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Trichosporon beigelli esterase (TBE): a versatile esterase for the resolution of economically important racemates

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Abstract—A hydrolase producing strain *Trichosporon beigelli* esterase (TBE) isolated from local cottage cheese in its native form has displayed versatility and high efficacy in the kinetic resolution of a wide range of economically important substrates, which include racemic secondary alcohols, such as 1-(6-methoxy-2-naphthyl)ethanol ($E \sim 316$), 1-(3,4-methylenedioxyphenyl)ethanol and pentanol ($E \sim 180$ and 156 resp.), and alkyl esters of carboxylic acids such as ibuprofen ($E \sim 340$), 2-(benzylthio)propanoic acid ($E \sim 1000$). In other substrates such as in the primary alcohol 2-(6-methoxy-2-naphthyl)propan-1-ol and carboxylic acids such as 2-(5-bromo-6-methoxy-2-naphthyl)propanoic acid, 2-(2-naphthyloxy)propanoic acid, and substituted 2-thiopropanoic acids, it displayed moderate to low selectivity. Commercial lipases such as CCL, PPL, and PSL were also used in the resolution of the substrates for comparative studies.

1. Introduction

Amongst the large variety of hydrolases currently being used for industrial applications, the availability of true esterases is limited to a few only. These applications have generally been performed by a handful of esterases available from animal sources such as pig and horse liver esterases,[1] acetylcholine esterase,[2] plant/animal cholesterol esterase,[3] etc. Microbial carboxyl-esterase NP[4] or proteases, such as α-Chymotrypsin[5] *Bacillus subtilisin*,[6] and papain[7] are also being used for hydrolysis. Despite the progress made in the area of genetic engineering, the supply of synthetically useful esterases still remains inadequate. Therefore, the search for and development of new esterases preferably from microbial sources capable of accepting a wide variety of non-natural substrates is always desirable. Biocatalytic properties especially those of esterases and lipases have been well documented and so have been their uses in organic synthesis.[8–10] These hydrolases are of general interest since they can be used without the requirement of expensive cofactors and are available at affordable prices. Most of the known hydrolases display a wide and varying range of substrate specificity and hydrolytic activity, which is not only a function of the structure of the substrate but may also be influenced by the reaction conditions. These hydrolytic enzymes have been extensively used in the generation of a large number of enantiomerically enriched molecules such as alcohols, esters, amides, acids, etc., which are valuable as fine chemicals, building blocks, or synthetic intermediates.[11–20] Their applications in the protecting group chemistry of highly functionalized molecules such as peptides, saccharides, and their conjugates have been a steadily growing area.[21]

The effectiveness of some of the most commonly known commercial enzyme preparations such as *Pseudomonas* sp., *Candida rugosa*, *Candida cylindracea*, and *Candida antarctica* A and B, Pig liver esterase, *Mucor miehei*, etc., lies in their broad substrate acceptability and high enantioselectivity. Several new biocatalysts, including lipases and esterases,[22] have been isolated from various sources through the screening of thousands of strains and added to the armory of organic chemists. Besides being advantageous in terms of activity and selectivity, a biocatalyst needs to fulfill the requirements of robustness,[23] such as that shown by the above mentioned commercial enzymes. We have been continuously engaged in the search for new sources of hydrolases and dehydrogenases and our efforts in this direction have been

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successful, as many of these new enzymes have found applications in the preparation of chiral auxiliaries, drug intermediates, and useful chemical entities.[24–28]

2. Results and discussion

Recently, we reported the activity of an enantioselective hydrolase *Trichosporon* sp. (DSMZ no. 11829, a yeast strain isolated from local cottage cheese) now identified as *Trichosporon beigelli*.[27,28] There, we successfully demonstrated the high enantioselectivity displayed by the strain in the resolution of the racemic chlorohydrin intermediate[27] of (*S*)-propranolol ($E > 550$) as well as in the preparation of (*S*)-naproxen by the kinetic resolution of its racemic methyl ester ($E \sim 500$).[28a] Lipases from other yeasts, especially from the genera *Candida* and *Saccharomyces* are well known including their applications in biotransformation reactions.[29–31] Herein, we report the general applicability of *T. beigelli* in the resolution of some economically important molecules, especially in the resolution of aryl alkyl carbinols (primary as well as secondary alcohols), arylpropanoic acids, aryloxypropanoic acid, and substituted 2-thiopropanoic acids. The present results together with data previously published by us[28b] show *T. beigelli* to be one of the more versatile hydrolases (as a whole cell preparation, cell free extract, or purified form), which is capable of accepting a wide range of non-natural substrates.[32] These studies also finally establish the identity of *T. beigelli* as a true esterase (now designated as TBE) and not a lipase as mentioned earlier.[27,28] These conclusions are based on the data obtained from *N*-terminal amino acids sequencing[32] together with the reactivity/selectivity profiles of TBE, taking into account the opposite selectivity observed during the hydrolysis of various racemic esters in comparison to the results obtained from some commercial lipases.

3. Hydrolysis of alkyl acylates of secondary and primary alcohols

3.1. Kinetic resolution of racemic 1-(6-methoxy-2-naphthyl)ethanol 1

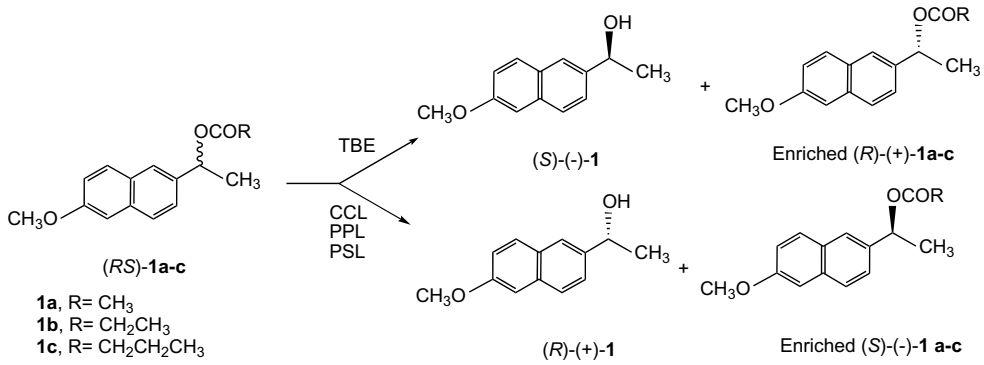
The kinetic resolution of alkyl acylates of racemic 1-(6-methoxy-2-naphthyl)ethanols **1a–c**, which are valuable chiral auxiliaries used in the synthesis of optically active compounds[33] and also in the fine chemical industry,[34] was efficiently carried out using TBE (Scheme 1). The hydrolase displayed a high enantioselectivity for the acetyl derivative ($E \sim 316$, 99% ee), showing a preference for the (*S*)-enantiomer, thus exhibiting an opposite selectivity to all the commercial lipases viz. CCL, PPL, and PSL, which afforded the (*R*)-alcohol. With an increase in the size of alkyl ester group from acetate to butyrate, there was a considerable decrease in reactivity/selectivity profiles of TBE, whereas commercial lipases displayed significant improvements with the exception of PSL, which did not show any noteworthy changes in the rates of hydrolysis. PSL also displayed the best selectivity for the acetyl derivative ($E \sim 492$, 99% ee) while CCL and PPL showed a preference for butyrate derivative ($E \sim 53$, 93.2% ee and $E \sim 159$, 99% ee, respectively). XAD resin immobilized *Pseudomonas* sp., PPL, and rabbit gastric juice have previously been reported for the preparation of (*R*)-**1**.[35a–c] Table 1 summarizes the results obtained from these studies.

3.2. Kinetic resolution of racemic 1-(3,4-methylenedioxyphenyl)ethanol 2 and 1-(3,4-methylenedioxyphenyl)pentanol 3

1-(3,4-Methylenedioxyphenyl)ethanol **2** and 1-(3,4-methylenedioxyphenyl)pentanol **3** are important members of a class of molecules that are used as chiral auxiliaries in organic synthesis. Alkyl acylates of racemic **2a–c** and **3a–c** (R = CH_3, C_2H_5, C_3H_7) were subjected to kinetic resolution using TBE and commercial lipases (Scheme 2). As expected, TBE exhibited preference for *S*-enantiomer of acetyl derivative ($E = 179.6$, 98% ee) and lower activity and selectivity for the higher homologues. Conversely, the commercial enzymes, which furnished *R*-alcohols, did not discriminate among the alkyl acylates appreciably in terms of activity and selectivity. Table 2 summarizes the results obtained from the above studies.

3.3. Kinetic resolution of racemic 2-(6-methoxy-2-naphthyl)propanol 4

After demonstrating an excellent resolution of secondary alcohols, we next decided to study the resolution potential of TBE with respect to a primary alcohol, which are generally hard to resolve with high enantio-

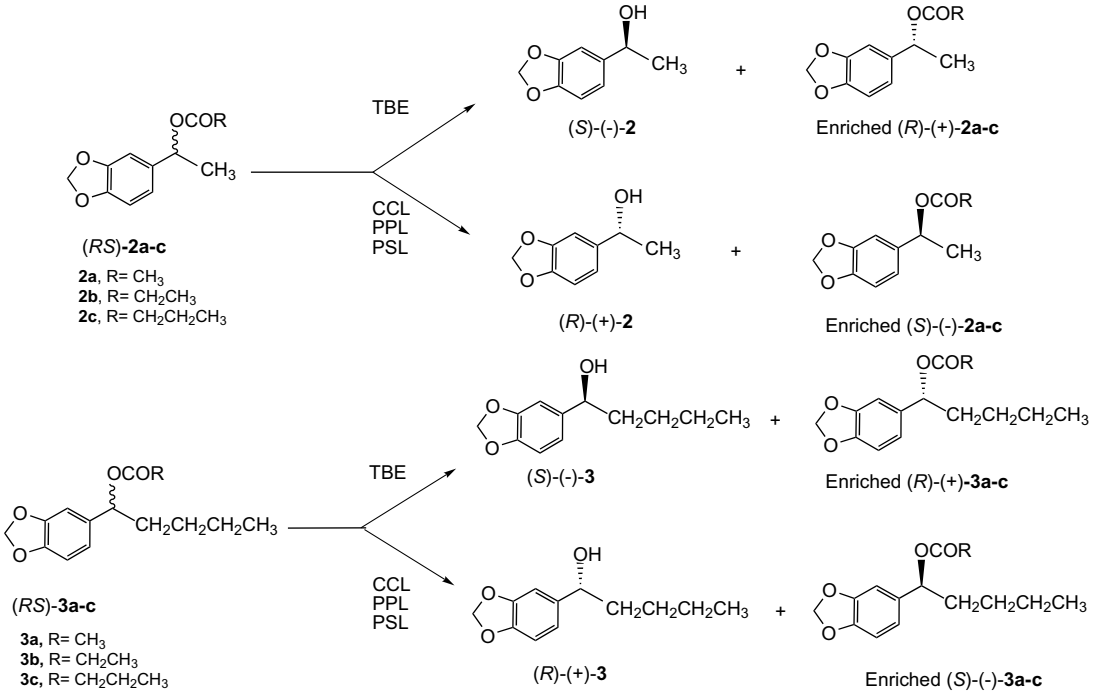


Scheme 1.

Table 1. Hydrolysis of racemic alkyl acylates of 1-(6-methoxy-2-naphthyl)ethanols **1a–c** using TBE and commercial lipases CCL, PPL, and PSL

Entry	TBE				CCL				PPL				PSL			
	Conv. (%)	ee (%)	Conf. prod.	*E* Factor	Conv. (%)	ee (%)	Conf. prod.	*E* Factor	Conv. (%)	ee (%)	Conf. prod.	*E* Factor	Conv. (%)	ee (%)	Conf. prod.	*E* Factor
1a	32.0	99.0	*S*	316	24.0	12.6	*R*	1.34	10.0	13.0	*R*	1.31	46.0	99.0	*R*	492
1b	10.0	69.6	*S*	2.2	32.0	14.8	*R*	1.44	32.0	54.0	*R*	4.26	43.0	64.0	*R*	7.3
1c	08.0	62.0	*S*	4.5	40.0	93.2	*R*	52.9	40.0	99.0	*R*	158.9	36.0	70.0	*R*	8.29

Substrate concn 40 g/L, incubation time 16 h, temp 20 °C, pH 7.0 (0.1 M sodium phosphate buffer) ratio of substrate/enzyme 1:0.3 (dry powder of commercial enzyme) and 1:0.75 (lyophilized TBE). Ee% determined by HPLC using Chiralcel-OD-H with hexane/2-propanol (95:5) as the mobile phase. The specific rotation of the resolved (*S*)-alcohol **1** (99% ee) was $[\alpha]_D^{25} = -39.6$ (*c* 0.8, $CHCl_3$). The absolute configuration was determined by comparison of the sign of the specific rotation with those reported.[36] Specific optical rotation of the resolved alcohol was determined after separation of the acyl ester and alcohol on silica gel column and elution with dichloromethane and methanol (19:1). The resolved bio-products were obtained in overall yields of 75–80%.



Scheme 2.

selectivity due to the presence of a carbon atom between hydroxyl group and the stereogenic center. Naproxol, that is, 2-(6-methoxy-2-naphthyl)propanol **4**, which was selected for these studies, is a commercially important molecule that is reported to possess more potent anti-inflammatory activity than (*S*)-naproxen.[37] (*S*)-Naproxol has reportedly been prepared by the use of lipases and dehydrogenases with low to moderate ees.[38,39] Its enantiomeric excess was further improved by a second kinetic resolution reaction, for example, PPL catalyzed double kinetic resolution.[39] Microbial oxidation of racemic naproxol using *Glomerella cingulata* has also been used for the preparation of (*R*)-(+)-naproxol.[40] Herein, racemic acylates of naproxols **4a–d** were subjected to kinetic resolution using TBE, CCL, PPL, and PSL (Scheme 3). Except for PPL, which showed somewhat better selectivity (ee ~ 77%), the other three enzymes including TBE proved to be ineffective in the high resolution of the substrates **4a–c**. Though long chain alkyl esters of primary alcohols are reported to afford products with higher ees,[41] an increase in the size of alkyl group such as in the acylate **4d**, further decreased the selectivity as well as the reactivity with the exception of PPL where octanoyl ester displayed a significant improvement in selectivity (ee ~ 91.4%). Here, both TBE and PSL showed preference for the (*R*)-enantiomer, while CCL and PPL hydrolyzed the (*S*)-enantiomer. Table 3 summarizes the results obtained from these studies. In these studies, standard (*S*)- and (*R*)-naproxol were prepared by lithium aluminum hydride (LAH) reduction of methyl esters of enantiomerically pure (*R*)- and (*S*)-naproxen, respectively.

