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Synthesis and lipase-catalyzed resolution studies on novel (±)-2-(2-acetoxyethyl)-4-arylmethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates

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Abstract

Five novel methyl (±)-2-(2-acetoxyethyl)-4-arylmethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates have been synthesized and their lipase-catalyzed resolution via stereoselective deacetylation of acetoxyethyl moiety present in the molecule studied. It has been observed that Novozyme®-435 in THF efficiently catalyses the enantioselective deacetylation of these acetoxyethyl dihydrobenzoxazines leading to the formation of optically enriched methyl (+)-4-arylmethyl-2-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates. The biocatalytic reaction was found to be chemoselective alongwith being enantioselective, because the lipase exclusively catalyses the deesterification of the ester function derived from the alcoholic hydroxy moiety in the molecule over the one derived from the aromatic carboxylic acid group.

Keywords: Synthesis; Lipase; Resolution; 1,4-Benzoxazines

1. Introduction

The charter of chemical industries and chemical research, in general is changing today because of the pressing need to develop environmentally benign methodologies for the synthesis of well-defined molecules. It is at this juncture that nature's catalysts "enzymes" come into the picture [1,2]. The discovery that enzymes are not restricted to their natural role, i.e. can accept a variety of substrates and reaction conditions, and can work in organic media has proved to be a boon for synthetic organic chemists [3–6]. Further, applications of enzymes in organic solvents has minimized the use of gene engineering for production of enzymes of desired catalytic properties, because the selectivity of the biocatalysts can be tuned by using them in different solvents [7]. Among the different biocatalytic processes, lipase-catalyzed selective acylation/deacylation reactions represent an important class of enzymatic transformations in organic synthesis, which is mainly attributed to low cost of lipases and their wide tolerance towards a variety of reaction conditions and substrates [8,9]. In the recent past, we have demonstrated the use of lipases for carrying out regio-, chemo-, enantio-, diastereo- and prochiral selective acylation/deacylation reactions on a variety of substrates, such as alcohols, polyphenolics, acids, sugars, nucleosides, etc. [10–16].

Benzoxazines are a class of heterocyclic compounds which have a benzene nucleus fused to the oxazine ring. Compounds having 1,4-oxazine skeletons show interesting biological activities, such as psychotropic [17], antitumour [18], antimicrobial [19,20], antifungal [21,22], calcium/potassium antagonist [23–25], antihypertensive [26], analgesic [27], etc. The biological activities of 2,4-disubstituted 3-oxo-2*H*-1,4-benzoxazines have been a major subject of research, because they are widely abundant in nature as cyclic hydroxamic acids [28,29]. The presence of these hydroxamic acid derivatives in several crops, like maize, wheat and rye play an important role in the chemical defense against deleterious pests [28–36]. Some of these com-

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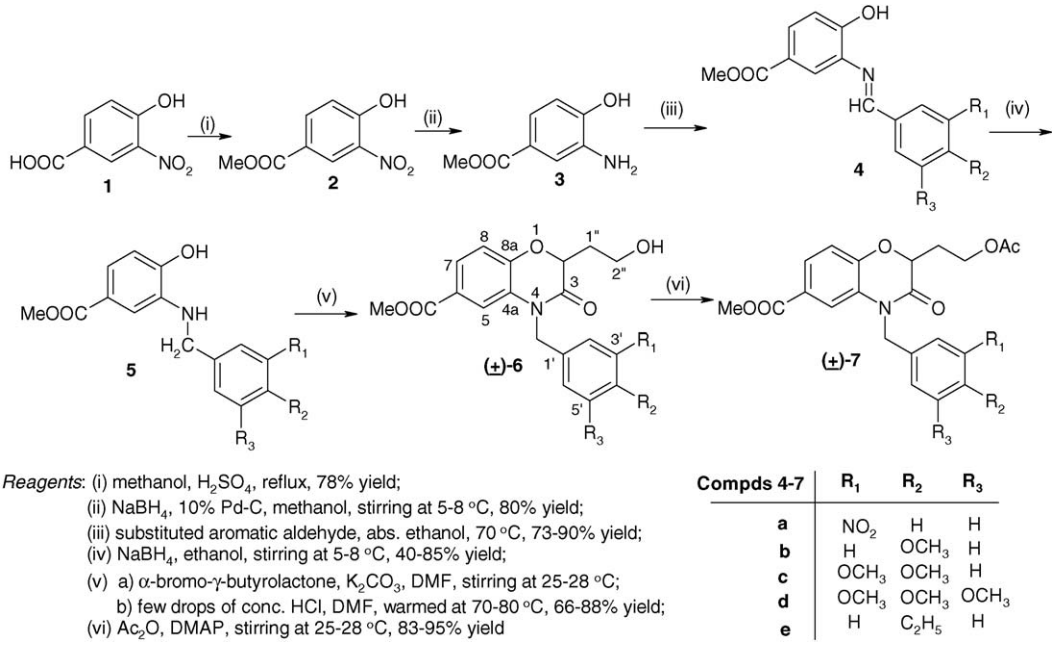


