hydroxymethyl-7-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one **5c** catalysed by different lipases, in different solvents by using different acylating agents at different temperatures

Solvent	**Lipase**	Acylating agent	**Time (hr)**	**Temp** °C
Toluene	CAL	Vinyl acetate	9	40
Toluene	CRL	Vinyl acetate	48	40
Toluene	Amano PS	Vinyl acetate	28	40
Toluene	Amano PY	Vinyl acetate	52	40
DIPE	CAL	Vinyl acetate	9	40
DIPE	CRL	Vinyl acetate	42	40
DIPE	Amano PS	Vinyl acetate	21	40
DIPE	Amano PY	Vinyl acetate	46	40
THF	CAL	Vinyl acetate	23	40
THF	CRL	Vinyl acetate	52	40
THF	Amano PS	Vinyl acetate	41	40
THF	Amano PY	Vinyl acetate	68	40
Toluene	CAL	Acetic anhydride	24	40
Toluene	CAL	Heptanoic anhydride	7	40
Toluene	CAL	TFEB	20	40
DIPE	CAL	Acetic anhydride	22	40
DIPE	CAL	Heptanoic anhydride	10	40
DIPE	CAL	TFEB	15	40
Diphenyl ether	CAL	Heptanoic anhydride	7	40
Diphenyl ether	CAL	Heptanoic anhydride	6	60
Diphenyl ether	CAL	Heptanoic anhydride	5	90
Diphenyl ether	CAL	Heptanoic anhydride	-	130
Diphenyl ether	CAL	Heptanoic anhydride	-	170

high resolution mass spectrometer in positive ion mode using matrix HEDS (*bis*-hydroxyethylsulfide) doped with sodium acetate.

General procedure for the preparation of 2,4-dihydroxyphenyl aryl ketones, 3a-c

The heterogenous mixture of fused $ZnCl_2$ (0.15 mol, 20 g) and the phenylacetic acid (**2a-c**, 0.15 mol) was heated slowly with stirring till the solution became homogeneous. Resorcinol (**1**, 0.15 mol, 11g) was added and the reaction mixture was kept for about 2 hr at 140-45°C, cooled to RT and poured into crushed ice containing concentrated hydrochloric acid (1:1). The solid that separated out was filtered and washed repeatedly with water and saturated sodium bicarbonate solution. The crude product was purified using column chromatography over silica gel, with a gradient solvent system of petroleum ether:ethyl acetate to obtain the corresponding pure phenyl aryl ketones **3a-c** in 75-80% yield. These were characterized from their spectral data and comparison with reported data[22-24].

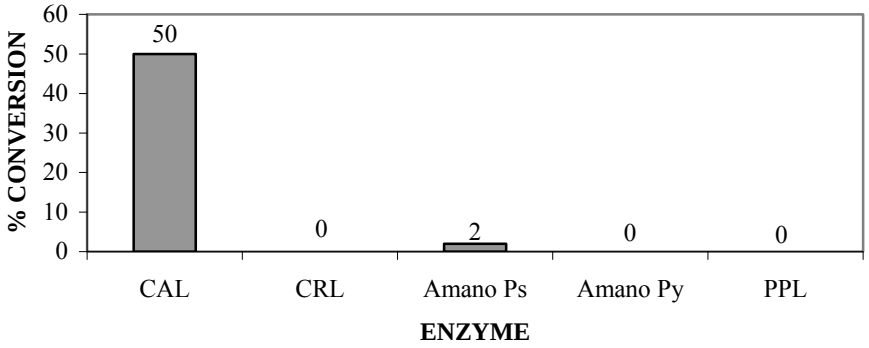


Figure 1 - Acetylation of **5c** with different lipases and vinyl acetate in toluene at 40°C