4. Hydrolysis of alkyl esters of carboxylic acids

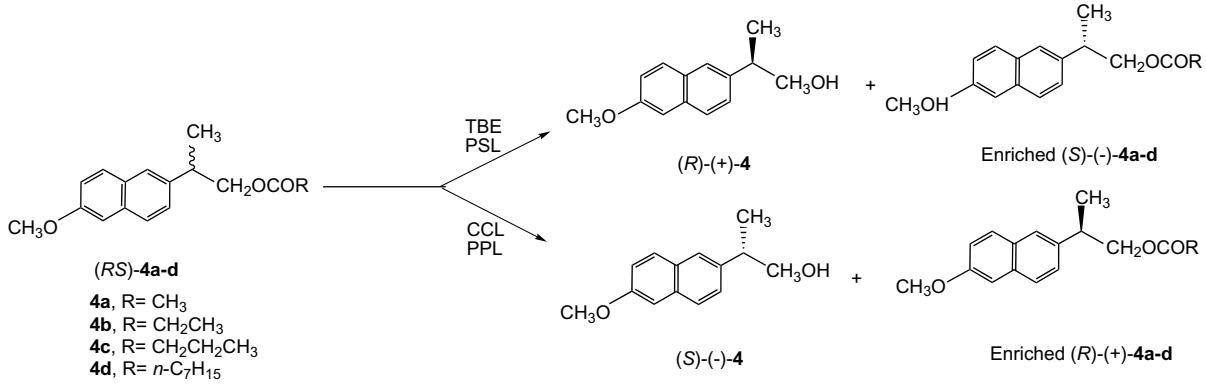
4.1. Kinetic resolution of racemic 2-(5-bromo-6-methoxy-2-naphthyl)propanoic acid 5

Racemic 2-(5-bromo-6-methoxy-2-naphthyl)propanoic acid **5** is a key intermediate in the synthesis of the anti-inflammatory drug naproxen.[42a,b] Generally,

Table 2. Hydrolysis of racemic acyl esters of 1-(3,4-methylenedioxyphenyl)ethanols **2a–c** and 1-(3,4-methylenedioxyphenyl)pentanols **3a–c** using TBE and commercial enzymes CCL, PPL, and PSL

Entry	TBE				CCL				PPL				PSL			
	Conv. (%)	ee (%)	Conf. prod.	*E* Factor	Conv. (%)	ee (%)	Conf. prod.	*E* Factor	Conv. (%)	ee (%)	Conf. prod.	*E* Factor	Conv. (%)	ee (%)	Conf. prod.	*E* Factor
2a	37.4	98	*S*	179.7	42.6	98	*R*	216.7	41.4	98	*R*	206.7	45.3	98	*R*	249.7
2b	33.6	28	*S*	2.0	43.2	98	*R*	222.8	38.5	98	*R*	185.9	43.2	98	*R*	222.8
2c	27.4	08	*S*	1.2	43.6	98	*R*	227.2	30.7	97	*R*	100.3	39.4	99	*R*	388.3
3a	31.8	98	*S*	155.7	31.8	98	*R*	155.7	31.8	96	*R*	76.3	39.3	98	*R*	190.8
3b	27.6	59	*S*	4.8	35.7	98	*R*	171.3	35.2	91	*R*	34.7	33.6	96	*R*	79.4
3c	21.7	33	*S*	2.2	39.6	98	*R*	192.8	39.8	84	*R*	20.0	32.7	95	*R*	61.5

Substrate concn 40 g/L, incubation time 16 h, temp 20 °C, pH 7.0 (0.1 M sodium phosphate buffer); ratio of substrate/enzyme 1:0.25 (dry powder of commercial enzyme) and 1:0.75 (lyophilized TBE). Ee% determined by HPLC using Chiralcel-OD-H chiral column with hexane/2-propanol (95:5) as the mobile phase. The specific rotation of the resolved (*R*)-alcohol **2** 99% ee $[\alpha]_D^{25} = +55.4$ (*c* 1, $CHCl_3$), (*S*)-alcohol **3** (98% ee) $[\alpha]_D^{25} = -61.9$ (*c* 0.54, $CHCl_3$). Absolute configuration was determined by comparison of the sign of the specific rotation with those reported in the literature.[26] The combined yields of the resolved bio-products were in the range of 75–85%.



Scheme 3.

Table 3. Hydrolysis of racemic acyl esters of 2-(6-methoxy-2-naphthyl)propan-1-ol **4a–d** using TBE and commercial enzymes CCL, PPL, and PSL

R	TBE				CCL				PPL				PSL			
	Conv. (%)	ee (%)	Conf. prod.	*E* Factor	Conv. (%)	ee (%)	Conf. prod.	*E* Factor	Conv. (%)	ee (%)	Conf. prod.	*E* Factor	Conv. (%)	ee (%)	Conf. prod.	*E* Factor
4a	39.0	42.8	*R*	3.2	41.0	13.0	*S*	1.3	43.0	77.0	*S*	14.3	43.0	14.0	*R*	1.5
4b	38.0	59.6	*R*	5.0	41.0	40.0	*S*	1.1	26.0	53.7	*S*	3.9	35.0	17.7	*R*	1.6
4c	33.7	24.1	*R*	1.9	45.0	5.0	*S*	1.1	23.0	75.0	*S*	10.1	27.0	20.0	*R*	1.7
4d	12.3	13.0	*R*	1.3	9.6	3.0	*S*	1.1	23.0	72.4	*S*	7.7	8.6	8.9	*R*	1.2
4d[*]	8.4	9.4	*R*	1.2	7.0	3.8	*S*	1.1	26.0	91.4	*S*	30.4	8.4	2.0	*R*	1.0

Authentic samples of (*R*)- and (*S*)-alcohols were prepared by LAH reduction of methyl esters of (*R*)-(−)- and (*S*)-(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid and purified by CC over SiO_2 with dichloromethane/ethyl acetate (9:1) as the eluent to give (*R*)-alcohol (99%ee) with $[\alpha]_D^{25} = +17.5$ (*c* 1, $CHCl_3$) and (*S*)-alcohol (ee 99%) $[\alpha]_D^{25} = -17.5$ (*c* 1, $CHCl_3$).

[*] Cosolvent 2-propanol 20% v/v with buffer (0.1 M phosphate), substrate concentration 35 g/L, incubation time 30 h, temp 26 °C, pH 7.0, ratio of substrate/enzyme 1:0.3 (dry powder of commercial enzyme) and 1:0.75 (lyophilized TBE). Ee% determined by HPLC using Whelk (*R*,*R*) O 1 chiral column with mobile phase hexane/2-propanol/acetic acid (90:10:0.1). The combined yields of the resolved bio-products were in the range of 75–80%.

(*S*)-(+)-naproxen is obtained through a final step resolution of its esters by enzymatic hydrolysis whereas the resolution at the penultimate stage, that is, the resolution of **5**, may provide an alternative route to the asymmetric synthesis thus making the process more simple and economically advantageous.[42c] Earlier, we have also successfully demonstrated the superiority of TBE in the resolution of racemic naproxen.[28] We, therefore, envisaged to undertake the resolution of racemic **5a–c** (Scheme 4) and study the effect of a bulkier group such as bromine on overall reactivity and selectivity. Most of the alkyl esters of **5** proved to be poor substrates for the two biocatalysts used in these transformations. TBE hydrolyzed the methyl ester of racemic **5** to the corresponding (*S*)-bromoacid with 43% enantiomeric excess. Ethyl and butyl esters also proved to be equally poor substrates for TBE both in terms of reactivity and selectivity. However, in our hands the commercial enzyme

Table 4. Hydrolysis of racemic esters of 2-(5-bromo-6-methoxy-2-naphthyl)propanoic acid **5a–c** using TBE and commercial enzyme CCL

Entry	Enzyme	Time (h)	Conv. (%)	ee (%)	Conf. prod.	*E* Factor
5a	TBE	150	31	42.8	*S*	2.9
	CCL	90	39	99	*S*	383
5b	TBE	150	12	28.6	*S*	2.4
	CCL	105	40	61.9	*S*	6.3
5c	TBE	150	8.0	17.8	*S*	1.5
	CCL	105	26.6	28.7	*S*	2.0

Substrate concentration 16 g/L, temp 30 °C, pH 8.0 (0.1 M phosphate buffer) ratio of substrate/enzyme 1:1 (dry powder of commercial enzyme, lyophilized TBE). Ee% determined by HPLC using Whelk (*S*,*S*)-O 1 chiral column (hexane/2-propanol/acetic acid, 90:10:0.1). The specific rotation of the resolved (*S*)-acid ee >99% was $[\alpha]_D^{25} = +42.5$ (*c* 1, $CHCl_3$). The combined yields of the resolved bioproducts were in the range of 60–70%.

CCL exhibited the highest enantioselectivity for the methyl ester **5a** affording (*S*)-**5** in an enantiomerically pure form ($E \sim 383$, >99% ee), while other esters **5b** and **5c** displayed moderate selectivity. This is an important outcome of these studies indicating that CCL can be advantageously used for the resolution of the precursor **5** for the preparation of (*S*)-naproxen. To the best of our knowledge this is the first report of the use of CCL for the preparation of (*S*)-**5** with high enantiopurity. The results of these studies are summarized in Table 4.

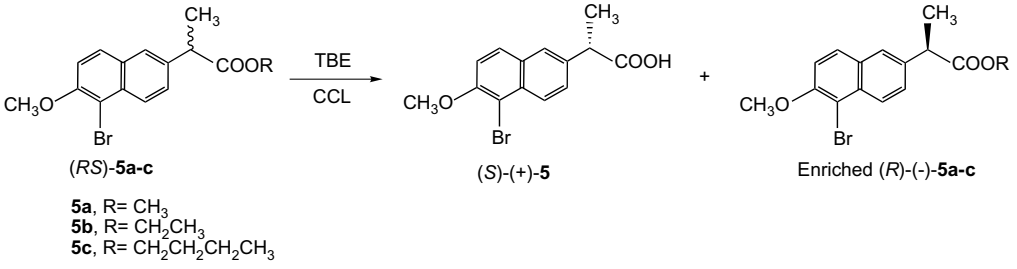
4.2. Kinetic resolution of racemic ibuprofen 6

Ibuprofen is one of the blockbuster drugs used in the treatment of inflammation, pain, and fever.[43] As in the case of (*S*)-naproxen, it is the (*S*)-ibuprofen, which is clinically active though it is reported that the (*R*)-enantiomer isomerizes to the bioactive form in the biological system.[44] The high selectivity of TBE for (*S*)-naproxen prompted us to explore the possibility of kinetic resolution of racemic ibuprofen, that is, 4-(2-methylpropyl)-α-methyl phenylacetic acid **6**. Therefore, racemic methyl, ethyl, and butyl esters **6a–c** were prepared and subjected to hydrolysis using TBE (Scheme 5) as well as CCL. As expected, TBE displayed high enantioselectivity for the methyl and ethyl ester giving an (*S*)-acid of high enantiomeric excess ($E \sim 117$ and 340, ee 98.5% and 99%, respectively) but showed poor selectivity for higher esters. Commercial lipase CCL, on the other hand was also selective for methyl ester ($E \sim 238$, 98% ee), which is in agreement with the results published earlier by McConville et al.[45] Table 5 summarizes the results of these experiments.

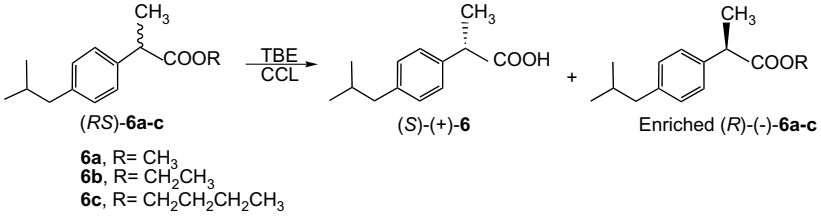
Resolution studies of racemic substrates **1–6** clearly showed the efficacy of TBE as an important esterase. In general, during the kinetic resolution studies of alkyl acylates of primary and secondary alcohols, it was observed that TBE and commercial lipases exhibited preferences for opposite enantiomers. However, in the hydrolysis of alkyl esters of carboxylic acids, both TBE and CCL displayed preference for the (*S*)-enantiomers.

4.3. Kinetic resolution of racemic 2-(2-naphthyloxy)-propanoic acid 7

In addition to naproxen and ibuprofen, we also studied the resolution of naphthyloxypropanoic acid as substrate for TBE (Scheme 6). Aryloxypropanoic acids are reportedly associated with several biological activities, such as herbicidal, hypocholesterolemic, microsomal, stearoyl-Coa-desaturase activity[46,47] thereby making them valuable substrates for kinetic resolution. In these experiments, TBE catalyzed hydrolysis of the alkyl esters of racemic 2-(2-naphthyloxy)propanoic acid **7a–c**



Scheme 4.



Scheme 5.

Table 5. Hydrolysis of racemic alkyl esters of 4-(2-methylpropyl)-α-methylphenylacetic acid **6a–c** using TBE and commercial enzymes CCL

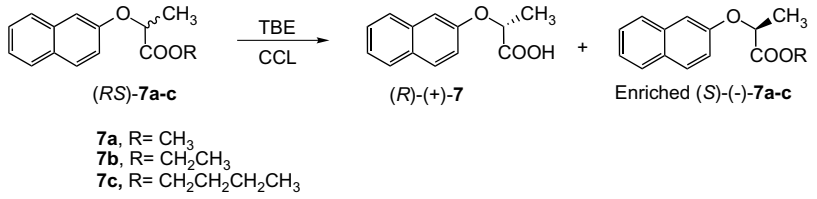
Entry	Enzyme	Time (h)	Conv. (%)	ee (%) product	Conf. (product)	*E* Factor
6a	TBE	96	34.8	98.5	*S*	117
	CCL	36	43.0	98.0	*S*	2.4
6b	TBE	96	32.2	99.0	*S*	340
	CCL	48	34.0	63.0	*S*	6
6c	TBE	96	7.2	17.4	*S*	0.9
	CCL	48	27.0	22.3	*S*	1.4

Substrate concentration 50 g/L, temp 29 °C, buffer pH 8.0 (0.1 M sodium phosphate) ratio of substrate/enzyme 1:0.7 (dry powder of commercial enzyme, lyophilized TBE). Ee% determined by HPLC using Chiradex column with mobile phase ($MeOH/H_2O$/TEA, 50:50:0.1). The combined yields of the resolved bio-products were in the range of 70–80%.