Compds 4-7	R_1	R_2	R_3
a	NO_2	H	H
b	H	OCH_3	H
c	OCH_3	OCH_3	H
d	OCH_3	OCH_3	OCH_3
e	H	C_2H_5	H

Scheme 1. Synthesis of methyl (±)-2-(2-acetoxyethyl)-4-arylmethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates.

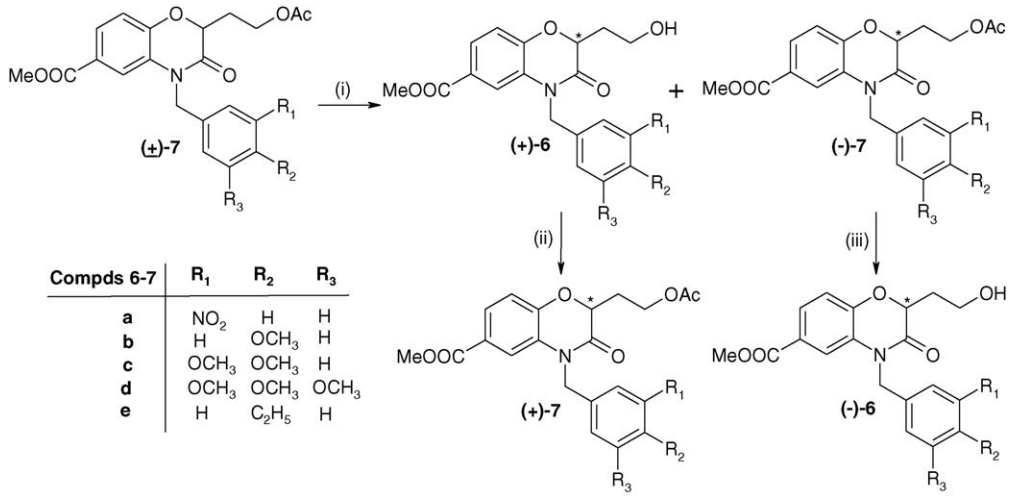
pounds also possess anti-inflammatory activity [37] together with other interesting biological activities, e.g. fibrinogen receptor antagonists and factor Xa inhibitors [38,39].

Various methods are known for the synthesis of 2-substituted 3-oxo-3,4-dihydo-2*H*-1,4-benzoxazines, however most of them lead to the formation of *racemic* products [40]. It is a well-established fact now that different enantiomers of a chiral compound react differently under physiological conditions and therefore exert different biological activities. Thus chiral drugs need to be prepared in enantiomerically pure forms. There is only one report of preparation of (*R*)- and (*S*)-2-substituted 3-oxo-3,4-dihydo-2*H*-1,4-benzoxazines in enantiomerically pure forms, where the precursor compound has been resolved to affect the enantioselective synthesis [41]. In view of the significant pharmacological and synthetic importance of these compounds, we herein report the synthesis of a series of novel racemic 2,4-disubstituted 3-oxo-3,4-dihydo-2*H*-1,4-benzoxazine-6-carboxylates and their lipase-catalyzed enantioselective resolution studies in organic solvents.