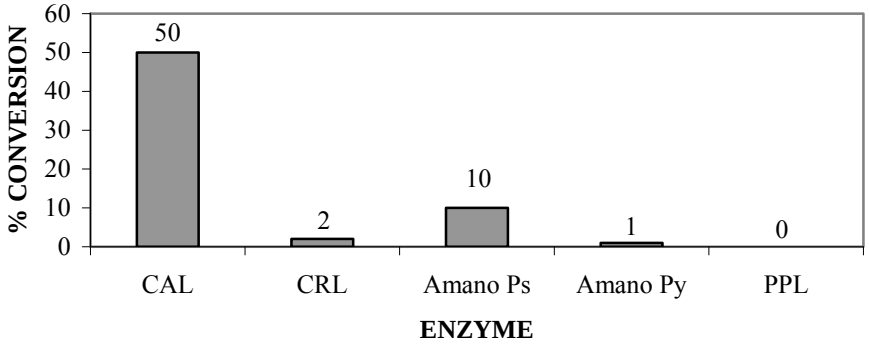


Figure 2 - Acetylation of **5c** with different lipases in diisopropylether (DIPE) at 40°C

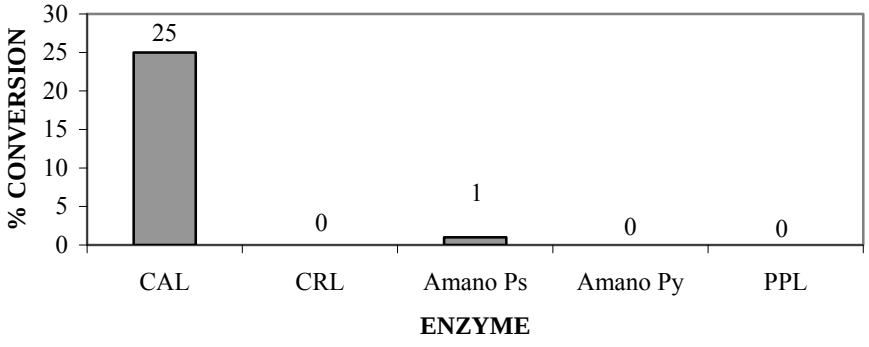
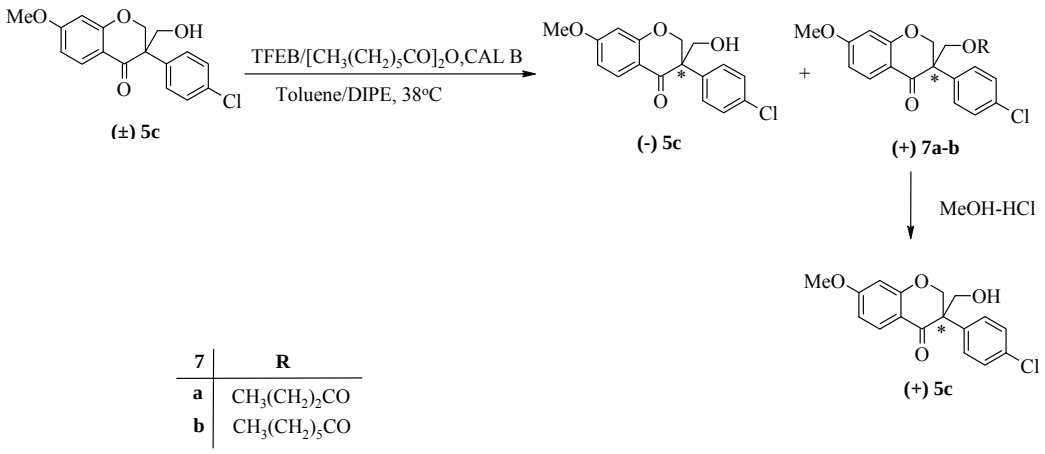


Figure 3 - Acetylation of **5c** with different lipases and vinyl acetate in THF at 40°C