Table 6. Hydrolysis of racemic alkyl esters of 2-(2-naphthyloxy)propanoic acid **7a–c** using TBE and commercial CCL

Entry	TBE				CCL			
	Conv. (%)	ee (%)	Conf. prod.	*E* Factor	Conv. (%)	ee (%)	Conf. prod.	*E* Factor
7a	38.0	79.0	*R*	14.1	44.0	33.9	*R*	2.6
7b	23.0	23.0	*R*	1.7	43.0	30.0	*R*	2.3
7c	07.3	34.0	*R*	2.0	44.0	43.0	*R*	1.3

Substrate concentration 35 g/L, incubation time 64 h, temp 25 °C, pH 7.5 (0.1 M sodium phosphate buffer), ratio of substrate/enzyme 1:0.6 (dry powder of commercial enzyme) and 1:1 (lyophilized TBE powder). Ee% determined by HPLC using Whelk (*S*,*S*)-O 1 chiral column with mobile phase (hexane/2-propanol/acetic acid, 90:10:0.1). The specific rotation of the resolved (*R*)-acid (ee 79%) was $[\alpha]_D^{25} = +49.2$ (*c* 1, $CHCl_3$,); ee 97% $[\alpha]_D^{25} = +60.1$ (*c* 1, $CHCl_3$) after crystallization. The combined yields of the resolved bio-products were in the range of 65–80%.



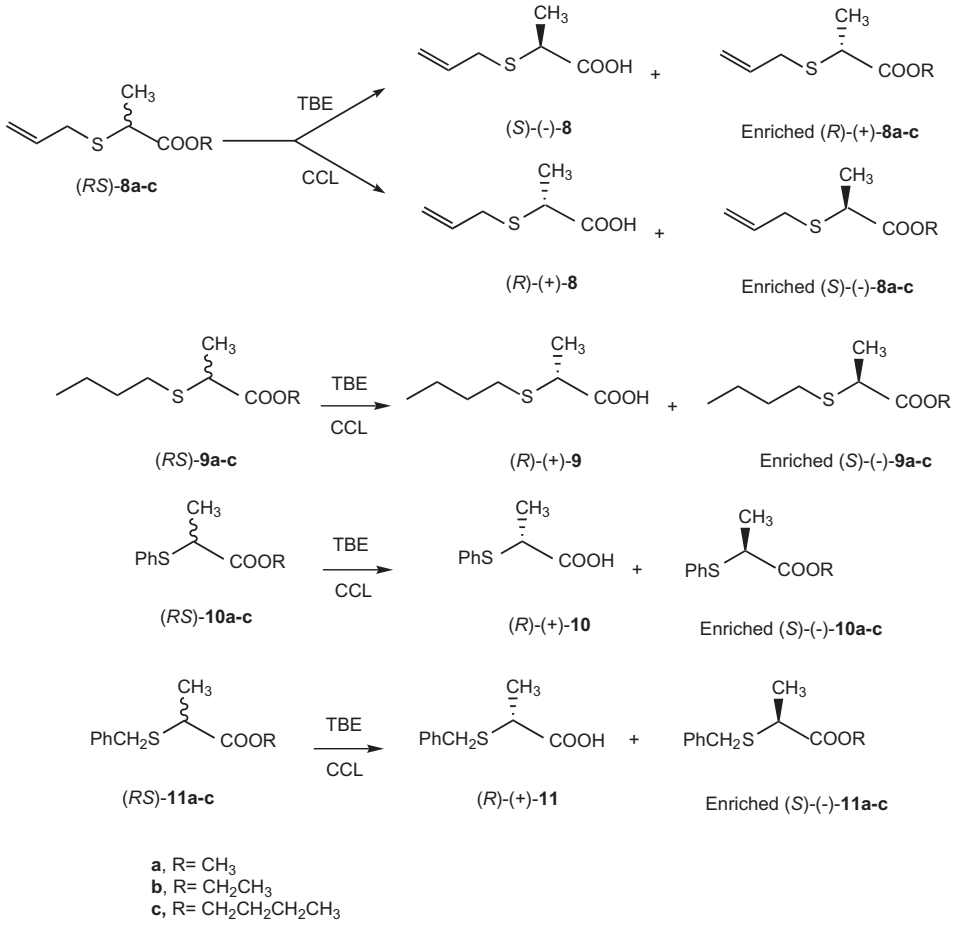
Scheme 6.

to afford the (*R*)-acid with 79% ee, which was highest for the methyl ester (after one crystallization enantiopurity improved to 97% and total yield 28%) but exhibited low activity as well as selectivity for the higher homologues. CCL also displayed poor selectivity for all the esters to give the (*R*)-(+)-acid. Resolution of racemic **7** has also been reported using microbial strains like *G. cingulata* and *Glocosporium olivarum*.[40] Table 6 summarizes the results of the above experiments.

5. Resolution of racemic 2-(allylthio)propanoic acid 8a–c, 2-(*n*-butylthio)propanoic acid 9a–c, 2-(phenylthio)propanoic acid 10a–c, and 2-(benzylthio)propanoic acid 11a–c

Herein, we also included the alkyl esters of 2-(allylthio)propanoic acid **8a–c**, 2-(*n*-butylthio)propanoic acid **9a–c**, 2-(phenylthio)propanoic acid **10a–c**, and 2-(benzylthio)propanoic acid **11a–c**, in order to explore the potential of TBE in accepting a diverse range of substrates. Enantiomerically enriched thio analogues, such as 2-methyl-3-mercaptopropanoic acid or related compounds, have found applications as chiral synthons including components of enzyme inhibitors[48,49] and also as precursors of ACE inhibitors like captopril, analpril, cizalapril, etc.[50] Herein, racemic alkyl esters of 2-(allylthio)propanoic acid **8a–c**, 2-(*n*-butylthio)propanoic acid **9a–c**, 2-(phenylthio)propanoic acid **10a–c**, and 2-(benzylthio)propanoic acid **11a–c** were synthesized and subjected to kinetic resolution using both TBE and CCL (Scheme 7). The two enzymes exhibited opposite selectivities for the esters of 2-(allylthio)propanoic acids **8a–c**, however, CCL gave products with higher enantiopurity (**8c**, $E \sim 19.38$, 86% ee) as compared to TBE. In all other substrates **9–11a–c**, both the enzymes displayed a preference for the same enantiomer producing (*R*)-acids. For the substrates 2-(*n*-butylthio)propanoic acid esters **9a–c** and 2-(phenylthio)propanoic acid esters **10a–c**, TBE showed low to moderate selectivities and CCL in comparison was even less effective. Tan et al. have reported the resolution of substrate **10a** using *Pseudomonas cepacia* with poor reactivity and high enantioselectivity.[51] Surprisingly, in the resolution of one of the substrates **11a**, that is, methyl ester of 2-(benzylthio)propanoic acid, TBE displayed exceptionally high enantioselectivity (E >1000, >99% ee) that steadily decreased with increase in size of alkyl ester group **11b**,**c**. Table 7 summarizes the results of the above experiments.

For the data obtained from the resolution studies of alkyl esters of **8–11a–c** and taking into consideration



Scheme 7.

Table 7. Hydrolysis of racemic alkyl esters of 2-(allylthio) propanoic acid **8a**–**c**, **2**-(*n*-butylthio) propanoic acids **9a**–**c**, 2-(phenylthio) propanoic acids **10a**–**c**, and 2-(benzylthio) propanoic acids **11a**–**c** using TBE and CCL

CCL						TBE					
Substrate (entry)	Time (h)	Conv. (%)	ee (%)	Conf. prod.	*E* Factor	Substrate (entry)	Time (h)	Conv. (%)	ee (%)	Conf. prod.	*E* Factor
8a	4	42.2	2.0	*R*	1.05	**8a**	12	34.6	41.2	*S*	2.94
8b	6	38.0	23.0	*R*	1.81	**8b**	12	40.0	8.0	*S*	1.12
8c	12	31.0	86.0	*R*	19.8	**8c**	12	42.0	6.5	*S*	1.18
9a	4	41.8	33.6	*R*	2.51	**9a**	12	15.4	63.9	*R*	5.08
9b	12	40.0	57.7	*R*	5.37	**9b**	12	27.0	1.5	*R*	1.0
9c	12	12.2	64.0	*R*	4.97	**9c**	12	33.5	11.5	*R*	1.33
10a	4	33.0	11.7	*R*	2.81	**10a**	12	23.4	33.5	*R*	2.21
10b	12	17.5	37.8	*R*	2.39	**10b**	12	35.9	20.7	*R*	1.68
10c	12	15.5	2.0	*R*	1.03	**10c**	12	8.3	3.3	*R*	1.07
11a	8	25.2	26.0	*R*	1.35	**11a**	12	36.5	>99.5	*R*	>1000
11b	12	18.6	2.6	*R*	1.05	**11b**	12	35.5	38.4	*R*	2.74
11c	12	20.8	2.0	*R*	1.02	**11c**	12	9.9	5.8	*R*	1.1

Substrate concentration 50 g/L, pH 8.0 (0.1 M sodium phosphate buffer), temp 25 °C, ratio of substrate/enzyme 1:0.45 (dry powder of commercial enzyme, lyophilized TBE). Ee% determined by HPLC using Diacel OD-H chiral column (hexane/2-propanol, 95:5) with flow rate 0.5 mL/min. The optical rotation of the resolved (*S*)-acid **8** $[\alpha]_D^{25} = -30.7$ {authentic sample $[\alpha]_D^{25} = +84.8$, (*c* 1, $CHCl_3$)}, (*R*)-acid **9** $[\alpha]_D^{25} = +18.3$ {authentic sample $[\alpha]_D^{25} = +28.6$, (*c* 1, $CHCl_3$)}, (*R*)-acid **10** $[\alpha]_D^{25} = +24.0$ {authentic sample +71.5, (*c* 1 $CHCl_3$)} and (*R*)-acid **11**, $[\alpha]_D^{25} = +129.1$ {authentic sample $[\alpha]_D^{25} = +130$, (*c* 0.9, $CHCl_3$)}. The absolute configuration was determined by a comparison of the sign of the specific rotation reported in the literature[51] as well as by the comparison of the sign of specific rotation with (*R*)-(+)-thio acid synthesized by condensation of enantiomerically pure (*S*)-(−)-2-bromopropanoic acid with respective mercaptol in dry acetone/potassium carbonate to furnish, after usual workup, substituted (*R*)-(+)-2-thiopropanoic acid. The combined yields of the resolved bio-products were in the range of 60–70%.

the selectivity factor *E* (which generally falls in the range 1–5) it can be concluded that most of them are poor substrates for both TBE and CCL with the exception of **8c** and **11a**. The determination of ee% and the absolute

configurations of the products was also established, which is based on the data obtained from their mandelate esters using a method described below.

The enantiopurity of resolved acid from the ester of 2-(benzylthio)propanoic acid was determined by HPLC (on a chiral column Whelk (*S*,*S*) O-1 using mobile phase of hexane/2-propanol/acetic acid, 90:10:0.2). The ee% of other kinetically resolved acids could not be determined directly by chiral HPLC due to the failure to achieve resolution on chiral columns available with us. Therefore, authentic (*R*)-mercaptoacids were synthesized by condensing (*S*)-(−)-2-bromopropanoic acid with alkyl mercaptols, with the resulting enantiomerically enriched (*R*)-mecaptoacids esterified with methyl (*R*)-mandelate. The synthetic mandelates were analyzed by ^{1}H NMR to rule out the possibility of racemization, which might have had occurred during the preparation of these (*R*)-acids. However, in all the synthetic samples enantiomerically >98% was recorded by ^{1}H NMR, which displayed a single methine proton signal for the diastereoisomer (*R*)-mecaptoacid mendalates whereas a clear splitting of methine protons signals at δ 5.9–6.0 for the (*R*)- and (*S*)-diastereoisomers was observed in a racemic sample. A comparison of the specific rotation values of synthesized (*R*)-acids and resolved acids enabled us to determine their ee% to a fair degree of accuracy besides establishing their absolute configurations by comparison of the signs of the specific rotations. Table 7 summarizes the results of these experiments.

6. Conclusion

The efficacy and high enantioselectivity of hydrolase producing strain TBE in its crude native form has been effectively demonstrated on a broad spectrum of molecules, which include the alkyl esters of alcohols and carboxylic acids, for example, 1-aryl alkanols, NSAIDS (such as Ibuprofen), and a variety of thiocarboxylic acids. Earlier, we have already demonstrated the high efficacy of TBE in the resolution of anti-inflammatory drug naproxen as well as chlorohydrin precursor of a β-blocker drug propranalol. There are currently very few esterases commercially available possessing such a broad and diverse range of substrate acceptability. TBE, which is fairly stable and robust, is another addition to such a small list of esterases.

7. Experimental

All the reagents used in the study were of analytical grade. The authenticity of racemic esters prepared during the study was confirmed by spectroscopic analysis including NMR, LC/MS, and that of the resolved enantiomers by specific rotation and chiral chromatographic methods. Melting points recorded by capillary method are uncorrected. The optical rotations were measured on a Perkin–Elmer 241 polarimeter with chloroform as the solvent. Chiral HPLC analysis carried out on Shimadzu LC10 AT model. TLCs were run on 0.25 mm silica gel 60 F_{254} plates (E. Merck) using UV light, or ceric sulfate solution for detection of the spots. All the compounds were characterized by spectral methods, which include FT-IR (Brucker 270-30), ^{1}H NMR (Brucker Avance DPX-200) at 200 MHz, and ^{13}C NMR at 50 MHz with $CDCl_3$ as solvent and TMS as internal standard, MS on JEOL MSD-300 and GCMS-QP 2000.

For 1-(6-methoxy-2-naphthyl)ethanol, 1-(3,4-methylenedioxyphenyl)ethanol, and 1-(3,4-methylenedioxyphenyl)pentanol, the resolution was effected on Chiralcel OD-H HPLC column (5 μm) using *n*-hexane/2-propanol (95:5) with a flow rate of 0.5 mL/min. The enantiopurity and progress of this reaction for aryloxypropanoic acid was monitored by analyzing on Lichro Cart-250-4, Whelk (*S*,*S*)- or (*R*,*R*)-O 1 (5 μm) chiral column (mobile phase hexane/2-propanol/acetic acid, 90:10:0.1) flow rate 0.8 mL/min and double array detector. For naproxol the resolution was effected on Whelk (*R*,*R*)-O 1 chiral column using hexane/2-propanol/acetic acid (90:10:0.1) with a flow rate of 0.5 mL/min. For 5-bromonaproxen the resolution was carried out on Whelk (*S*,*S*)–O 1 chiral column (hexane/2-propanol/acetic acid, 90:10:0.1) with a flow rate of 0.8 mL/min. For ibuprofen the resolution was effected on a Chiradex column (5 μm) using methanol/water/triethylamine (50:50:0.1) with a flow rate of 0.8 mL/min. Commercial lipases like porcine pancreatic lipase (PPL 2.33 U/mg solid) *C. cylindraceae* lipase (CCL 1.5 U/mg solid) was procured from Sigma Chemical, USA. *Pseudomonas* sp. (PSL, 13 U/mg solid) was obtained as a gift from Amano, Japan. The procured lipases were used as such without further purification. Metrohm pH stat-718 was used for maintaining pH during biotransformations.