2. Results and discussion

Five (±)1,4-benzoxazines **6a**–**6e** have been synthesized starting from reduction of the corresponding Schiff's bases **4a**–**4e**, followed by the condensation of the resulting secondary amines **5a**–**5e** with (±)-α-bromo-γ-butyrolactone in 66–88% yields by the modified procedure of Tawada et al. (Scheme 1) [42]. The condensation of amines **5a**–**5e** with α-bromo-γ-butyrolactone to afford 1,4-benzoxazines **6a**–**6e** probably takes place in two steps. In the first step, the phenoxide ion generated from the amines **5a**–**5e** in the presence of K_2CO_3 in DMF may substitute the bromide ion on the α-bromo-γ-butyrolactone and in the second step, intramolecular nucleophilic attack by NH of the intermediate amine at carbonyl carbon of lactone moiety leads to cyclisation affording benzoxazines in the presence of HCl in DMF. Schiff's bases, methyl 3-arylmethylideneamino-4-hydroxybenzoates **4a**–**4e** in turn have been synthesized in three steps starting from the esterification of 4-hydroxy-3-nitrobenzoic acid, with methanol and catalytic amount of concentrated sulfuric acid, followed by the reduction of nitro into amino group and condensation of resulting amine with corresponding substituted aromatic aldehydes in high to moderate yields (Scheme 1). Two Schiff's bases **4a** and **4d** derived from 3-nitrobenzaldehyde and 3,4,5-trimethoxybenzaldehyde crystallized out from the reaction mixtures as yellow solids. Other three Schiff's bases, viz. **4b**, **4c** and **4e** were obtained as light brown oils and used directly in the next step. Acetylation of (±)-2-(2-hydroxyethyl)-1,4-benzoxazines **6a**–**6e** to prepare the corresponding acetates (±)-**7a**–**7e** was achieved by treating the hydroxy compounds with acetic anhydride in the presence of catalytic amount of 4-*N*,*N*-dimethylaminopyridine (DMAP) in excellent yields. The structures of all the novel compounds, i.e. Schiff's bases **4a** and **4d**, secondary amines **5a**–**5e**, hydroxybenzoxazines **6a**–**6e** and their corresponding acetates **7a**–**7e** were unambiguously established on the basis of their spectral (IR, ^{1}H-, ^{13}C NMR and HRMS) analysis. Structures of known compounds **2** and **3** were further confirmed by comparison of their physical and/or spectral data with those reported in the literature [43,44].

Four different lipases, i.e. Novozyme®-435, Amano *PS*, porcine pancreatic lipase (PPL) and *Candida rugosa* lipase (CRL) were screened for the enantioselective deacetylation of (±)-2-(2-acetoxyethyl)-1,4-benzoxazines **7a**–**7e** in four different organic solvents, i.e. tetrahydrofuran (THF), dioxane, diisopropyl ether (DIPE) and acetonitrile in the presence of *n*-butanol as acetyl acceptor. The deacetylation of the 2-acetoxyethyl-1,4-benzoxazines **7a**–**7e** catalyzed by CRL and PPL was too slow to be of any practical utility. It was observed that both, Novozyme®-435 in THF and Amano *PS* in acetonitrile catalyse



Scheme 2. Novozyme-435 catalysed enantioselective deacetylation of (±)-**7**.

Table 1
Novozyme®-435 catalyzed enantioselective deacetylation of (±)-2-(2-acetoxyethyl)-1,4-benzoxazines **7a**–**7e** in THF at 45 °C[a]

Entry	Substrate	Reaction time (h)	Conversion (%)	Products	Isolated yields[b] (%)
1	(±)-**7a**	4.5	47	(+)-**6a** and (−)-**7a**	64 and 90
2	(±)-**7b**	4.0	45	(+)-**6b** and (−)-**7b**	80 and 70
3	(±)-**7c**	3.5	45	(+)-**6c** and (−)-**7c**	86 and 66
4	(±)-**7d**	3.0	48	(+)-**6d** and (−)-**7d**	70 and 88
5	(±)-**7e**	4.5	50	(+)-**6e** and (−)-**7e**	90 and 84

[a] All these reactions when performed under identical conditions but without adding Novozyme-435 lipase did not yield any product.
[b] Yields were calculated by assuming corresponding single enantiomer as 100% in the starting methyl (±)-2-(2-acetoxyethyl)-4-arylmethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates **7a**–**7e**.

the deacetylation of these compounds leading to the formation of identical products, however the rate of deacetylation catalyzed by Novozyme®-435 was about 2.5–3 times faster than the rate of deacetylation catalyzed by Amano *PS*. On the basis of the results of screening test, Novozyme®-435—THF system was selected for further studies. In a typical reaction, racemic 2-(2-acetoxyethyl)-1,4-benzoxazines **7a**–**7e** were incubated with Novozyme®-435 in THF in the presence of 3–4 equivalents of *n*-butanol at 45 °C and the reaction was monitored by TLC. The reaction was stopped by filtering off the enzyme after about 45–50% conversion of the starting acetate to the deacetylated 4-arylmethyl-2-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates **6a**–**6e** (Scheme 2). The deacetylated 1,4-benzoxazines **6a**–**6e** and the unreacted 1,4-benzoxazines **7a**–**7e** were separated by column chromatography on silica gel with a gradient solvent system of petroleum ether-ethyl acetate in 64–90 and 66–90% yields, respectively; calculated by assuming the corresponding single

Table 2
Optical rotation values of Novozyme-435-catalyzed deacetylated products (+)-**6a**–**6e**; recovered, unreacted acetates (−)-**7a**–**7e**; acetates (+)-**7a**–**7e** obtained by chemical acetylation of enzymatically deacetylated compounds (+)-**6a**–**6e** and hydroxy compounds (−)-**6a**–**6e** obtained by deacetylation of unreacted acetates (−)-**7a**–**7e**[a]