General procedure for the partial methylation/benzylation of compounds 3a-c

In a 250 mL round bottom flask containing dry and distilled acetone (100 mL), the phenyl aryl ketone (**3a-c**, 0.05 mol) was added and the contents allowed to stir for a while at 25°C. To the stirred solution, freshly ignited potassium carbonate (3 g) and distilled dimethyl sulphate/benzyl chloride (1.1 equiv for one –OH group) was added. The reaction mixture was refluxed for 4-6 hr and the progress of the reaction was monitored on TLC (ethyl acetate in petroleum ether, 1:4). On completion, the reaction mixture was



Scheme III

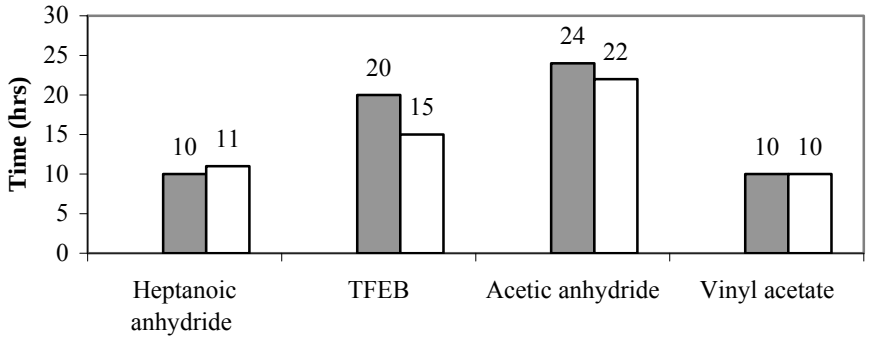


Figure 4 - Acetyaltion of **5c** with different acid anhydrides and activated esters with CAl at 40°C

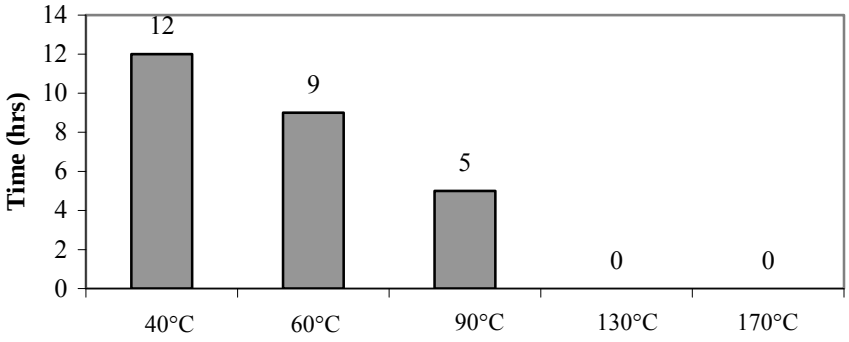


Figure 5 - Acetylation of **5c** with CAL using heptanoic anhydride at different temperatures

cooled to RT, potassium carbonate filtered off, the solvent removed under reduced pressure and ice flakes added to the concentrated mass. The crude product so obtained was filtered, washed thoroughly with water, dried and subjected to column chromatography over silica gel using gradient solvent system of petroleum ether:ethyl acetate. The corresponding pure methoxy/benzyloxy phenyl aryl

ketones **4a-d** were obtained in 82-92% yield. These were characterized from their spectral data and comparison with reported data[22,24-26].

General procedure for the preparation of 7-methoxy/benzyloxy-3-aryl-3-hydroxymethyl-2,3-dihydro-4*H*-1-benzopyran-4-ones, 5a-d

A solution of methoxy/benzyloxy phenyl aryl ketone (**4a-d**, 0.016 mol) in sodium hydroxide (0.5 N, 0.064 mol) and formaldehyde (37%, 0.072 mol) was stirred at 25-28°C. The progress of the reaction was followed by TLC (ethyl acetate in petroleum ether, 2:3). On completion, reaction mixture was acidified with dilute hydrochloric acid and the product was extracted with ether (3 × 60 mL). The combined ethereal layers were washed with brine (3 × 30 mL) and dried over anhyd. Na_2SO_4. The solvent was removed under reduced pressure to afford a gummy residue. This was purified by column chromatography over silica gel using a gradient solvent system of petroleum ether-ethyl acetate (3:2) to afford pure hydroxymethylisoflavanones **5a-d** in 65-70% yield.