The crude cell pellet of *T. beigelli* esterase (45 U/g dry cell mass) was prepared by the method as reported earlier.[28] The cell biomass (TBE) was centrifuged, washed twice with distilled water, and stored at −40 °C in small aliquots for future use. The wet cell biomass (150 g) was lyophilized to a dry powder (38 g) and used directly for kinetic resolution studies. Protein estimation was made according Lowry's method using BSA as the reference protein.[52]

7.1. Preparation of 1-(6-methoxy-2-naphthyl)ethanol 1

Sodium borohydride (0.6 g, 15.8 mmol) in four installments was added at −5 to 0 °C in 30 min to a stirring methanolic solution (100 mL) of 2-acetyl-6-methoxynaphthalene (10 g, 50 mmol) and the reaction monitored by TLC. After the completion of the reaction, the contents were concentrated in vacuo and the residue poured in ice-water (100 mL), extracted with dichloromethane (4 × 30 mL), organic layer washed with brine (2 × 15 mL), dried over anhydrous sodium sulfate, and concentrated. The crude reaction product was purified by chromatography over silica gel using toluene/ethyl acetate (7:3) as the eluant to afford the carbinol **1** as a crystalline white solid, mp 113–114 °C (9.54 g, 47.2 mmol, 94.4%); IR (KBr): 3333, 3007, 1632, 1606, 1504, 1487, 1463, 1390, 1348, 1260, 1235, 1217, 1195, 1163, 1119, 1073, 1028, 961, 930, 892, 853, 815 cm^{-1}; ^{1}H NMR (200 MHz): δ 1.57 (3H, d, $J = 6.4$ Hz, CH_3),

3.92 (3H, s, –OCH_3), 5.03 (1H, q, $J = 6.3$ Hz, CHOH), 7.13 (2H, m, Ar–H), 7.50 (1H, dd, $J = 8.0$ and 1.2 Hz, Ar–H), 7.70 (3H, m, Ar–H); ^{13}C NMR (50 MHz): δ 24.9, 55.2, 70.0, 105.7, 118.9, 123.7, 124.4, 127.0, 128.7, 129.4, 133.9, 141.0, 157.5; MS (*m*/*z*) (%): 202 (70), 188 (13), 187 (100), 159 (73), 144 (84), 141 (10), 127 (22), 115 (44), 93 (9), 89 (6), 64 (11); Anal. Calcd for $C_{13}H_{14}O_2$: C, 83.83; H, 7.57. Found: C, 83.99; H, 7.58.

7.2. Preparation of 1-(3,4-methylenedioxyphenyl)ethanol 2 and 1-(3,4-methylenedioxyphenyl)pentanol 3

Dry diethyl ether solution (100 mL) of piperonal (7.5 g each, 50 mmol each) under nitrogen current was added to Grignard reagent prepared by reacting methyl iodide/*n*-butyl bromide (65 mmol) and magnesium metal (70 mmol) in dry ether (120 mL) at 0–5 °C. After the completion of the reaction and usual workup, it was purified by column chromatography over silica gel using pet. ether/ethyl acetate (17:3) as eluant to give corresponding carbinols **2** (a colorless liquid, 8.1 g, 48.8 mmol, 96.4%) and **3** (a colorless liquid, 9.8 g, 47.1 mmol, 94.2%).

7.2.1. 1-(3,4-Methylenedioxyphenyl)ethanol 2. A colorless liquid; IR (KBr): 3374, 2973, 1609, 1503, 1488, 1442, 1371, 1320, 1244, 1189, 1130, 1101, 1074, 1039, 1008, 926, 877, 813 cm^{-1}; ^{1}H NMR (200 MHz): δ 1.41 (3H, d, $J = 6.3$ Hz, CH_3), 4.76 (1H, q, $J = 6.3$ Hz, CHOH), 5.9 (2H, s, CH_2O), 6.73 (1H, d, $J = 8.0$ Hz, Ar–H), 6.83 (1H, d, $J = 8.6$ Hz, Ar–H), 6.85 (1H, s, Ar–H); ^{13}C NMR (50 MHz): δ 25.1, 70.0, 100.9, 106.0, 108.1, 118.6, 140.1, 141.7, 147.6; MS (*m*/*z*) (%): 166 (38), 151 (53), 123 (24), 121 (8), 93 (100), 75 (15), 66 (56), 65 (58); Anal. Calcd for $C_9H_{10}O_3$: C, 65.05; H, 6.065. Found: C, 65.43; H, 6.071.

7.2.2. 1-(3,4-Methylenedioxyphenyl)pentanol 3. A colorless liquid; IR (neat): 3381, 2931, 1630, 1609, 1503, 1487, 1442, 1379, 1326, 1245, 1187, 1109, 1091, 1041, 935, 866, 811, 777, 640 cm^{-1}; ^{1}H NMR (200 MHz): δ 0.87 (3H, t, $J = 6.7$ Hz, CH_3), 1.16–1.33 (4H, m, $2 \times$ CH_2), 1.60–1.77 (2H, m, CH_2), 4.51 (1H, t, $J = 6.4$, CHOH), 5.92 (2H, s, CH_2O), 6.74 (2H, s, Ar–H), 6.77 (1H, s, Ar–H); ^{13}C NMR (50 MHz): δ 14.0, 22.6, 28.0, 38.7, 74.5, 100.9, 106.4, 108.0, 119.3, 139.1, 146.8, 147.7; MS (*m*/*z*) (%): 208 (100), 190 (7), 152 (98), 149 (37), 148 (10), 135 (19), 123 (95), 121 (31), 93 (98), 89 (12), 77 (42), 66 (26), 65 (97); Anal. Calcd for $C_{12}H_{16}O_3$: C, 69.20; H, 7.74. Found: C, 69.69; H, 7.72.

7.3. General method for the preparation of acyl derivatives of compounds 1-(6-methoxy-2-naphthyl)ethanol 1, 1-(3,4-methylenedioxyphenyl)ethanol 2, and 1-(3,4-methylenedioxyphenyl)pentanol 3: preparation of 1a–c, 2a–c, and 3a–c

Acyl derivatives of **1**–**3** were prepared by subjecting secondary alcohols (10 mmol) to react with respective alkanoic anhydrides (15 mmol) in the presence of dimethylaminopyridine (DMAP, 5 mg) and the contents heated for 5 min at 50 °C. After the completion of the reaction (monitored by TLC), the mixture was poured in ice-water and extracted with dichloromethane (3×50 mL). The organic layer separated and washed with water (2×20 mL), dried over sodium sulfate, concentrated in vacuo, and purified by chromatography over silica gel using toluene/ethyl acetate in increasing proportion as eluant to give the following acyl esters; **1a** (2.24 g, 9.2 mmol, 91.8%): **1b** (2.35 g, 9.1 mmol, 90.18%); **1c** (2.46 g, 9.04 mmol, 90.0%). **2a** (2.95 g, 14.2 mmol, 94.8%); **2b** (3.04 g, 13.7 mmol, 91.2%); **2c** (3.23 g, 13.7 mmol, 92.1%). **3a** (3.60 g, 14.4 mmol, 96.2%); **3b** (3.8 g, 14.4 mmol, 96.4%), and **3c** (3.94 g, 14.2 mmol, 94.4%).

7.3.1. 1-Acetoxy-1-(6-methoxy-2-naphthyl)ethane 1a. A white solid; mp 66 °C; IR (KBr): 2972, 2939, 1732, 1631, 1606, 1506, 1486, 1461, 1420, 1391, 1309, 1238, 1179, 1120, 1107, 1083, 1025, 994, 962, 930, 900, 855, 808 cm^{-1}; ^{1}H NMR (200 MHz): δ 1.64 (3H, d, $J = 7.2$ Hz, CH_3), 2.08 (3H, s, OCOCH_3), 3.92 (3H, s, OCH_3), 6.0 (1H, q, $J = 7.2$ Hz, CH–O), 7.08 (1H, s, Ar–H), 7.15 (1H, d, $J = 8.1$ Hz, Ar–H), 7.40 (1H, d, $J = 8.5$ Hz, Ar–H), 7.48–7.72 (3H, m, Ar–H); ^{13}C NMR (50 MHz): δ 21.4, 22.1, 55.3, 72.5, 105.7, 119.1, 124.8, 125.1, 127.2, 128.7, 129.5, 134.3, 136.7, 157.9, 170.4; MS (*m*/*z*) (%): 244 (58), 201 (13), 187 (49), 185 (9), 171 (10), 169 (100), 153 (32), 141 (38), 128 (15), 127 (19), 114 (12); Anal. Calcd for $C_{15}H_{16}O_3$: C, 78.91; H, 7.06. Found: C, 79.02; H, 7.11.

7.3.2. 1-Propanoyloxy-1-(6-methoxy-2-naphthyl)ethane 1b. A white solid; mp 58–59 °C; IR (KBr): 2974, 2941, 1733, 1632, 1607, 1505, 1486, 1460, 1419, 1391, 1363, 1328, 1309, 1269, 1237, 1179, 1122, 1105, 1084, 1056, 1025, 994, 962, 925, 856, 821, 807 cm^{-1}; ^{1}H NMR (200 MHz): δ 1.16 (3H, t, $J = 7.5$ Hz, CH_3), 1.62 (3H, d, $J = 6.5$ Hz, CH_3), 2.38 (2H, q, $J = 7.4$ Hz, COCH_2), 3.92 (3H, s, OCH_3), 6.05 (1H, q, $J = 6.5$ Hz, CHCH$_3$), 7.14–7.26 (2H, m, Ar–H), 7.46 (1H, dd, $J = 1.4$ and 8.3 Hz, Ar–H), 7.72–7.76 (3H, m, Ar–H); ^{13}C NMR (50 MHz): δ 9.15, 22.2, 27.9, 55.3, 72.3, 106.7, 119.1, 124.7, 124.9, 127.2, 128.6, 129.5, 134.2, 136.9, 157.8, 173.8; MS (*m*/*z*) (%): 258 (39), 202 (38), 187 (15), 185 (100), 170 (18), 153 (10), 141 (27), 115 (19), 58 (23); Anal. Calcd for $C_{16}H_{18}O_3$: C, 79.91; H, 7.06. Found: C, 79.98; H, 7.08.

7.3.3. 1-Butanoyloxy-1-(6-methoxy-2-naphthyl)ethane 1c. A white solid; mp 35 °C; IR 2964, 2934, 1731, 1633, 1608, 1547, 1506, 1485, 1461, 1418, 1391, 1308, 1265, 1238, 1220, 1173, 1063, 1032, 951, 924, 892, 851, 810 cm^{-1}; ^{1}H NMR (200 MHz): δ 0.95 (3H, t, $J = 7.3$ Hz, CH_3), 1.57 (3H, d, $J = 6.2$ Hz, CHCH_3), 1.60–1.72 (2H, m, CH_2), 2.32 (2H, t, $J = 7.2$ Hz, CH_2CO), 3.87 (3H, s, OCH_3), 6.05 (1H, q, $J = 6.2$ Hz, O–CH), 7.11 (1H, s, Ar–H), 7.15 (1H, d, $J = 8.5$ Hz, Ar–H), 7.46 (1H, dd, $J = 8.5$ and 1.42 Hz, Ar–H), 7.69–7.73 (3H, m, Ar–H); ^{13}C NMR (50 MHz): δ 13.6, 18.2, 22.1, 36.1, 55.2, 72.3, 105.6, 119.0, 124.7, 124.9, 127.2, 128.6, 129.5, 134.2, 136.8, 157.8, 173.3; MS (*m*/*z*) (%): 272 (32), 202 (40), 185 (100), 170 (16), 154 (8), 141 (26), 115 (17), 45 (39); Anal. Calcd for $C_{17}H_{20}O_3$: C, 79.65; H, 7.86. Found: C, 79.88; H, 7.83.

7.3.4. 1-Acetoxy-1-(3,4-methylenedioxyphenyl)ethane 2a. A colorless liquid; IR (KBr): 2982, 1739, 1609, 1489, 1444, 1360, 1244, 1173, 1124, 1097, 1080, 1039, 1015, 933, 867, 842, 808, 745, 727, 717, 699 cm^{-1}; ^1H NMR (200 MHz): δ 1.49 (3H, d, $J = 6.5$ Hz, CH_3), 2.06 (3H, s, CH_3), 5.79 (1H, q, $J = 6.5$ Hz, CH), 5.93 (2H, s, OCH_2O), 6.75 (1H, d, $J = 8.5$ Hz, Ar–*H*), 6.78 (1H, d, $J = 8.5$ Hz, Ar–*H*), 6.85 (1H, s, Ar–*H*); ^{13}C NMR (50 MHz): δ 21.3, 22.1, 72.1, 101.0, 106.7, 108.1, 119.8, 135.6, 147.2, 147.8, 170.2; MS (*m*/*z*) (%): 208 (23), 152 (12), 148 (100), 135 (27), 133 (42), 105 (14), 93 (28), 77 (49), 65 (12); Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.580. Found: C, 63.91; H, 5.813.

7.3.5. 1-Propanoyloxy-1-(3,4-methylenedioxyphenyl)ethane 2b. A colorless liquid; IR (KBr): 2981, 1734, 1610, 1505, 1490, 1445, 1361, 1324, 1249, 1186, 1137, 1104, 1081, 10389, 1009, 937, 921, 894, 861, 809, 728 cm^{-1}; ^1H NMR (200 MHz): δ 1.09 (3H, t, $J = 7.5$ Hz, CH_3), 1.51 (3H, d, $J = 6.3$ Hz, CH_3), 2.31 (2H, q, $J = 7.5$ Hz, $COCH_2$), 5.8 (1H, q, $J = 6.5$ Hz, $CHCH_3$), 5.89 (2H, s, OCH_2O), 6.73 (1H, d, $J = 8.0$ Hz, Ar–*H*), 6.77 (1H, d, $J = 8.0$ Hz, Ar–*H*), 6.81 (1H, s, Ar–*H*); ^{13}C NMR (50 MHz): δ 8.96, 22.1, 27.7, 71.9, 101.0, 106.6, 108.0, 119.6, 135.7, 147.1, 147.7, 173.6; MS (*m*/*z*) (%): 222 (83), 166 (82), 151 (46), 149 (100), 148 (98), 121 (10), 119 (45), 93 (12), 89 (21), 77 (11), 65 (64); Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.349. Found: C, 65.01; H, 6.352.