Substrate	$[\alpha]_D^{25}$			
	Novozyme®-435 catalyzed deacetylated products (+)-**6a**–**6e**	Recovered, unreacted acetates (−)-**7a**–**7e**	Acetates (+)-**7a**–**7e** obtained by chemical acetylation of enzymatically deacetylated compounds (+)-**6a**–**6e**	Hydroxy compounds (−)-**6a**–**6e** obtained by deacetylation of unreacted acetates (−)-**7a**–**7e**
(±)-**7a**	**6a**-(+)-9.8	**7a**-(−)-19.5	**7a**-(+)-24.9	**6a**-(−)-32.8
(±)-**7b**	**6b**-(+)-9.7	**7b**-(−)-4.8	**7b**-(+)-17.8	**6b**-(−)-17.8
(±)-**7c**	**6c**-(+)-9.5	**7c**-(−)-5.9	**7c**-(+)-9.4	**6c**-(−)-4.9
(±)-**7d**	**6d**-(+)-14.9	**7d**-(−)-30.0	**7d**-(+)-12.5	**6d**-(−)-6.8
(±)-**7e**	**6e**-(+)-9.6	**7e**-(−)-11.5	**7e**-(+)-16.7	**6e**-(−)-14.6

[a] Optical rotation values of all the compounds have been measured either in $CHCl_3$ or MeOH at concentrations of 0.20–0.25 g/100 ml.

enantiomer as 100% in the starting racemic 2-(2-acetoxyethyl)-1,4-benzoxazines **7a**–**7e** (Table 1). Optical rotation values of deacetylated 1,4-benzoxazines **6a**–**6e** and recovered, unreacted acetates **7a**–**7e** were measured; we found that both series of compounds are optically active (Scheme 2 and Table 2). This revealed that Novozyme®-435 in THF preferentially catalyses the deacetylation of one enantiomer of acetylated racemic 1,4-benzoxazines **7a**–**7e** over the other. All these reactions when performed under identical conditions but without addition of any enzyme did not yield any product.

It is noteworthy that Novozyme®-435 catalysed deesterification of 1,4-benzoxazine acetates **7a**–**7e** in THF is chemoselective together with being enantioselective. This is because the lipase exclusively catalyses the deesterification at the acetoxyethyl moiety present at the C-2 position in the 1,4-benzoxazines **6a**–**6e**, whereas the other ester moiety in the compound, viz. methoxycarbonyl group present at C-6 position remains intact. It was also observed that the presence of methoxyl group(s) on the phenyl ring of arylmethyl substituent at C-4 position of 1,4-benzoxazines increases the efficiency of lipase-catalyzed deacetylation of acetylated benzoxazines (Table 2). Thus the reaction time for about 45–50% deacetylation of 1,4-benzoxazines **7b**–**7d** containing methoxyl group(s) is less than the time of deacetylation of compounds **7a** and **7e** bearing nitro and ethyl group, respectively on the benzene ring. Among the three 1,4-benzoxazines **7b**, **7c** and **7d** containing methoxyl group(s) on the aromatic ring, the rate of lipase-catalyzed deacetylation of **7d** containing three methoxyl groups is 1.16 times faster than the rate of deacetylation of **7c** containing two methoxyl groups, which in turn is 1.14 times faster than the rate of deacetylation of **7b** containing only one methoxyl group. This difference in the rate of deacetylation of acetylated 1,4-benzoxazines containing methoxyl, nitro and ethyl groups may be because of the difference in accommodation of the different substituents in the binding site of the lipase.

In order to determine the enantiomeric excess (ee) of enzymatically deacetylated (+)-2-(2-hydroxyethyl)-1,4-benzoxazines **6a**–**6e** and recovered, unreacted (−)-2-(2-acetoxyethyl)-1,4-benzoxazines **7a**–**7e**, chiral shift reagent ^{1}H NMR study was done using (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol [(+)-TFAE] shift reagent. The ^{1}H NMR spectral study of diastereomeric complexes between (±)-hydroxy-/(±)-acetoxy-1,4-benzoxazines and chiral shift reagent (+)-TFAE did not result in the separation of any of the signals in their spectra and therefore this technique could not be employed in determining the ee values of optically enriched compounds. Further, ee determination of enzymatically deacetylated (+)-2-(2-hydroxyethyl)-1,4-benzoxazines **6a**–**6e** was attempted by synthesis of *O*-acetylmandelic acid esters of (+)- and (±)-**6a**–**6e** with *S*-(+)-*O*-acetylmandelic acid in dichloromethane according to the procedure of Whitesell and Reynolds [45]. The ^{1}H NMR spectral analysis of the diastereomeric mandelates obtained from esterification of (±)-**6a**–**6e** were analysed to find out the splitting in chemical shift values of diastereomeric protons; none of the peaks in the ^{1}H NMR spectra of diastereomeric mandelates showed sufficient separation of the signals. Thus enantiomeric excess values of deacetylated, optically enriched (+)-2-(2-hydroxyethyl)-1,4-benzoxazines **6a**–**6e** or recovered, unreacted (−)-2-(2-acetoxyethyl)-1,4-benzoxazines **7a**–**7e** could not be determined by NMR techniques.