(±)-3-Hydroxymethyl-7-methoxy-3-phenyl-2,3-dihydro-4*H*-1-benzopyran-4-one, 5a: It was obtained as a thick colorless oil in 68% yield, R_f 0.35 (petroleum ether-ethyl acetate, 3:2); IR (KBr): 3455 (OH), 1671 (C=O), 1608, 1578, 1441, 1257, 1164, 1066, 1028, 925, 837, 756, 699, 541 cm^{-1}; UV-Vis (CH_3OH): nm 320, 280, 240; ^1H NMR ($CDCl_3$): δ 2.55 (brs, 1H, -OH), 3.72 (1H, d, *J* = 12.2 Hz), 3.79 (3H, s), 4.31 (1H, d, *J* = 11.8 Hz), 4.87 (1H, d, *J* = 12.2 Hz), 5.10 (1H, d, *J* = 12.2 Hz), 6.34 (1H, d, *J* = 2.2 Hz), 6.56 (1H, dd, *J* = 8.8 and 2.2 Hz), 7.28-7.48 (5H, m), 7.88 (1H, d, *J* = 8.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 55.0 , 56.0, 66.4, 71.7, 100.8, 110.7, 114.9, 127.2, 128.4, 129.2, 129.7, 135.4, 163.6, 166.6, 193.6; FABHRMS: Calcd for $C_{17}H_{16}O_4$: $(M+H)^+$ 285.1127. Found: *m/z* 285.1162.

(±)-3-Hydroxymethyl-7-methoxy-3-(4'-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one, 5b: It was obtained as a white solid in 65% yield, m.p. 98-100°C, R_f 0.32 (petroleum ether-ethyl acetate, 3:2); IR (KBr): 3459 (OH), 1672 (C=O), 1608, 1578, 1513, 1441, 1383, 1255, 1164, 1066, 1024, 926, 832, 774, 656, 547 cm^{-1}; UV-Vis (CH_3OH): nm 320, 280, 240; ^1H NMR ($CDCl_3$): δ 2.53 (brs, 1H), 3.68 (1H, d, *J* = 11.8 Hz), 3.77 (3H, s), 3.79 (3H, s), 4.28 (1H, d, *J* = 11.7 Hz), 4.85 (1H, d, *J* = 12.2 Hz), 5.05 (1H, d, *J* = 12.2 Hz), 6.34 (1H, d, *J* = 2.2 Hz), 6.55 (1H, dd, *J* = 8.8 and 2.2 Hz), 6.87 (2H, d, *J* = 8.7 Hz), 7.38 (2H, d, *J* = 8.7 Hz), 7.87 (1H, d, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$): δ 54.3, 55.5, 55.9, 66.4, 71.8, 100.7, 110.6, 114.6, 114.8, 127.2, 128.4, 129.6, 159.6, 163.4, 166.5, 193.6; FABHRMS: Calcd for $C_{18}H_{18}O_5Na$: $(M+Na)^+$ 337.1052. Found: *m/z* 337.1104.