7.3.6. 1-Butanoyloxy-1-(3,4-methylenedioxyphenyl)ethane 2c. A colorless liquid; IR (KBr): 2968, 1733, 1610, 1505, 1490, 1445, 1373, 1325, 1248, 1180, 1137, 1103, 1039, 1005, 937, 810 cm^{-1}; ^1H NMR (200 MHz): δ 0.91 (3H, t, $J = 7.4$ Hz, CH_3), 1.48 (3H, d, $J = 6.6$ Hz, CH_3), 1.77 (2H, h, $J = 7.3$ Hz, CH_2), 2.25 (2H, t, $J = 7.2$ Hz, $COCH_2$), 5.81 (1H, q, $J = 6.5$ Hz, CH), 5.90 (2H, s, OCH_2O), 6.75 (1H, d, $J = 8.0$ Hz, Ar–*H*), 6.77 (1H, d, $J = 8.0$ Hz, Ar–*H*), 6.84 (1H, s, Ar–*H*); ^{13}C NMR (50 MHz): δ 13.5, 18.5, 22.2, 36.4, 71.8, 101.0, 106.6, 108.2, 119.7, 135.7, 147.1, 147.7, 172.8; MS (*m*/*z*) (%): 236 (84), 166 (100), 151 (40), 149 (100), 148 (98), 121 (11), 119 (50), 91 (94), 89 (22), 77 (12), 71 (54), 65 (68); Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.08; H, 6.825. Found: C, 66.77; H, 6.849.

7.3.7. 1-Acetoxy-1-(3,4-methylenedioxyphenyl)pentane 3a. A colorless liquid; IR (KBr): 3074, 2957, 1731, 1631, 1610, 1504, 1445, 1370, 1335, 1237, 1109, 1096, 1040, 935, 907, 861, 811, 621 cm^{-1}; ^1H NMR (200 MHz): δ 0.86 (3H, t, $J = 7.0$ Hz, CH_3), 1.15–1.32 (4H, m, $2 \times CH_2$), 1.71–2.0 (2H, m, CH_2), 2.04 (3H, s, $COCH_3$), 5.64 (1H, t, $J = 7.0$ Hz, $CHOH$), 5.90 (2H, s, CH_2O), 6.70–6.82 (3H, m, Ar–*H*); ^{13}C NMR (50 MHz): δ 13.9, 21.2, 22.4, 27.7, 35.9, 76.0, 101.0, 106.9, 108.0, 120.4, 134.7, 147.2, 147.7, 170.3; MS (*m*/*z*) (%): 250 (100), 208 (56), 193 (52), 161 (47), 152 (98), 148 (58), 135 (99), 131 (77), 103 (35), 93 (96), 77 (47), 65 (90); Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.248. Found: C, 67.31; H, 7.255.

7.3.8. 1-Propanoyloxy-1-(3,4-methylenedioxyphenyl)pentane 3b. A colorless liquid; IR (KBr): 2957, 1737, 1631, 1610, 1504, 1490, 1445, 1380, 1359, 1335, 1248, 1185, 1108, 1080, 1040, 935, 891, 862, 728, 639 cm^{-1}; ^1H NMR (200 MHz): 0.84 (3H, t, $J = 7.0$ Hz, CH_3), 1.05–1.31 (7H, m, $2 \times CH_2$ and CH_3), 1.63–1.93 (2H, m, CH_2), 2.30 (2H, q, $J = 7.0$ Hz, $COCH_2$), 5.67 (1H, t, $J = 7.0$ Hz, OCH), 5.89 (2H, s, OCH_2), 6.69–6.80 (3H, m, Ar–*H*); ^{13}C NMR (50 MHz): δ 9.0, 13.9, 22.4, 27.7, 27.8, 36.0, 75.8, 101.0, 126.9, 108.0, 120.3, 135.0, 147.1, 147.7, 173.8; MS (*m*/*z*) (%): 264 (26), 208 (21), 191 (12), 161 (13), 151 (100), 135 (51), 131 (17), 103 (8), 93 (10), 77 (12), 65 (16), 58 (89); Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.626. Found: C, 68.63; H, 7.666.

7.3.9. 1-Butanoyloxy-1-(3,4-methylenedioxyphenyl)pentane 3c. A colorless liquid; IR (KBr): 2960, 1734, 1610, 1504, 1490, 1381, 1303, 1247, 1178, 1095, 1040, 967, 936, 909, 861, 728, 638 cm^{-1}; ^1H NMR (200 MHz): δ 0.90 (3H, t, $J = 7.1$ Hz, CH_3), 1.0 (3H, t, $J = 7.4$ Hz, CH_3), 1.09–1.34 (4H, m, $2 \times CH_2$), 1.55–1.73 (4H, m, $2 \times CH_2$), 2.30 (2H, q, $J = 7.7$ Hz, $COCH_2$), 5.68 (1H, t, $J = 7.0$ Hz, CH–O), 5.96 (2H, s, OCH_2O), 6.78 (1H, d, $J = 7.7$ Hz, Ar–*H*), 6.83 (1H, dd, $J = 1.1$ and 7.9 Hz, Ar–*H*), 6.84 (1H, s, Ar–*H*); ^{13}C NMR (50 MHz): δ 13.56, 13.62, 18.16, 22.40, 27.69, 35.90, 36.50, 75.79, 101.0, 106.9, 108.0, 120.3, 134.9, 147.1, 147.7, 173.0; MS (*m*/*z*) (%): 278 (4), 221 (12), 208 (35), 191 (16), 161 (17), 152 (9), 151 (100), 149 (21), 135 (71), 131 (20), 103 (10), 77 (14), 71 (94), 65 (17); Anal. Calcd for $C_{16}H_{22}O_4$: C, 69.04; H, 7.966. Found: C, 69.22; H, 7.972.

7.4. Preparation of 2-(6-methoxy-2-naphthyl)propan-1-ol 4

Lithium aluminum hydride (LAH) (1.2 g) was added slowly to a solution of racemic methyl 2-(6-methoxy-2-naphthyl)propanoic acid (9.4 g, 41 mmol) in dry diethyl ether (100 mL) and after the completion of the reaction (monitored by TLC), the reaction was worked up by quenching the excess of LAH by the addition of ethyl acetate. The contents were diluted with water, the organic layer separated and aqueous layer extracted with solvent ether (2×50 mL). The combined organic layer was washed with water (2×15 mL) dried over sodium sulfate and concentrated to give crude product, which on column chromatography over silica gel using dichloromethane/ethyl acetate (9:1) gave **4** (8.02 g, 37.12 mmol, 92.3%); A white solid; mp 88 °C; IR (KBr): 3346, 2961, 2936, 1734, 1633, 1605, 1502, 1484, 1461, 1414, 1392, 1323, 1264, 1212, 1164, 1121, 1102, 1029, 959, 927, 882, 854, 813 cm^{-1}; ^1H NMR (200 MHz): δ 1.38 (3H, d, $J = 7.0$ Hz, CH_3), 3.0–3.11 (1H, m, CH), 3.74 (2H, d, $J = 6.8$ Hz, CH_2OH), 3.90 (3H, s, OCH_3), 7.13 (1H, d, $J = 2.4$ Hz, Ar–*H*), 7.16 (1H, d, $J = 8.39$ Hz, Ar–*H*), 7.36 (1H, dd, $J = 8.4$ and 1.6 Hz, Ar–*H*), 7.6 (1H, s, Ar–*H*), 7.77 (2H, dd, $J =$ 2.4 and 8.5 Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 17.7, 42.4, 51.2, 55.3, 68.6, 105.6, 118.9, 125.9, 126.3, 127.2, 129.1, 133.6, 138.7, 157.5; MS (*m*/*z*) (%): 216 (12), 198 (8), 189 (19), 177 (21), 161 (100), 140 (13), 134 (37), 118 (20), 82 (10), 80 (12), 63 (21); Anal. Calcd for $C_{14}H_{16}O_2$: C, 77.74; H, 7.45. Found: C, 77.99; H, 7.44.

7.5. General method for the preparation of alkyl acyl derivatives of compounds 2-(6-methoxy-2-naphthyl)-propan-1-ol 4: preparation of 4a–d

The esters of racemic **4** were prepared by taking 10 mmol each of racemic **4** and adding alkanoic anhydrides (acetic anhydride, propanoic anhydride, butyric anhydride, 15 mmol each) in the presence of catalytic amounts of DMAP and the contents heated to 50 °C for half an hour and thereafter stirred at room temperature for a further 24 h. The reaction mixture was poured into ice-water and the crude solids washed several times with cold water and then dissolved in dichloromethane (50 mL), dried over sodium sulfate, concentrated, and crystallized from pet. ether/ethylacetate (17:3) to give **4a** (2.50 g, 9.7 mmol, 97.2%), **4b** (2.61 g, 9.6 mmol, 96.6%), and **4c** (2.74 g, 9.6 mmol, 95.8%).

The octanoate derivative **4d** was prepared by stirring a dichloromethane solution (50 mL) of racemic **4** (2.2 g, >10 mmol), DCC (2.5 g, 12 mmol), and octanoic acid (1.8 g, 13 mmol) in the presence of a catalytic amount of DMAP (10 mg) for 12 h. The contents diluted with *n*-hexane (20 mL), the precipitate filtered, and washed with *n*-hexane/dichloromethane (9:1, 30 mL). The combined filtrate was concentrated and dissolved in ethyl acetate (60 mL), washed with water (2×10 mL), the organic layer dried, concentrated, and the product chromatographed over silica gel and eluted with pet. ether/ethyl acetate mixture (19:1) to give **4d** (3.29 g, 9.6 mmol, 96.2%).

7.5.1. 1-Acetoxy-2-(6-methoxy-2-naphthyl)propane 4a. A white solid; mp 64–65 °C; IR 2969, 1739, 1630, 1606, 1505, 1486, 1463, 1390, 1367, 1233, 1182, 1162, 1032, 980, 855, 816, 675 cm^{-1}; ^1H NMR (200 MHz): δ 1.37 (3H, d, $J = 7.0$ Hz, CH_3), 2.06 (3H, s, $COCH_3$), 3.17–3.27 (1H, m, CH), 3.91 (3H, s, OCH_3), 4.24 (2H, ddd, $J = 17.8$, 10.8, and 7.3 Hz, CH_2O), 7.11 (1H, s, Ar–H), 7.13 (1H, d, $J = 8.2$ Hz, Ar–H), 7.33 (1H, d, $J = 8.5$ Hz, Ar–H), 7.59 (1H, s, Ar–H), 7.70 (2H, d, $J = 8.5$ Hz, Ar–H); ^{13}C NMR (50 MHz): δ 18.2, 20.9, 38.8, 55.3, 69.5, 105.6, 118.9, 125.5, 126.3, 127.0, 129.0, 129.2, 133.5, 138.4, 157.5, 171.1; MS (*m*/*z*) (%): 258 (12), 198 (100), 185 (50), 170 (15), 153 (10), 141 (13), 128 (40), 115 (10), 45 (43); Anal. Calcd for $C_{16}H_{18}O_3$: C, 74.39; H, 7.02. Found: C, 74.88; H, 7.08.

7.5.2. 1-Propanoyloxy-2-(6-methoxy-2-naphthyl)propane 4b. A white solid; mp 60–62 °C; IR (KBr): 2977, 1733, 1631, 1606, 1506, 1460, 1418, 1392, 1379, 1369, 1267, 1233, 1181, 1164, 1030, 897, 817, 671 cm^{-1}; ^1H NMR (200 MHz): δ 1.07 (3H, t, $J = 7.5$ Hz, CH_3), 1.40 (3H, d, $J = 7.0$ Hz, CH_3), 2.27 (2H, q, $J = 7.5$ Hz, $COCH_2$), 3.17–3.27 (1H, m, $CHCH_3$), 3.89 (3H, s, OCH_3), 4.24 (2H, ddd, $J = 18.3$, 10.5, and 7.2 Hz, OCH_2), 7.10 (1H, s, Ar–H), 7.13 (1H, dd, $J = 2.4$ and 8.2 Hz, Ar–H), 7.32 (1H, dd, $J = 8.45$ and 1.67 Hz, Ar–H), 7.58 (1H, d, $J = 8.4$ Hz, Ar–H), 7.73 (2H, d, $J = 8.2$ Hz, Ar–H); ^{13}C NMR (50 MHz): δ 9.18, 18.2, 27.6, 38.9, 55.3, 69.3, 105.6, 118.9, 125.6, 126.3, 126.9, 129.1, 129.2, 133.5, 138.4, 157.5, 174.5; MS (*m*/*z*) (%): 272 (63), 171 (100), 144 (92), 127 (44), 115 (39), 89 (4), 77 (8), 58 (8); Anal. Calcd for $C_{17}H_{20}O_3$: C, 74.97; H, 7.40. Found: C, 75.11; H, 7.43.

7.5.3. 1-Butanoyloxy-2-(6-methoxy-2-naphthyl)propane 4c. A white solid; mp 37 °C; IR (KBr): 2976, 1734, 1506, 1486, 1458, 1392, 1368, 1267, 1233, 1182, 1163, 1140, 1083, 1030, 872, 817, 670 cm^{-1}; ^1H NMR (200 MHz): δ 1.07 (3H, t, $J = 7.5$ Hz, CH_3), 1.35 (3H, d, $J = 7.0$ Hz, CH_3CH), 1.42–1.46 (2H, m, CH_2), 2.26 (2H, t, $J = 7.5$ Hz, $COCH_2$), 3.24 (1H, q, $J = 7.0$ Hz, CH_3CH), 3.88 (3H, s, OCH_3), 4.23 (2H, ddd, $J = 18.2$, 10.8, and 7.2 Hz, OCH_2), 7.1 (1H, s, Ar–H), 7.12 (1H, d, $J = 8.0$ Hz, Ar–H), 7.32 (1H, dd, $J = 1.4$ and 8.4 Hz, Ar–H), 7.57 (1H, s, Ar–H), 7.68 (2H, d, $J = 8.3$ Hz, Ar–H); ^{13}C NMR (50 MHz): δ 13.2, 18.2, 27.6, 38.9, 55.3, 69.3, 105.6, 118.9, 125.6, 126.3, 127.0, 129.0, 129.2, 133.5, 138.4, 157.5, 174.5; MS (*m*/*z*) (%): 286 (4), 198 (100), 185 (33), 170 (10), 153 (7), 141 (10), 115 (7), 45 (26); Anal. Calcd for $C_{18}H_{22}O_3$: C, 75.49; H, 7.743. Found: C, 75.92; H, 7.77.

7.5.4. 1-Octanoyloxy-2-(6-methoxy 2-naphthyl)propane 4d. A white solid; mp 24 °C; IR (KBr): 2967, 1731, 1634, 1607, 1506, 1485, 1463, 1418, 1319, 1266, 1106, 1033, 961, 886, 851, 811, 674 cm^{-1}; ^1H NMR (200 MHz): δ 0.85 (3H, t, $J = 6.8$ Hz, CH_3), 1.20–1.27 (8H, m, $4 \times CH_2$), 1.36 (3H, d, $J = 7.0$ Hz, CH_3CH), 1.50–1.56 (2H, m, CH_2), 2.24 (2H, t, $J = 7.4$ Hz, $COCH_2$), 3.17–3.27 (1H, m, $CHCH_3$), 3.89 (3H, s, OCH_3), 4.24 (2H, ddd, $J = 20.2$, 10.8, and 7.2 Hz, CH_2CH), 7.10 (1H, s, Ar–H), 7.13 (1H, dd, $J = 2.3$ and 8.5 Hz, Ar–H), 7.33 (1H, dd, $J = 1.5$ and 8.5 Hz, Ar–H), 7.58 (1H, s, Ar–H), 7.68 (2H, d, $J = 8.4$ Hz, Ar–H); ^{13}C NMR (50 MHz): δ 14.1, 18.2, 22.6, 25.0, 28.9, 29.1, 31.7, 34.4, 38.9, 55.3, 69.2, 105.6, 118.9, 125.6, 126.3, 127.0, 129.1, 129.2, 133.6, 138.41, 157.5, 173.9; MS (*m*/*z*) (%): 342 (2), 199 (100), 186 (19), 155 (3), 141 (4), 116 (3), 58 (7); Anal. Calcd for $C_{22}H_{30}O_3$: C, 77.15; H, 8.828. Found: C, 77.86; H, 8.833.