However, to have an idea of extent of enantioselectivity, the enzymatically deacetylated (+)-2-(2-hydroxyethyl)-1,4-benzoxazines **6a**–**6e** were chemically acetylated with acetic anhydride in the presence of DMAP to the corresponding acetates (+)-**7a**–**7e** and recovered, unreacted (−)-2-(2-acetoxyethyl)-1,4-benzoxazines **7a**–**7e** were chemically deacetylated to the corresponding alcohols (−)-**6a**–**6e** (Scheme 2). The comparison of sign and specific rotation values of enzymatically deacetylated (+)-1,4-benzoxazines **6a**–**6e** and (−)-1,4-benzoxazines **6a**–**6e** obtained from chemical deacetylation of unreacted acetates (−)-**7a**–**7e** revealed that they are opposite in sign (Table 2). Similarly, the comparison of sign and specific rotation values of recovered, unreacted (−)-1,4-benzoxazines **7a**–**7e** and (+)-**7a**–**7e** obtained from chemical acetylation of enzymatically deacetylated compounds (+)-**6a**–**6e** revealed that they are opposite in sign (Table 2). These results indicate that Novozyme®-435-catalyzed deacetylation of (±)-**7a**–**7e** is stereoselective and leads to the formation of (+)-(2-hydroxyethyl)-1,4-benzoxazines **6a**–**6e** and (−)-2-(2-acetoxyethyl)-1,4-benzoxazines **7a**–**7e** of variable optical enrichment. All the 20 optically enriched 1,4-benzoxazines, i.e. enzymatically deacetylated alcohols (+)-**6a**–**6e**, recovered, unreacted acetates (−)-**7a**–**7e**, acetates (+)-**7a**–**7e** obtained from chemical acetylation of enzymatically deacetylated alcohols and alcohols (−)-**6a**–**6e** obtained from chemical deacetylation of recovered, unreacted acetates were unambiguously identified on the basis of their spectral (IR, ^{1}H NMR, ^{13}C NMR and HRMS) analysis. The spectral data of (+)-and (−)-**6a**–**6e** were identical with that of the data of (±)-**6a**–**6e**, similarly the spectral data of (−)- and (+)-**7a**–**7e** were identical with the data of (±)-**7a**–**7e**.

3. Conclusion

We have demonstrated the application of *Candida antarctica* lipase B for the preparation of optically enriched 4-arylmethyl-2-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates and their corresponding acetates, these compounds belong to a class of compounds exhibiting interesting biological activities. As it is difficult to synthesize compounds of this class in enantiomerically pure forms, the enzymatic methodology reported herein may find utility in the synthesis of optically enriched compounds of this class. During the course of this study, we have synthesized seventeen novel compounds, i.e. two Schiff's bases **4a** and **4d**, five amines **5a**–**5e**, five hydroxyethylated 1,4-benzoxazines **6a**–**6e** and five acetoxyethylated 1,4-benzoxazines **7a**–**7e**. Studies on biological activity evaluation of these compounds are in progress.

4. Experimental

Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. Column chromatography was carried out using silica gel (100–200 mesh). Melting points were determined using H_2SO_4 bath and are uncorrected. Analyt-