(±)-3-(4'-Chlorophenyl)-3-hydroxymethyl-7-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one, 5c: It was obtained as a white solid in 70% yield, m.p. 108-10°C, R_f 0.30 (petroleum ether-ethyl acetate, 3:2); IR (KBr): 3439 (OH), 1671 (C=O), 1608, 1576, 1494, 1442, 1383, 1338, 1258, 1164, 1096, 1065, 1011, 1026, 835, 750, 539 cm^{-1}; UV-Vis (CH_3OH): nm 320, 280, 240; ^1H NMR ($CDCl_3$): δ 3.70 (1H, d, *J* = 11.8 Hz), 3.80 (3H, s), 4.27 (1H, d, *J* = 11.8 Hz), 4.85 (1H, d, *J* = 12.4 Hz), 5.04 (1H, d, *J* = 12.2 Hz), 6.34 (1H, d, *J* = 2.4 Hz), 6.56 (1H, dd, *J* = 8.8 and 2.4 Hz), 7.28-7.42 (4H, m), 7.85 (1H, d, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$): δ 54.5, 56.0, 66.2, 71.5, 100.0, 110.8, 114.7, 128.1, 129.0, 129.7, 133.9, 134.4, 163.5, 166.7, 193.1; FABHRMS: Calcd for $C_{17}H_{15}O_4Cl$: $(M+H)^+$ 319.0737. Found: *m/z* 319.0779.

(±)-7-Benzyloxy-3-hydroxymethyl-3-phenyl-2,3-dihydro-4*H*-1-benzopyran-4-one, 5d: It was obtained as a light yellow solid in 60% yield, m.p. 74-76°C, R_f 0.30 (petroleum ether-ethyl acetate, 3:2). IR (KBr): 3465 (OH), 1672 (C=O), 1607, 1440, 1379, 1253, 1171, 1096, 1015, 835, 697, 587 cm^{-1}; UV-Vis (CH_3OH): nm 325, 280, 240; ^1H NMR ($CDCl_3$): δ 2.41 (1H, brs), 3.73 (1H, d, *J* = 11.8 Hz), 4.32 (1H, d, *J* = 11.8 Hz), 4.88 (1H, d, *J* = 12.2 Hz), 5.05 (2H, s), 5.10 (1H, d, *J* = 12.2 Hz), 6.44 (1H, d, *J* = 2.2 Hz), 6.65 (1H, dd, *J* = 8.8 and 2.3 Hz), 7.30-7.49 (m, 10H, aromatic protons), 7.91(1H, d, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$): δ 55.0, 66.5, 70.7, 71.7, 101.8, 111.1, 115.1, 127.3, 127.9, 128.4, 128.7, 129.1, 129.3, 129.7, 135.3, 136.2, 163.5, 165.7, 193.6; FABHRMS: Calcd for $C_{23}H_{20}O_4Na$: $(M+Na)^+$ 383.1218. Found: *m/z* 383.1259.

General procedure of enzymatic acylation of (±)-3-hydroxymethyl-3-aryl-benzopyran-4-ones, 5a-d

To a solution of the (±)-hydroxymethylisoflavanone (**5a-d**, 1 mmol) in anhydrous toluene (5 mL), the acylating agent vinyl acetate/acetic anhydride/TFEB/heptanoic anhydride (1.1 equiv) was added, followed by the addition of *Candida antarctica* lipase (CAL, 150 mg). The suspension was stirred at 35-38ºC in an incubator and progress of the reaction was monitored periodically by TLC. After about 50% conversion of the starting material into the product, the reaction was quenched by filtering off the

enzyme and the solvent evaporated to dryness *in vacuo* to afford a gummy residue. This was purified by column chromatography over silica gel using a gradient solvent system of petroleum ether-ethyl acetate to obtain optically enriched (+)-3-aryl-3-acetoxymethyl-benzopyran-4-ones **6a-d** and (-)-3-hydroxymethyl-3-aryl-benzopyran-4-ones **5a-d** in 63-79 and 55-72% yields, respectively (yields were calculated by assuming single enantiomer as 100% in starting (±)-**5a-d**). The (-)-hydroxymethyliso-flavanones **5a-d** and (+)-acetoxymethylisoflavanone **6a-d** were identified on the basis of their spectral data. Control reaction mixture kept under identical conditions, but without the addition of CAL did not indicate any acetylation even after prolonged periods of incubation (2-4 days).