7.6. Preparation of compounds 2-(5-bromo-6-methoxy-2-naphthyl)propanoic acid 5, 4-(2-methylpropyl)-α-methylphenylacetic acid 6, and 2-(2-naphthyloxy)propanoic acid 7

Compound **5** was received as a gift sample from an Indian Pharma company while commercially available compound **6** was used directly for the preparation of its alkyl esters. Compound **7** was prepared by refluxing an acetone solution of β-naphthol (15 g, 104 mmol, 160 mL) and 2-bromopropanoic acid (18 mL, 120 mmol) for 46 h. After completion of reaction it was concentrated in vacuo followed by dilution with dilute HCl to pH 2. The contents were extracted with ethyl acetate (4×100 mL), the organic layer washed with water (2×50 mL) dried over sodium sulfate, and concentrated to give crude product, which on purification by cc over silica gel and elution with pet. ether/ethyl acetate (4:1) afforded **7** (20.4 g, 94.5 mmol, 90%).

7.6.1. 2-(5-Bromo-6-methoxy-2-naphthyl)propanoic acid 5. A white solid; mp 177–179 °C; IR (KBr): 3320,

2973, 2942, 1701, 1627, 1598, 1555, 1495, 1480, 1457, 1440, 1414, 1378, 1352, 1335, 1273, 1252, 1229, 1200, 1181, 1158, 1061, 973, 820, 800 cm^{-1}; ^1H NMR (200 MHz): δ 1.56 (3H, d, $J = 6.6$ Hz, CH_3), 3.9 (1H, q, $J = 7.2$ Hz, CH), 3.99 (3H, s, OCH_3), 7.22 (1H, d, $J = 8.8$ Hz, Ar–*H*), 7.50 (1H, d, $J = 8.4$ Hz, Ar–*H*), 7.66 (1H, s, Ar–*H*), 7.72 (1H, d, $J = 8.8$ Hz, Ar–*H*), 8.16 (1H, d, $J = 8.3$ Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 18.4, 45.3, 57.1, 108.5, 114.0, 126.4, 126.6, 127.2, 127.8, 129.0, 132.4, 136.8, 153.8, 177.3; MS (*m*/*z*) (%): 308 (M−1) (30), 278 (6), 264 (100), 227 (3), 197 (9), 183 (35), 169 (29), 153 (40); Anal. Calcd for $C_{14}H_{13}BrO_3$: C, 54.57; H, 4.238. Found: C, 54.940; H, 4.246.

7.6.2. 4-(2-Methylpropyl)-α-methylphenylacetic acid 6. A white solid; mp 75 °C; IR (KBr): 3210, 2956, 1721, 1508, 1461, 1420, 1380, 1365, 1321, 1268, 1230, 1183, 1123, 1092, 1067, 1008, 969, 935, 900, 866, 834, 820, 779, 690 cm^{-1}; ^1H NMR (200 MHz): δ 0.89 (6H, d, $J = 6.5$ Hz, $2 \times CH_3$), 1.49 (3H, d, $J = 7.8$ Hz, CH_3), 1.77–1.90 (1H, m, CH), 2.44 (2H, d, $J = 7.0$ Hz, $CHCH_2$), 3.70 (1H, q, $J = 7.0$ Hz, CH_3CH), 7.1 (2H, d, $J = 8.0$ Hz, Ar–*H*), 7.22 (2H, d, $J = 8.0$ Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 18.1, 22.4, 30.2, 45.0, 45.0, 127.3, 129.4, 137.0, 140.9, 181.2; MS (*m*/*z*) (%): 206 (47), 163 (100), 119 (55), 107 (51), 91 (82), 77 (13); Anal. Calcd for $C_{13}H_{18}O_2$: C, 75.69; H, 8.794. Found: C, 75.81; H, 8.801.

7.6.3. 2-(2-Naphthyloxy)propanoic acid 7. A white solid; mp114 °C; IR (KBr): 3651, 3060, 2988, 1705, 1629, 1599, 1510, 1467, 1421, 1391, 1370, 1359, 1335, 1312, 1257, 1244, 1217, 1184, 1139, 1121, 1099, 1048, 1020, 971, 837, 813, 745, 681 cm^{-1}; ^1H NMR (200 MHz): δ 1.70 (3H, d, $J = 6.8$ Hz, CH_3), 4.92 (1H, q, $J = 6.8$ Hz, CH), 7.07 (1H, d, $J = 1.6$ Hz, Ar–*H*), 7.13 (1H, dd, $J = 8.9$ and 2.4 Hz, Ar–*H*), 7.30–7.46 (2H, m, Ar–*H*), 7.73 (1H, d, $J = 8.1$ Hz, Ar–*H*), 7.70 (2H, d, $J = 8.5$ Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 18.5, 72.1 107.9, 118.8, 124.2, 126.6, 126.9, 127.7, 129.5, 129.9, 134.3, 155.2, 177.9; MS (*m*/*z*) (%): 216 (100), 202 (10), 194 (5), 173 (5), 159 (16), 148 (3), 121 (3); Found: M^+, 216.2333 $C_{13}H_{12}O_3$ requires M 216.2348.

7.7. General method for the preparation of alkyl esters of 2-(5-bromo-6-methoxy-2-naphthyl)propanoic acid 5, 4-(2-methylpropyl)-α-methylphenyl acetic acid 6, and 2-(2-naphthyloxy)propanoic acid 7: preparation of 5a–c, 6a–c, and 7a–c

The alkyl ester derivatives of **5**–**7** were prepared by refluxing a benzene solution of a mixture of the respective carboxylic acid (30 mmol), thionylchloride (35 mmol) in the presence of catalytic amounts of pyridine (0.5 mL) for 2–3 h. The contents were concentrated and the resulting acid chloride allowed to react with dry alcohols (methanol/ethanol/*n*-butanol) in dichloromethane. After the usual workup, the alkyl esters were purified by chromatography over silica gel using *n*-hexane/ethyl acetate in increasing proportion as eluent to give following esters, respectively, **5a** (3.1 g, 9.6 mmol, 95.6%), **5b** (3.18 g, 9.4 mmol, 90%), **5c** (3.44 g, 9.4 mmol, 93.8%); **6a** (2.18 g, 9.9 mmol, 94.7%), **6b** (2.26 g, 9.7 mmol, 93.4%), **6c** (2.51 g, 9.6 mmol, 92.3%); **7a** (2.10 g, 9.1 mmol, 95.4%), **7b** (2.2 g, 9.0 mmol, 94.9%), and **7c** (2.50 g, 9.2 mmol, 95.8%).

7.7.1. Methyl 2-(5-bromo-6-methoxy-2-naphthyl)propanoic acid 5a. A white solid; mp 72–73 °C; IR (KBr): 3068, 1731, 1647, 1629, 1597, 1557, 1494, 1458, 1440, 1376, 1356, 1340, 1275, 1252, 1213, 1180, 1140, 1090, 68, 944, 912, 881, 872, 851, 823, 801, 762, 750 cm^{-1}; ^1H NMR (200 MHz): δ 1.55 (3H, d, $J = 7.1$ Hz, CH_3), 3.63 (3H, s, OCH_3), 3.84 (1H, q, $J = 7.0$ Hz, CH), 3.97 (3H, s, OCH_3), 7.17 (1H, d, $J = 9.0$ Hz, Ar–*H*), 7.47 (1H, dd, $J = 1.5$ and 8.8 Hz, Ar–*H*), 7.61 (1H, s, Ar–*H*), 7.70 (1H, d, $J = 9.0$ Hz, Ar–*H*), 8.15 (H, d, $J = 8.8$ Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 18.5, 45.1, 52.1, 57.0, 108.5, 113.9, 126.3, 126.7, 127.7, 128.9, 129.8, 132.3, 136.5, 153.8, 174.8; MS (*m*/*z*) (%): 324 (M+1) (13), 322 (37), 266 (13), 265 (95), 264 (9), 263 (100), 184 (65), 169 (19), 153 (18), 141 (40), 139 (17), 115 (20), 64 (12); Anal. Calcd for $C_{15}H_{15}BrO_3$: C, 55.74; H, 4.677. Found: C, 56.04; H, 4.683.

7.7.2. Ethyl 2-(5-bromo-6-methoxy-2-naphthyl)propanoic acid 5b. A white solid; mp 69–70 °C; IR (KBr): 2976, 1727, 1628, 1599, 1497, 1479, 1451, 1405, 1375, 1354, 1336, 1325, 1273, 1253, 1226, 1178, 1157, 1070, 975, 897, 856, 800, 762, 753, 690, 662, 627 cm^{-1}; ^1H NMR (200 MHz): δ 1.40 (3H, t, $J = 7.1$ Hz, CH_3), 1.55 (3H, d, $J = 7.1$ Hz, CH_3CH), 3.83 (1H, q, $J = 7.2$ Hz, CH), 3.94 (3H, s, OCH_3), 4.10 (2H, q, $J = 7.1$ Hz, OCH_2), 7.18 (1H, d, $J = 9.0$ Hz, Ar–*H*), 7.54 (1H, d, $J = 8.9$ Hz, Ar–*H*), 7.65 (1H, s, Ar–*H*), 7.72 (1H, d, $J = 9.0$ Hz, Ar–*H*), 8.15 (1H, d, $J = 8.8$ Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 14.2, 18.5, 45.3, 57.0, 60.9, 108.5, 113.9, 126.2, 126.6, 127.7, 128.9, 129.8, 132.3, 136.6, 153.7, 174.4; MS (*m*/*z*) (%) 338 (M+1) (37), 336 (38), 265 (100), 263 (100), 184 (48), 169 (16), 153 (16), 141 (33), 139 (15), 115 (15); Anal. Calcd for $C_{16}H_{17}BrO_3$: C, 56.98; H, 5.081. Found: C, 56.83; H, 5.079.

7.7.3. Butyl 2-(5-bromo-6-methoxy-2-naphthyl)propanoic acid 5c. A white solid; mp 55–56 °C; IR (KBr): 2960, 1731, 1629, 1601, 1558, 1496, 1481, 1461, 1377, 1355, 1327, 1273, 1252, 1211, 1179, 1117, 1067, 1024, 975, 942, 920, 888, 862, 824, 800 cm^{-1}; ^1H NMR (200 MHz): δ 0.82 (3H, t, $J = 7.3$ Hz, CH_3), 1.16–1.31 (2H, m, CH_2), 1.45–1.57 (2H, m, CH_2CH_2), 1.55 (3H, d, $J = 7.2$ Hz, CH_3CH), 3.84 (1H, q, $J = 7.1$ Hz, CH_3CH), 3.98 (3H, s, OCH_3), 4.05 (2H, t, $J = 6.5$ Hz, OCH_2), 7.20 (1H, d, $J = 9.0$ Hz, Ar–*H*), 7.45 (1H, dd, $J = 1.5$ and 8.4 Hz, Ar–*H*), 7.65 (1H, s, Ar–*H*), 7.72 (1H, d, $J = 9.0$ Hz, Ar–*H*), and 8.16 (1H, d, $J = 8.8$ Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 13.7, 18.5, 19.1, 30.6, 45.3, 57.1, 64.8, 108.5, 113.9, 126.2, 126.6, 127.8, 128.8, 129.8, 132.3, 136.7, 153.7, 174.5; MS (*m*/*z*) (%): 366 (M+1) (30), 364 (3), 265 (100), 263 (100), 184 (38), 169 (12), 153 (12), 141 (23), 115 (9), 58 (7); Anal. Calcd for $C_{18}H_{21}BrO_3$: C, 58.13; H, 5.452. Found: C, 58.77; H, 5.449.

7.7.4. Methyl 4-(2-methylpropyl)-α-methylphenylacetic acid 6a. A colorless liquid; IR (neat): 2954, 2869,

1740, 1613, 1512, 1459, 1434, 1376, 1366, 1334, 1249, 1206, 1165, 1122, 1095, 1070, 1022, 1010, 969, 921, 859, 800 cm^{-1}; ^1H NMR (200 MHz): δ 0.94 (6H, d, $J = 6.6$ Hz, $2 \times CH_3$), 1.52 (3H, d, $J = 7.2$ Hz, CH_3), 1.32–1.45 (1H, m, $CHCH_2$), 2.49 (2H, d, $J = 7.2$ Hz, CH_2), 3.66 (3H, s, $COOCH_3$), 3.73 (1H, q, $J = 7.0$ Hz, CH_3CH), 7.1 (2H, d, $J = 8.0$ Hz, Ar–*H*), 7.22 (2H, d, $J = 8.0$ Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 18.6, 22.4, 30.2, 45.0, 45.1, 51.8, 127.1, 129.3, 137.8, 140.5, 175.1; MS (*m*/*z*) (%): 220 (37), 161 (100), 121 (29), 117 (64), 105 (24), 91 (58), 77 (18), 60 (20); Anal. Calcd for $C_{14}H_{20}O_2$: C, 76.32; H, 9.149. Found: C, 76.74; H, 9.143.

7.7.5. Ethyl 4-(2-methylpropyl)-α-methylphenylacetic acid 6b. A colorless liquid; IR (neat): 2956, 2933, 1735, 1512, 1464, 1421, 1367, 1330, 1245, 1166, 1095, 1072, 1024, 848, 799, 728 cm^{-1}; ^1H NMR (200 MHz): δ 0.92 (6H, d, $J = 6.6$ Hz, $2 \times CH_3$), 1.22 (3H, t, $J = 7.1$ Hz, CH_3CH_2), 1.50 (3H, d, $J = 7.16$ Hz, CH_3CH), 1.30–1.44 (1H, m, $CHCH_2$), 2.49 (2H, d, $J = 7.1$ Hz, $CHCH_2$), 3.70 (1H, q, $J = 7.0$ Hz, CH_3CH), 4.13 (2H, q, $J = 7.1$ Hz, CH_2O), 7.11 (2H, d, $J = 8.1$ Hz, Ar–*H*), 7.23 (2H, d, $J = 8.1$ Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 14.1, 18.6, 22.4, 30.2, 45.1, 45.2, 60.6, 127.1, 129.3, 137.9, 140.4, 174.7; MS (*m*/*z*) (%): 234 (52), 162 (100), 119 (93), 115 (34), 105 (46), 91 (85), 77 (25), 58 (32); Anal. Calcd for $C_{15}H_{22}O_2$: C, 76.88; H, 9.462. Found: C, 77.11; H, 9.471.