ical TLCs were performed on pre-coated Merck silica gel $60F_{254}$ plates; the spots were detected either using UV light or with 4% alcoholic $FeCl_3$ solution. The IR spectra were recorded on a Perkin-Elmer model 2000 FT-IR spectrophotometer. The optical rotations were measured with Bellingham-Stanley AD 220 polarimeter. The optical rotations of synthetic 1,4-benzoxazines **6a**–**6e** and their corresponding acetates **7a**–**7e** prepared by acetic anhydride-DMAP method were measured and found to be zero. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance AC-300 spectrometer at 300 and 75.5 MHz, respectively. The chemical shift values are reported as δ ppm relative to TMS used as internal standard and the coupling constants (J) are measured in Hz. The FAB-HRMS spectra of all the compounds were recorded on a JEOL JMS-AX505W high-resolution mass spectrometer in positive ion mode using the matrix HEDS (bishydroxyethylsulphide) doped with sodium acetate. Novozyme®-435 was a gift from Novozymes A/S (Copenhagen, Denmark) and Amano PS was a gift from Dr. Yogesh S. Sanghvi, Rasayan, Inc. (CA, USA), whereas *C. rugosa* lipase (CRL) and porcine pancreatic lipase (PPL) were purchased from Sigma Chemical Co. (USA). These lipases were used after storing in vacuo over P_2O_5 for 24 h. The chiral shift reagents (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol [(+)-TFAE] and chiral derivatizing agent (*S*)-(+)-*O*-acetylmandelic acid were purchased from Aldrich Chemical Co. (USA). The organic solvents THF, dioxane and diisopropyl ether were used after drying and distillation over sodium pieces, and CH_3CN and *n*-butanol were used after distillation over ignited potassium carbonate.

5. General procedure for the preparation of Schiff's bases, methyl 3-arylmethylideneamino-4-hydroxybenzoates 4a–4e

To a solution of methyl 3-amino-4-hydroxybenzoate (**3**, 10.02 g, 60 mmol)) in dry EtOH (100 ml) was added corresponding aromatic aldehyde (1 equiv.) and the reaction mixture was heated to 65–70 °C for 30–60 min. When TLC examination revealed completion of reaction, the reaction mixture was allowed to cool to 25–28 °C. In case of reaction of amine **3** with *m*-nitrobenzaldehyde and 3,4,5-trimethoxybenzaldehyde, solid products crystallized out from the reaction mixture on cooling, which were filtered and washed with EtOH to afford pure Schiff's bases **4a** and **4d** in 73 and 85% yields, respectively. In case of other three aldehydes, i.e. 4-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde and 4-ethylbenzaldehyde crystallization of products from the reaction mixture on cooling was not observed and attempt to purify the compounds by CC led to the disintegration of the product. The solvent was removed from the reaction mixture under vacuum in these three cases and the crude products **4b**, **4c** and **4e**, obtained in quantitative yields were used directly in the next step.

*5.1. Methyl 4-hydroxy-3-(3-nitrophenylmethylidineamino) benzoate (**4a**)*

It was obtained as a yellow crystalline solid (13.14 g) in 73% yield, mp 165–168 °C. R_f: 0.40 (25% ethyl acetate in petroleum ether); IR (KBr): 3427 (OH), 1711 (CO), 1592 (N=C), 1526, 1436, 1353, 1284, 1225, 800, 767 and 740 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (3H, s, $COOCH_3$), 7.01 (1H, d, J = 8.2 Hz, C-5H), 7.69 (1H, s, C-5′H), 7.85 (1H, d, J = 7.1 Hz, C-6H), 7.93 (1H, s, C-2H), 8.30 and 8.32 (2H, 2s, C-4′H and C-6′H), 8.83 (1H, s, N=CH), 8.92 (1H, s, C-2′H) and 9.07 (1H, br s, OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 50.64 ($COOCH_3$), 114.81 (C-5), 118.44, 120.58, 122.10, 124.39, 128.65, 129.23 and 133.72 (C-1, C-2, C-6, C-2′, C-4′, C-5′ and C-6′), 134.91, 136.52 and 147.49 (C-3, C-1′ and C-3′), 155.17 (C-4), 155.63 (N=C) and 165.34 (CO); ESMS *m/z* calculated for $C_{15}H_{12}N_2O_5^+$ 300.0746, observed 300.2917.

*5.2. Methyl 4-hydroxy-3-(3,4,5-trimethoxyphenylmethylidineamino)benzoate (**4d**)*

It was obtained as a yellow crystalline solid (17.60 g) in 85% yields, mp 171–173 °C. R_f: 0.40 (25% ethyl acetate in petroleum ether); IR (KBr): 3394 (OH), 1712 (CO), 1622, 1581 (N=C), 1505, 1458, 1437, 1420, 1382, 1331, 1288, 1220, 1128, 1000, 826 and 766 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (9H, s, 3× OCH_3), 3.92 (3H, s, $COOCH_3$), 7.00 (1H, d, J = 8.3 Hz, C-5H), 7.08 (2H, s, C-2′H and C-6′H), 7.87 (1H, d, J = 8.3 Hz, C-6H), 7.93 (1H, s, C-2H) and 8.54 (1H, s, N=CH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 51.81 ($COOCH_3$), 56.07 (C-3′ and C-5′ OCH_3), 60.83 (C-4′ OCH_3), 106.11 (C-2′ and C-6′), 114.82 (C-5), 118.04 (C-2), 122.13 (C-1), 130.17 and 130.60 (C-6 and C-1′), 135.71 (C-4′) and 142.50 (C-3), 153.41 (C-3′ and C-5′), 155.91 (C-4), 158.87 (N=C) and 166.60 (CO); ESMS *m/z* calculated for $C_{18}H_{19}NO_6^+$ 345.1212, observed 345.1659.