(+)-3-Acetoxymethyl-7-methoxy-3-phenyl-2,3-dihydro-4*H*-1-benzopyran-4-one, 6a:

It was obtained as a white solid in 62% yield, m.p. 92-94°C, R_f 0.30 (petroleum ether- ethyl acetate, 4:1); $[\alpha]^{25}_D$ + 2.00° (*c* 0.01, $CHCl_3$); IR (KBr): 1743 (-$OCOCH_3$), 1677 (C=O), 1609, 1442, 1383, 1235, 1169, 1100, 1036, 929, 711, 699 cm^{-1}; UV-Vis (CH_3OH): nm 320, 280, 240; ^1H NMR ($CDCl_3$): δ 2.02 (3H, s), 3.80 (3H, s), 4.38 (1H, d, *J* = 11.8 Hz), 4.71 (1H, d, *J* = 12.1 Hz), 4.72 (1H, d, *J* = 12.1 Hz), 5.09 (1H, d, *J* = 12.2 Hz), 6.35 (1H, d, *J* = 2.3 Hz), 6.59 (1H, dd, *J* = 8.8 and 2.2 Hz), 7.28-7.49 (5H, m), 7.90 (1H, d, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$): δ 21.1, 52.7, 55.9, 66.2, 71.3, 100.8, 110.6, 114.6, 127.3, 128.6, 129.26, 129.9, 134.6, 163.2, 166.5, 170.9, 190.5; FABHRMS: Calcd for $C_{19}H_{18}O_5$: $(M+H)^+$ 327.1223. Found: *m/z* 327.1232.

(+)-3-Acetoxymethyl-7-methoxy-3-(4'-methoxy-phenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one, 6b: It was obtained as a yellowish solid in 71% yield, m.p. 86-88°C; R_f 0.35 (petroleum ether-ethyl acetate, 4:1); $[\alpha]^{25}_D$ + 1.20° (*c* 0.01, $CHCl_3$); IR (KBr): 1742 (-$OCOCH_3$), 1669 (C=O), 1610, 1550, 1490, 1380, 1255, 1233, 1160, 1025, 1012, 830, 745, 535 cm^{-1}; UV-Vis (CH_3OH): nm 320, 280, 240; ^1H NMR ($CDCl_3$): δ 2.01 (3H, s), 3.75 (3H, s), 3.78 (3H, s), 4.32 (1H, d, *J* = 11.5 Hz), 4.68 (1H, d, *J* = 11.0 Hz), 4.69 (1H, d, *J* = 11.8 Hz), 5.03 (1H, d, *J* = 12.1 Hz), 6.34 (1H, s, *J* = 2.5 Hz), 6.56 (1H, dd, *J* = 6.5 and 2.5 Hz), 6.86 (2H, d, *J* = 9.0 Hz), 7.40 (2H, d, *J* = 9.0 Hz), 7.89 (1H, d, *J* = 9.5 Hz); ^{13}C NMR ($CDCl_3$): δ 21.1, 52.0, 55.5, 55.9, 66.2, 71.3, 100.8, 110.6, 114.5, 114.6, 126.4, 128.5, 129.9, 159.8, 163.1, 166.4, 170.9, 190.7; FABHRMS: Calcd for $C_{20}H_{20}O_6Na$: $(M+Na)^+$ 379.1158. Found: *m/z* 379.1147.