7.7.6. Butyl 4-(2-methylpropyl)-α-methylphenylacetic acid 6c. A colorless liquid; IR (neat): 2958, 2933, 1737, 1512, 1464, 1421, 1383, 1331, 1243, 1201, 1167, 1120, 1093, 1071, 1022, 941, 817, 798, 726 cm^{-1}; ^1H NMR (200 MHz): δ 0.88 (3H, t, $J = 7.0$ Hz, CH_3), 0.92 (6H, d, $J = 6.6$ Hz, $2 \times CH_3$), 1.24–1.35 (2H, m, CH_2), 1.42–1.62 (3H, m, CH_2 and CH), 1.50 (3H, d, $J = 7.2$ Hz, CH_3CH), 2.42 (2H, d, $J = 7.2$ Hz, CH_2CH), 3.71 (1H, q, $J = 7.2$ Hz, CH_3CH), 4.09 (2H, t, $J = 6.5$ Hz, CH_2O), 7.21 (2H, d, $J = 8.1$ Hz, Ar–*H*), 7.24 (2H, d, $J = 8.1$ Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 13.6, 18.4, 19.3, 20.3, 30.6, 45.1, 45.2, 64.4, 127.1, 129.2, 137.9, 140.4, 174.7; MS (*m*/*z*) (%): 262 (27), 219 (13), 162 (39), 161 (100), 145 (5), 119 (52), 105 (17), 91 (42), 77 (11), 58 (72), 45 (44), 43 (90); Anal. Calcd for $C_{17}H_{26}O_2$: C, 77.81; H, 9.987. Found: C, 77.98; H, 9.982.

7.7.7. Methyl 2-(2-naphthyloxy)propanoic acid 7a. A creamish solid; mp 55–56 °C; IR (KBr): 2954, 1759, 1630, 1599, 1510, 1468, 1446, 1390, 1375, 1257, 1133, 1096, 1052, 981, 839, 812, 749 cm^{-1}; ^1H NMR (200 MHz): δ 1.67 (3H, d, $J = 6.8$ Hz, CH_3), 3.74 (3H, s, $COOCH_3$), 4.92 (1H, q, $J = 6.8$ Hz, CH), 7.04 (1H, s, Ar–*H*), 7.17 (1H, dd, $J = 2.2$ and 8.88 Hz, Ar–*H*), 7.32–7.42 (2H, m, Ar–*H*), 7.69 (1H, d, $J = 8.0$ Hz, Ar–*H*), 7.83 (2H, d, $J = 8.8$ Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 18.7, 52.4, 72.6, 107.7, 118.9, 124.1, 126.5, 126.9, 127.7, 129.4, 129.8, 134.4, 155.5, 172.7; MS (*m*/*z*) (%): 230 (67), 171 (65), 144 (100), 127 (39), 115 (60), 60 (12); Anal. Calcd for $C_{14}H_{14}O_3$: C, 73.07; H, 6.117. Found: C, 73.66; H, 6.123.

7.7.8. Ethyl 2-(2-naphthyloxy)propanoic acid 7b. A creamish solid; mp 52–53 °C; IR (KBr): 2985, 1753, 1630, 1600, 1510, 1468, 1445, 1390, 1257, 1216, 1182, 1130, 1095, 1017, 971, 839, 812, 749 cm^{-1}; ^1H NMR (200 MHz): δ 1.23 (3H, t, $J = 7.1$ Hz, CH_3), 1.67 (3H, d, $J = 6.8$ Hz, CH_3CH), 4.25 (2H, q, $J = 7.2$ Hz, CH_2O), 4.88 (1H, q, $J = 6.8$ Hz, CH_3CH), 7.05 (1H, d, $J = 2.4$ Hz, Ar–*H*), 7.22 (1H, dd, $J = 2.5$ and 8.9 Hz, Ar–*H*), 7.29–7.46 (2H, m, Ar–*H*), 7.63 (1H, d, $J = 8.0$ Hz, Ar–*H*), 7.75 (2H, d, $J = 8.8$ Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 14.2, 18.6, 61.3, 72.7, 107.8, 118.9, 124.0, 126.5, 126.9, 127.7, 129.7, 129.9, 134.3, 155.6, 172.3; MS (*m*/*z*) (%): 244 (61), 171 (88), 144 (100), 127 (40), 115 (39), 77 (7); Anal. Calcd for $C_{15}H_{16}O_3$: C, 73.75; H, 6.601. Found: C, 74.00; H, 6.609.

7.7.9. Butyl 2-(2-naphthyloxy)propanoic acid 7c. A viscous liquid; IR (neat): 2960, 1755, 1630, 1600, 1511, 1408, 390, 1356, 1257, 1216, 1182, 1095, 1048, 1019, 972, 944, 839, 811, 748 cm^{-1}; ^1H NMR (200 MHz): δ 0.83 (3H, t, $J = 7.0$ Hz, CH_3), 1.18–1.31 (2H, m, CH_2CH_3), 1.46–1.66 (2H, m, CH_2), 1.68 (3H, d, $J = 6.9$ Hz, CH_3CH), 4.19 (2H, t, $J = 6.5$ Hz, OCH_2CH_2), 4.92 (1H, q, $J = 6.8$ Hz, CH_3–CHO), 7.05 (1H, br s, Ar–*H*), 7.20 (1H, dd, $J = 2.3$ and 8.9 Hz, Ar–*H*), 7.29–7.45 (2H, m, Ar–*H*), 7.68 (1H, d, $J = 8.1$ Hz, Ar–*H*), 7.74 (2H, d, $J = 8.7$ Hz, Ar–*H*); ^{13}C NMR (50 MHz): δ 13.6, 18.6, 19.0, 30.6, 65.2, 72.7, 107.7, 118.9, 124.0, 126.5, 126.9, 127.7, 129.4, 134.4, 155.6, 172.4; MS (*m*/*z*) (%): 272 (62), 171 (100), 144 (97), 127 (43), 115 (36), 77 (7), 43 (16); Anal. Calcd for $C_{17}H_{20}O_3$: C, 74.97; H, 7.401. Found: C, 75.21; H, 7.394.

7.8. General method for the preparation of alkyl esters of 2-(allylthio) propanoic acid 8; preparation of 8a–c

A mixture of thiolactic acid (15 mL, 168 mmol), allyl bromide (15.6 mL, 180 mmol) in acetone (200 mL), and anhydrous potassium carbonate (15 g) was refluxed for 4 h. After the completion of reaction, the contents were concentrated and then diluted with ice-water (200 mL). The reaction mixture was extracted with dichloromethane (3×100 mL). The aqueous portion after acidification with dil. HCl was extracted with ethyl acetate (4×100 mL). The organic layer was washed with water, dried over anhydrous sodium sulfate, and concentrated in vacuo to give crude 2-allylthiolactic acid as a liquid (16.2 g). The crude acid was dissolved in dichloromethane (150 mL), and thionyl chloride (11 mL, >150 mmol) and pyridine (1.6 mL, 20 mmol) were added and the contents refluxed for 50 min, concentrated, and reconstituted in dichloromethane (90 mL) to divide into three equal parts. Dry methanol, ethanol, and *n*-butanol (5 mL each) were separately added to each part. Each of the reaction mixtures were heated at 60–70 °C for 10 min, concentrated and after usual workup, purification by cc over silica gel using *n*-hexane/ethyl acetate (99:1) as eluant to give the respective esters **8a** (6.8 g, 42.5 mmol, 78.6%), **8b** (7.3 g, 41.9 mmol, 74.9%), and **8c** (8.2 g, 40.6 mmol, 72.5%).

7.8.1. Methyl 2-(allylthio)propanoic acid 8a. A light yellowish liquid; IR (neat): 2925, 1736, 1632, 1402, 1255, 1156, 1021, 923, 794 cm^{-1}; ^1H NMR (200 MHz): δ 1.39 (3H, d, $J = 7.3$ Hz, CH_3), 3.1–3.28 (2H, m, CH_2S), 3.29 (1H, q, $J = 7.2$ Hz, $CHCH_3$), 3.70 (3H, s, $COOCH_3$), 5.06–5.7 (2H, m, CH_2=CH), 5.66–5.86 (1H, m, =CH); ^{13}C NMR (50 MHz): δ 17.0, 34.6, 39.7, 52.1, 117.8, 133.5, 173.6; MS (*m*/*z*) (%): 161 (M+1) (33), 137 (20), 128 (12), 117 (10), 115 (10), 101 (29), 99 (11), 88 (100), 78 (11), 75 (45), 74 (34), 68 (22); Found: M^+, 160.2366 $C_7H_{12}O_2S$ requires M 160.2358.

7.8.2. Ethyl 2-(allylthio)propanoic acid 8b. A yellowish liquid; IR (neat): 2980, 2934, 1734, 1635, 1448, 1428, 1375, 1324, 1257, 1233, 1160, 1096, 1062, 1022, 990, 921, 859, 741 cm^{-1}; ^1H NMR (200 MHz): δ 1.25 (3H, t, $J = 7.1$ Hz, CH_3), 1.38 (3H, d, $J = 7.2$ Hz, $CHCH_3$), 3.12–3.38 (3H, m, CH_2 and CH), 4.15 (2H, q, $J = 7.0$ Hz, OCH_2), 5.05–5.16 (2H, m, CH_2=CH), 5.70–5.78 (1H, m, =CH); ^{13}C NMR (50 MHz): δ 14.2, 17.0, 34.6, 39.8, 61.0, 117.8, 133.5, 173.2; MS (*m*/*z*) (%): 174 (30), 102 (100), 101 (40), 85 (5), 75 (8), 74 (63), 67 (28), 61 (15), 60 (94), 57 (12), 47 (46), 43 (96); Found: M^+, 174.2632 $C_8H_{14}O_2S$ requires M 174.2626.

7.8.3. *n*-Butyl 2-(allylthio)propanoic acid 8c. A light brownish liquid; IR (neat): 2960, 2934, 1733, 1455, 1377, 1322, 1256, 1164, 1066, 1018, 996, 938 cm^{-1}; ^1H NMR (200 MHz): δ 0.94 (3H, t, $J = 7.2$ Hz, CH_3), 1.42 (3H, d, $J = 7.2$ Hz, CH_3CH), 1.35–1.46 (2H, m, CH_2CH_3), 1.56–1.68 (2H, m, CH_2CH_2), 3.21–3.42 (3H, m, CH_2S and CH), 4.14 (2H, t, $J = 6.5$ Hz, OCH_2), 5.10–5.22 (2H, m, CH_2=CH), 5.70–5.86 (1H, m, CH_2=CH); ^{13}C NMR (50 MHz): δ 13.7, 17.1, 19.2, 30.7, 34.6, 39.9, 64.9, 117.8, 133.6, 173.3; MS (*m*/*z*) (%): 204 (M+2) (16), 130 (18), 101 (33), 85 (4), 75 (16), 74 (100), 67 (17), 61 (10), 60 (55), 58 (21), 47 (22), 43 (89); Found: M^+, 202.3171 $C_{10}H_{18}O_2$ requires M 202.3162.

7.9. General method for the preparation of alkyl esters of 2-(*n*-butylthio)propanoic acid 9; preparation of 9a–c

The title compound was prepared by refluxing an acetone solution (200 mL) of thiolactic acid (15 mL, 168 mmol) and *n*-butyl bromide (19.5, 180 mmol) in the presence of potassium carbonate (15 g) for 50 h and the resulting crude acid that was obtained after the usual workup was subjected to esterification by the procedure as described in Section 7.8 to give **9a** (7.2 g, 40.9 mmol, 73%), **9b** (7.5 g, 39.5 mmol, 70%), and **9c** (8.92 g, 40.9 mmol, 73.1%).

7.9.1. Methyl 2-(*n*-butylthio)propanoic acid 9a. A yellowish liquid; IR (neat): 2956, 1738, 1454, 1374, 1333, 1260, 1062, 1009, 995, 854, 776, 695 cm^{-1}. ^1H NMR (200 MHz): δ 0.92 (3H, t, $J = 7.2$ Hz, CH_3), 1.34–1.61 (4H, m, $2 \times CH_2$), 1.40 (3H, d, $J = 7.2$ Hz, CH_3CH), 2.58–2.66 (2H, m, CH_2S), 3.41 (1H, q, $J = 7.1$ Hz, CH_3CH), 3.76 (3H, s, $COOCH_3$); ^{13}C NMR (50 MHz): δ 13.5, 17.1, 21.4, 30.9, 31.3, 40.8, 52.0, 173.5; MS (*m*/*z*) (%): 176 (27), 147 (1), 137 (25), 130 (12), 117 (16), 109 (14), 89 (59), 75 (100), 74 (19.4), 57 (43); Found: M^+, 176.2791 $C_8H_{16}O_2S$ requires M 176.2784.

7.9.2. Ethyl 2-(*n*-butylthio)propanoic acid 9b. A yellowish liquid; IR (neat): 2961, 2933, 1733, 1450, 1373, 1321, 1255, 1221, 1160, 1095, 1063, 1021, 897 cm^{-1}; ^1H NMR (200 MHz): δ 0.90 (3H, t, $J = 7.1$ Hz, CH_3), 1.28 (3H, t, $J = 7.0$ Hz, CH_3CH_2O), 1.42 (3H, d, $J = 7.2$ Hz, CH_3CH), 1.34–1.63 (4H, m, $2 \times CH_2$), 2.62 (2H, m, SCH_2), 3.38 (1H, q, $J = 7.2$ Hz, CH_3CH), 4.14 (2H, q, $J = 7.1$ Hz, OCH_2); ^{13}C NMR (50 MHz): δ 13.7, 14.2, 17.2, 22.0, 31.0, 31.4, 41.7, 61.0, 173.3; MS (*m*/*z*) (%): 190 (23), 134 (2), 117 (33), 102 (100), 91 (4), 89 (6), 77 (5), 75 (88), 74 (43), 61 (55), 57 (16); Found: M^+, 190.3055 $C_9H_{18}O_2S$ requires M 190.3052.

7.9.3. Butyl 2-(*n*-butylthio)propanoic acid 9c. A yellowish liquid; IR (neat): 2960, 2933, 1733, 1453, 1376, 1321, 1253, 1219, 1163, 1070, 1018 cm^{-1}; ^1H NMR (200 MHz): δ 0.84 (3H, t, $J = 7.2$ Hz, CH_3), 1.21 (3H, t, $J = 7.1$ Hz, CH_2CH_3), 1.35 (3H, d, $J = 7.1$ Hz, $CHCH_3$), 1.27–1.63 (8H, m, $4 \times CH_2$), 2.55 (2H, m, CH_2S), 3.29 (1H, q, $J = 7.1$ Hz, CH_3CH), 4.11 (2H, t, $J = 7.2$ Hz, OCH_2); ^{13}C NMR (50 MHz): δ 13.5, 13.6, 17.1, 18.8, 19.0, 21.9, 34.7, 41.1, 62.3, 64.9, 173.5; MS (*m*/*z*) (%): 218 (14), 130 (20), 117 (32), 89 (9), 74 (100), 62 (35), 59 (37), 56 (24), 47 (11), 43 (57); Found: M^+, 218.3597 $C_{11}H_{22}O_2S$ requires M 218.3588.