6. General procedure for the preparation of methyl 3-arylmethylamino-4-hydroxybenzoates 5a–5e

To a solution of Schiff's bases (**4a**–**4e**, 15 mmol) in dry methanol (100 ml) was added sodium borohydride (1.1 g, 30 mmol) in small lots and the reaction was stirred at 5–8 °C for 1–2 h till TLC showed completion of the reaction. The solvent was removed under reduced pressure and ice-cold water (100 ml) was added to the residue. Compound was extracted with ethyl acetate (3× 50 ml), the combined organic phase was washed with sodium bicarbonate solution (100 ml) and dried over anhydrous sodium sulphate. Solvent removed under reduced pressure and the resulting crude yellow solid was crystallized from ethyl acetate to afford pure amines **5a**–**5e** in 40–85% yields.

*6.1. Methyl 4-hydroxy-3-(3-nitrophenylmethylamino)benzoate (**5a**)*

It was obtained as a pale-yellow crystalline solid (7.68 g) in 85% yield, mp 146–148 °C. R_f: 0.40 (25% ethyl acetate in petroleum ether); IR (KBr): 3380 (br, OH and NH), 1687 (CO), 1600, 1528, 1450, 1348, 1311, 1267, 1202, 1132 and 765 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (3H, s, $COOCH_3$), 4.51 (2H, s, NCH_2), 4.56 (1H, br s, OH/NH), 5.76 (1H, br s, NH/OH), 6.75 (1H, d, J = 8.1 Hz, C-5H), 7.24 (1H, s, C-2H), 7.38 (1H, dd, J = 8.1 and 1.3 Hz, C-6H), 7.50 (1H, t, J = 7.8 Hz, C-5′H), 7.71

(1H, d, J = 7.6 Hz, C-6′H), 8.12 (1H, d, J = 8.2 Hz, C-4′H) and 8.24 (1H, s, C-2′H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 46.40 (COO*C*H$_3$), 52.23 (NCH$_2$), 110.90, 113.78, 119.89, 121.69, 122.24, 122.52, 130.62 and 134.50 (C-1, C-2, C-5, C-6, C-2′, C-4′, C-5′ and C-6′), 137.39, 143.85, 148.84 and 149.93 (C-3, C-4, C-1′ and C-3′) and 167.39 (CO); HRMS *m*/*z* calculated for $C_{15}H_{14}N_2O_5Na^+$ 325.0800, observed 325.0788.

*6.2. Methyl 4-hydroxy-3-(4-methoxyphenylmethylamino)benzoate (**5b**)*

It was obtained as a yellowish-white crystalline solid (3.45 g) in 40% yield, mp 168–169 °C. R_f: 0.40 (25% ethyl acetate in petroleum ether); IR (KBr): 3415 (NH), 3268 (br, OH), 1673 (CO), 1603, 1516, 1443, 1262, 1201, 1130, 1003 and 766 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$ + few drops of DMSO-d_6): δ 3.80 and 3.83 (6H, 2s, 3H each, OCH$_3$ and COOCH$_3$), 4.30 (2H, s, NCH$_2$), 4.50 (1H, br s, OH/NH), 6.78 (1H, d, J = 8.0 Hz, C-5H), 6.87 (2H, d, J = 8.2 Hz, C-3′H and C-5′H), 7.29 (4H, m, C-2′H, C-6′H, C-2H and C-6H) and 9.45 (1H, br s, NH/OH); ^{13}C NMR (75.5 MHz, CDCl$_3$ + few drops of DMSO-d_6): δ 47.06 (COO*C*H$_3$), 51.38 (OCH$_3$), 55.10 (NCH$_2$), 110.71, 112.88, 113.85, 119.41, 121.41 and 128.67 (C-1, C-2, C-5, C-6, C-2′, C-3′, C-5′ and C-6′), 131.34 and 137.00 (C-3 and C-1′), 148.86 (C-4), 158.62 (C-4′) and 167.23 (CO); HRMS *m*/*z* calculated for $C_{16}H_{17}NO_4Na^+$ 310.1055, observed 310.1070.