(+)-3-Acetoxymethyl-3-(4'-chlorophenyl)-7-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one, 6c: It was obtained as a white solid in 79% yield, m.p. 120-122°C; R_f 0.30 (petroleum ether-ethyl acetate, 4:1); $[\alpha]^{25}_D$ + 1.50° (*c* 0.01, $CHCl_3$); IR (KBr): 1744 (-$OCOCH_3$), 1678 (C=O), 1609, 1577, 1494, 1442, 1383, 1257, 1234, 1165, 1097, 1028, 1097, 1028, 1013, 929, 835, 749, 542 cm^{-1}; UV-Vis (CH_3OH): nm 320, 280, 220; ^1H NMR ($CDCl_3$): δ 2.04 (3H, s, -$OCOCH_3$), 3.82 (3H, s), 4.39 (1H, d, *J* =11.6 Hz, C-1'H_α), 4.63 (1H, d, *J* = 11.8 Hz, C-1'H_β), 4.73 (1H, d, *J* = 11.8 Hz, C-2H_α), 5.06 (1H, d, *J* =12.2 Hz, C-2H_β), 6.36 (1H, d, *J* = 2.2 Hz), 6.59 (1H, dd, *J* = 8.8 and 2.2 Hz), 7.30-7.47 (4H, m), 7.89 (1H, d, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$): δ 21.1, 52.27, 56.0, 66.0, 71.1, 100.8, 110.9, 114.3, 128.8, 129.4, 130.0, 133.21, 134.7, 163.1, 166.6, 170.8, 190.1; FABHRMS: Calcd for $C_{19}H_{17}O_5ClNa$: $(M+Na)^+$ 383.0662. Found: *m/z* 383.0630.

(+)-3-Acetoxymethyl-7-benzyloxy-3-phenyl-2,3-dihydro-4*H*-1-benzopyran-4-one, 6d: It was obtained as a yellowish solid in 67% yield, m.p. 102-04°C, R_f 0.32 (petroleum ether-ethyl acetate, 4:1); $[\alpha]^{25}_D$ + 1.90° (*c* 0.01, $CHCl_3$); IR (KBr): 1742 (-$OCOCH_3$), 1678 (C=O), 1608, 1576, 1497, 1439, 1379, 1233, 1172, 1101, 1060, 1038, 838, 739, 698, 543 cm^{-1}; UV-Vis (CH_3OH): nm 280; ^1H NMR ($CDCl_3$): δ 2.03 (3H, s), 4.38 (1H, d, *J* = 11.8 Hz), 4.70 (1H, d, *J* = 11.9 Hz), 4.73 (1H, d, *J* = 11.9 Hz), 5.05 (2H, s), 5.09 (1H, d, *J* = 12.2 Hz), 6.44 (1H, d, *J* = 2.2 Hz), 6.67 (1H, dd, *J* = 8.8 and 2.1 Hz), 7.28-7.50 (10H, m), 7.92 (1H, d, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$): δ 21.1, 52.7, 66.2, 71.3, 101.9, 111.2, 70.7, 114.8, 127.4, 127.9, 128.6, 128.7, 129.1, 129.2, 130.0, 134.6, 136.2, 163.1, 165.6, 170.9, 190.5; FABHRMS: Calcd $C_{25}H_{22}O_5Na$: $(M+Na)^+$ 425.1365. Found: *m/z* 425.1337.

(+)-3-Butanoyloxymethyl-3-(4'-chlorophenyl)-7-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one, 7a: It was obtained as a white solid in 67% yield, m.p. 90-92°C; R_f 0.38 (petroleum ether-ethyl acetate, 4:1); $[\alpha]^{25}_D$ + 1.30° (*c* 0.01, $CHCl_3$); IR (KBr): 1740 , 1678 (C=O), 1609, 1577, 1494, 1442, 1389, 1385, 1256, 1164, 1097, 1029, 930, 835, 749, 543 cm^{-1}; UV-Vis (CH_3OH): nm 320, 280, 240; ^1H NMR ($CDCl_3$): δ 0.91 (3H, t, *J* = 8.0 Hz), 1.59 (2H, m), 2.26 (2H, t, *J* = 7.2 Hz), 3.82 (3H, s), 4.43 (1H, d, *J* = 12.0 Hz), 4.65 (2H, m), 5.06 (1H, d, *J* = 12.0 Hz), 6.37 (1H, d, *J* = 2.2 Hz), 6.59 (1H, dd, *J* = 8.8 and 2.2 Hz), 7.30-7.47 (4H, m), 7.89 (1H, d, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$): δ 13.9, 18.7, 36.3, 52.3, 56.0, 65.8, 71.2, 110.9, 100.8, 114.3, 128.8, 129.4, 130.0, 133.3, 134.6, 163.1, 166.6, 173.4, 190.1; FABHRMS: Calcd for

$C_{21}H_{21}O_5ClNa$: $(M+Na)^+$ 411.0975. Found: *m/z* 411.0981.