7.10. General method for the preparation of alkyl esters of 2-(phenylthio)propanoic acid 10; preparation of 10a–c

The title compound was obtained by stirring an acetone solution (200 mL) of thiolactic acid (15 mL, 168 mmol), bromobenzene (19 mL, 180 mmol), and potassium carbonate (15 g) at room temp till the completion of reaction. The crude acid obtained was directly esterified by the method as described in Section 7.8 above to give ester **10a** (16.3 g, 42 mmol, 80%), **10b** (8.3 g, 39.5 mmol, 70.4%), and **10c** (9.7, 40.7 mmol, 72.4%).

7.10.1. Methyl 2-(phenylthio)propanoic acid 10a. A yellowish liquid; IR (neat): 2989, 1736, 1583, 1475, 1438, 1375, 1329, 1260, 1228, 1192, 1161, 1067, 1025, 965, 854, 749, 691 cm^{-1}; ^1H NMR (200 MHz): δ 1.47 (3H, d, $J = 7.1$ Hz, CH_3), 3.66 (3H, s, $COOCH_3$), 3.79 (1H, q, $J = 7.1$ Hz, CH_3CH), 7.40–7.47 (5H, m, Ar–H); ^{13}C NMR (50 MHz): δ 17.5, 45.2, 52.3, 128.1, 128.9, 133.1, 173.0; MS (*m*/*z*) (%): 196 (46), 137 (100), 135 (17), 109 (45), 103 (7), 77 (11), 69 (11); Found: M^+, 196.2693 $C_{10}H_{12}O_2S$ requires M 196.2688.

7.10.2. Ethyl 2-(phenylthio)propanoic acid 10b. A yellowish liquid; IR (neat): 2981, 2934, 1733, 1584, 1476, 1440, 1374, 1323, 1256, 1225, 1095, 1067, 1024, 748, 692 cm^{-1}; ^1H NMR (200 MHz): δ 1.17 (3H, t, $J = 7.1$ Hz, CH_3), 1.48 (3H, d, $J = 7.1$ Hz, $CHCH_3$), 3.29 (1H, q, $J = 7.1$ Hz, CH), 4.13 (2H, q, $J = 7.1$ Hz, OCH_2), 7.18–7.26 and 7.40–7.49 (5H, m, Ar–H); ^{13}C NMR (50 MHz): δ 14.0, 17.4, 45.3, 61.2, 126.9, 128.9, 132.9, 133.9, 172.7; MS (*m*/*z*) (%): 210 (51), 137 (100), 135 (19), 109 (43), 103 (8), 77 (12), 65 (17), 60 (23), 52

(13), 47 (12); Found: M^+, 210.2962 $C_{11}H_{14}O_2S$ requires M 210.2956.

7.10.3. Butyl 2-(phenylthio)propanoic acid 10c. A yellowish liquid; IR (neat): 2982, 2933, 1733, 1584, 1475, 1440, 1374, 1322, 1256, 1225, 1160, 1095, 1066, 1024, 776, 748, 692 cm^{-1}; ^1H NMR (200 MHz): δ 0.86 (3H, t, $J = 7.1$ Hz, CH_3), 1.29 (3H, d, $J = 7.2$ Hz, $CHCH_3$), 1.34–1.60 (4H, m, $2 \times CH_2$), 3.20 (1H, q, $J = 7.2$ Hz, $CHCH_3$), 4.0 (2H, t, $J = 6.5$ Hz, OCH_2), 7.26–7.33 and 7.43–7.49 (5H, m, Ar–*H*); ^{13}C NMR (50 MHz): δ 13.7, 16.9, 30.1, 35.9, 40.5, 65.0, 127.1, 128.5, 129.0, 137.6, 173.2; MS (*m*/*z*) (%): 238 (32), 137 (100), 135 (13), 110 (9), 109 (29), 103 (5), 65 (11), 60 (15), 58 (7), 43 (17), 41 (11); Found: M^+, 238.2499 $C_{13}H_{18}O_2S$ requires M 238.2492.

7.11. General method for the preparation of alkyl esters of 2-(benzylthio)propanoic acid 11; preparation of 11a–c

The title compound was obtained by reacting an acetone solution (200 mL) of thiolactic acid (15 mL, 168 mmol), benzylbromide (21.5 mL, 180 mmol), and potassium carbonate (15 g) for 24 h. The crude acid obtained thus was directly esterified by the method described in Section 7.8 above to give the products **11a** (9.4 g, 44.7 mmol, 80%), **11b** (10.6 g, 47.3 mmol, 84.4%), and **11c** (11.7 g, 46.4 mmol, 81.25%).

7.11.1. Methyl 2-(benzylthio)propanoic acid 11a. A colorless liquid; IR (neat): 3029, 1736, 1602, 1495, 1453 cm^{-1}; ^1H NMR (200 MHz): δ 1.38 (3H, d, $J = 7.2$ Hz, CH_3), 3.31 (1H, q, $J = 7.1$ Hz, C*H*), 3.70 (3H, s, $COOCH_3$), 3.82 (2H, d, $J = 18.48$ Hz, CH_2Ph), 7.21–7.36 (5H, m, Ar–*H*); ^{13}C NMR (50 MHz): δ 16.9, 36.0, 40.3, 52.2, 127.2, 128.5, 129.1, 137.6, 173.5; MS (*m*/*z*) (%): 210 (3), 194 (39), 164 (5), 151 (4), 123 (35), 121 (3), 107 (9), 91 (100), 88 (6), 77 (5), 65 (12); Found: M^+, 210.2359 $C_{11}H_{14}O_2$ S requires M 210.2356.

7.11.2. Ethyl 2-(benzylthio)propanoic acid 11b. A yellowish liquid; IR (neat): 2980, 2934, 1733, 1602, 1495, 1453, 1375, 1323, 1258, 1222, 1159, 1093, 1065, 1023, 858, 768, 702 cm^{-1}; ^1H NMR (200 MHz): δ 1.25 (3H, t, $J = 7.1$ Hz, CH_3), 1.37 (3H, d, $J = 7.2$ Hz, $CHCH_3$), 3.27 (1H, q, $J = 7.2$ Hz, C*H*), 3.82 (2H, d, $J = 20.42$ Hz, $PhCH_2$), 4.16 (2H, q, $J = 7.2$ Hz, OCH_2), 7.20–7.36 (5H, m, Ar–*H*); ^{13}C NMR (50 MHz): δ 14.2, 16.9, 35.9, 40.4, 61.1, 127.1, 128.5, 129.0, 137.6, 173.0; MS (*m*/*z*) (%): 224 (14), 151 (13), 124 (14), 123 (100), 102 (52), 92 (19), 91 (94), 77 (11), 74 (44), 65 (37), 61 (12), 52 (11), 47 (55), 41 (18); Found: M^+, 224.3231 $C_{12}H_{16}O_2S$ requires M 224.3224.

7.11.3. Butyl 2-(benzylthio)propanoic acid 11c. A yellowish liquid: IR (KBr): 2960, 1733, 1602, 1494, 1454, 1377, 1323, 1257, 1164, 1069, 1028, 767, 701 cm^{-1}; ^1H NMR (200 MHz): δ 0.93 (3H, t, $J = 7.1$ Hz, CH_3), 1.38 (3H, d, $J = 7.2$ Hz, $CHCH_3$), 1.32–1.43 (2H, m, CH_2CH_3), 1.52–1.61 (2H, m, OCH_2CH_2), 3.27 (1H, q, $J = 7.2$ Hz, C*H*), 3.82 (2H, d, $J = 19.4$ Hz, $PhCH_2$), 4.12 (2H, t, $J = 6.5$ Hz, OCH_2), 7.21–7.36 (5H, m, Ar–*H*); ^{13}C NMR (50 MHz): δ 13.7, 16.9, 30.1, 35.9, 40.5, 65.0, 127.1, 128.5, 129.0, 137.6, 173.2; MS (*m*/*z*) (%): 252 (11), 151 (10), 130 (25), 124 (13), 123 (100), 117 (6), 92 (16), 91 (98), 77 (8), 75 (15), 74 (98), 61 (11), 58 (19), 57 (22), 43 (28); Found: M^+, 252.3766 $C_{14}H_{20}O_2S$ requires M 252.3760.

7.12. Preparation of (*R*)-2-(benzylthio)propanoic acid 11

A mixture of acetone solution of (*S*)-(−)-2-bromopropanoic acid (0.46 g, 3.0 mmol), benzylmercaptol (0.4 g, 3.2 mmol), and fused potassium carbonate (0.5 g) was stirred at 20 °C for 15 h. After completion of the reaction, the contents were filtered, the solid material washed with acetone (2×15 mL). The combined organic layer concentrated in vacuo, reconstituted in chloroform (30 mL) and the contents diluted with water and pH adjusted to 4, the organic layer separated, washed with water (2×10 mL), dried, concentrated, and cc over SiO_2 using *n*-hexane/EtOAc (9:1) as eluant gave *R*-2-(benzylthio)propanoic acid as a white solid (mp 66 °C) (0.51 g, 2.6 mmol, 86.7% yield) $[\alpha]_D^{25} = +130.1$ (*c* 1, $CHCl_3$).

7.13. General procedure for the hydrolysis of racemic acylates of primary and secondary alcohols

Racemic acylate of primary/secondary alcohol (~1 mmol) in sodium phosphate buffer (0.1 M) at pH 7 and specified temperature was stirred after the addition of crude enzyme in a specified ratio (as given in Tables 1–4). The pH of the reaction maintained by pH-stat using 0.05 M NaOH solution. The samples were analyzed on chiral HPLC after the extraction of the aliquots (25–50 μL) with HPLC grade ethyl acetate (100 μL), centrifugation at 8000*g* and filtration of the organic layer through 0.45 μm pore size filter. After the termination of the reaction, the contents were extracted with organic solvents, concentrated in vacuo, and separated on a silica gel column to get optically enriched ester and the alcohol.

7.14. Typical example of kinetic resolution of 1-acetoxy-(6-methoxynaphthyl)ethane 1a

In a typical experiment, a mixture of **1a** (250 mg, ⩾1 mmol) and TBE (190 mg) in sodium phosphate buffer (0.1 M, pH 7.0, 6 mL) was stirred at 20 °C and after completion of the reaction (16 h) and usual workup, the bio-products were separated on a silica gel column after elution with dichloromethane/methanol (19:1) to give **1a** (119 mg, 0.49 mmol), $[\alpha]_D^{25} = +41.6$ (*c* 0.56, $CHCl_3$) and **1** (52 mg, 0.27 mmol), $[\alpha]_D^{25} = -39.6$ (*c* 0.8, $CHCl_3$).

7.15. Typical example of kinetic resolution of 1-butanoyloxy-1-(3,4-methylenedioxyphenyl)ethane 2c

In a typical experiment, a mixture of **2c** (236 mg, 1 mmol) and PSL (60 mg) in sodium phosphate buffer (0.1 M, pH 7.0, 5 mL) was stirred at 20 °C and after completion of the reaction (16 h) and usual workup, the bio-products were separated on a silica gel column after elution with pet. ether/ethyl acetate (19:1) to give **2c** (110 mg, 0.46 mmol), $[\alpha]_D^{25} = -96.6$ (*c* 0.8, $CHCl_3$) and **2** (55 mg, 0.33 mmol), $[\alpha]_D^{25} = +55.4$ (*c* 1.0, $CHCl_3$).

7.16. Typical example of kinetic resolution of 1-acetoxy-1-(3,4-methylenedioxyphenyl)pentane 3a

In a typical experiment, a mixture of **3a** (250 mg, 1 mmol) and TBE (188 mg) in sodium phosphate buffer (0.1 M, pH 7.0, 6.5 mL) was stirred at 20 °C and after completion of the reaction (16 h) and usual workup, the bio-products separated on a silica gel column after elution with pet. ether/ethyl acetate (19:1) to give **3a** (133 mg, 0.53 mmol), $[\alpha]_D^{25} = +47.6$ (*c* 0.25, $CHCl_3$) and **3** (57 mg, 0.27 mmol), $[\alpha]_D^{25} = -61.9$ (*c* 0.54, $CHCl_3$).

7.17. General procedure for the hydrolysis of racemic alkyl esters of carboxylic acids

Suspension of racemic ester (⩽1 mmol) in sodium phosphate buffer (0.1 M) at a specified pH and temperature was stirred in the presence of a crude enzyme in a specified ratio (as given in Tables 5–7) and the pH maintained by a pH-stat using 0.1 M NaOH solution. Except for substrate **8**–**10a**–**c**, the progress of the reaction was monitored on chiral HPLC after the extraction of the aliquots (25–50 μL, acidified to pH 3 with dil. HCl) with HPLC grade ethyl acetate (100 μL), centrifugation at 8000*g* and filtration of the organic layer through 0.45 μm pore size filter. After completion of the reaction, the contents were adjusted to pH 3, extracted with ethyl acetate, concentrated in vacuo, and the bio-products separated on a silica gel column to give optically enriched alkyl ester and acid.

7.18. Typical example of kinetic resolution of methyl 2-(5-bromo-6-methoxy-2-naphthyl)propanoic acid 5a

In a typical experiment, a mixture of **5a** (140 mg, 0.5 mmol) and CCL (140 mg) in sodium phosphate buffer (pH 8.0, 10 mL) was stirred at 30 °C and after completion of the reaction (90 h) and usual workup, the bio-products were separated on a silica gel column after elution with a pet. ether/ethyl acetate to give optically enriched **5a** (62 mg, 0.19 mmol), $[\alpha]_D^{25} = -27.2$ (*c* 0.8, $CHCl_3$) and **5** (40 mg, 0.13 mmol), $[\alpha]_D^{25} = +42.5$ (*c* 1, $CHCl_3$).

7.19. Typical example of kinetic resolution of methyl 2-(benzylthio)propanoic acid 11a

In a typical experiment, a mixture of **11a** (210 mg, 1 mmol) and TBE (100 mg) in sodium phosphate buffer (pH 8.0, 4 mL) was stirred at 25 °C and after completion of the reaction (24 h) and usual workup, the bio-products were separated on a silica gel column after elution with pet. ether/ethyl acetate (90:10) to give optically enriched **11a** (85 mg, 0.4 mmol), $[\alpha]_D^{25} = -98.7$ (*c* 1, $CHCl_3$) and **11** (44 mg, 0.22 mmol), $[\alpha]_D^{25} = +129.1$ (*c* 0.9, $CHCl_3$).

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