(+)-3-(4'-Chlorophenyl)-3-heptanoyloxymethyl-7-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one, 7b: It was obtained as a white solid in 69% yield, m.p. 104-06°C; R_f 0.35 (petroleum ether: ethyl acetate, 4:1), $[\alpha]^{25}_D$ + 1.00° (*c* 0.01, $CHCl_3$), IR (KBr): 1743 , 1670 (C=O), 1442, 1235, 1162, 1035, 544 cm^{-1}; UV-Vis (CH_3OH): nm 300, 280; ^1H NMR ($CDCl_3$): δ 0.86 (3H, t, *J* = 6.8 Hz), 1.27 (6H, brs), 1.60 (2H, m), 2.27 (2H, t, *J* = 6.8 Hz), 3.81 (3H, s, OCH_3), 4.44 (1H, d, *J* = 11.4 Hz), 4.65 (2H, m), 5.06 (1H, d, *J* = 11.4 Hz), 6.37 (1H, d, *J* = 2.2 Hz), 6.60 (1H, dd, *J* = 8.8 and 2.2 Hz), 7.30-7.48 (4H, m), 7.89 (1H, d, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$): δ 14.4, 22.8, 25.1, 29.1, 31.7, 34.4, 52.3 , 56.0, 65.8, 71.2, 100.8, 110.9, 114.3, 128.8, 129.4, 130.0, 133.3, 134.6, 163.1, 166.6, 173.6, 190.1; FABHRMS: Calcd for $C_{24}H_{27}O_5ClNa$: $(M+Na)^+$ 453.1445. Found: *m/z* 453.1413.

General procedure for chemical deacetylation of biocatalytically obtained acetates (+)-6a-d, and butanoate 7a and the heptanoate, 7b

The (+)-acyloxymethylated isoflavanone **6a-d** or **7a-b** (1 mmol) was dissolved in MeOH (5 mL) containing 1-2 drops of hydrochloric acid. The reaction mixture was stirred for 4 hr at 25-28ºC, quenched by the addition of ice-cold water (5 mL) and extracted with ethyl acetate (2 × 10 mL). The combined ethyl acetate layer was washed with brine, dried over anhyd. Na_2SO_4 and concentrated under reduced pressure to afford the (+)-hydroxymethylisoflavanones **5a-d** in 80-90% yield. These were identified on the basis of their spectroscopic data, which were found identical to the spectroscopic data of corresponding (-)- and (±)-hydroxymethylisoflavanones reported earlier in this paper.

Conclusions

The present study has shown interesting and potentially useful enantioselective capabilities of *Candida antarctica* lipase (CAL B) for the enantiomeric separation of racemic (±)-3-aryl-3-hydroxymethyl-2,3-dihydro-4*H*-1-benzopyran-4-ones. Heptanoic anhydride was found to be the most efficient acylating agent to transform the starting racemic substrate into the esters. In studies involving optimization of reaction conditions for resolution with CAL, it was found that no enzymatic resolution occurs at temperatures higher than 90°C. As it is difficult to synthesize such compounds in enantiomerically enriched forms by purely chemical methods, the efficient and effective biocatalytic approach reported herein may find utility in the synthesis of optically enriched compounds of this class.

Acknowledgements

Financial assistance from Danish International Development Agency (DANIDA, Denmark) and Council for Scientific and Industrial Research (CSIR, New Delhi, India) is gratefully acknowledged.

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