*6.3. Methyl 4-hydroxy-3-(3,4-dimethoxyphenylmethylamino)benzoate (**5c**)*

It was obtained as a yellowish-white crystalline solid (4.84 g) in 51% yield, mp 117–119 °C. R_f: 0.30 (25% ethyl acetate in petroleum ether); IR (KBr): 3390 (OH and NH), 1706 (CO), 1596, 1516, 1442, 1304, 1259, 1224, 1143, 1106, 1023 and 768 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.71 and 3.73 (9H, 2s, 2× OCH$_3$ and COOCH$_3$), 4.25 (2H, s, NCH$_2$), 5.35 (1H, br s, OH/NH), 6.75 (1H, d, J = 8.1 Hz, C-5H), 6.88 (2H, br s, C-2H and C-2′H), 7.01 (2H, d, J = 10.1 Hz, C-6H and C-6′H), 7.14 (1H, d, J = 8.1 Hz, C-5′H) and 10.25 (1H, br s, NH/OH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 46.61 (COO*C*H$_3$), 51.75 (NCH$_2$), 55.77 and 55.85 (2× OCH$_3$), 110.60, 111.67, 112.21, 113.05, 118.98 and 119.42 (C-2, C-5, C-6, C-2′, C-5′ and C-6′), 121.14 (C-1), 132.72 and 137.41 (C-3 and C-1′), 148.03, 149.10 and 149.21 (C-4, C-3′ and C-4′) and 166.99 (CO); HRMS *m*/*z* calculated for $C_{17}H_{19}NO_5Na^+$ 340.1161, observed 340.1156.

*6.4. Methyl 4-hydroxy-3-(3,4,5-trimethoxyphenylmethylamino)benzoate (**5d**)*

It was obtained as off-white crystalline solid (6.76 g) in 65% yield, mp 128–130 °C. R_f: 0.30 (25% ethyl acetate in petroleum ether); IR (KBr): 3456 (NH), 3286 (br, OH), 1707 (CO), 1601, 1523, 1459, 1285, 1243, 1128, 999, 827 and 764 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.71, 3.76 and 3.79 (12H, 3s, 3× OCH$_3$ and COOCH$_3$), 4.28 (2H, s, NCH$_2$), 5.01 (1H, br s, OH/NH), 6.66 (2H, s, C-2′H and C-6′H), 6.75 (1H, d, J = 7.8 Hz, C-5H), 7.13 (1H, s, C-2H), 7.19 (1H, d, J = 7.6 Hz, C-6H) and 10.00 (1H, br s, NH/OH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 48.26 (COO*C*H$_3$), 51.96 and 56.51 (3× OCH$_3$), 60.82 (NCH$_2$), 105.26, 111.37, 113.49 and 119.89 (C-2, C-5, C-6, C-2′ and C-6′), 121.78 (C-1), 136.11 and 137.65 (C-3 and C-1′), 149.60 and 153.69 (C-4, C-3′, C-4′ and C-5′) and 167.51 (CO); HRMS *m*/*z* calculated for $C_{18}H_{21}NO_6Na^+$ 370.1267, observed 370.1283.

*6.5. Methyl 3-(4-ethylphenylmethylamino)-4-hydroxybenzoate (**5e**)*

It was obtained as a yellowish-white crystalline solid (6.44 g) in 75% yield, mp 140–142 °C. R_f: 0.35 (25% ethyl acetate in petroleum ether); IR (KBr): 3423 (NH), 3245 (br, OH), 1682 (CO), 1595, 1523, 1447, 1385, 1310, 1262, 1203, 1125, 1001, 826 and 766 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.22 (3H, t, J = 7.6 Hz, CH$_2$C*H*$_3$), 2.62 (2H, q, J = 7.6 Hz, C*H*$_2$CH$_3$), 3.79 (3H, s, COOCH$_3$), 4.31 (2H, s, NCH$_2$), 4.72 (1H, br s, OH/NH), 6.76 (1H, d, J = 8.1 Hz, C-5H), 7.14 (1H, s, C-2H), 7.15 (2H, d, J = 8.4 Hz, C-3′H and C-5′H), 7.23 (1H, d, J = 8.1 Hz, C-6H), 7.28 (2H, d, J = 8.4 Hz, C-2′H and C-6′H) and 9.80 (1H, s, NH/OH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 16.11 (CH$_2$*C*H$_3$), 28.80 (*C*H$_2$CH$_3$), 47.89 (COO*C*H$_3$), 51.91 (NCH$_2$), 111.16, 113.41, 119.93 and 121.93 (C-1, C-2, C-5 and C-6), 128.00 and 128.41 (C-2′, C-3′, C-5′ and C-6′), 137.08 and 137.56 (C-1′ and C-4′), 143.36 (C-3), 149.37 (C-4) and 167.74 (CO); HRMS *m*/*z* calculated for $C_{17}H_{19}NO_3Na^+$ 308.1263, observed 308.1281.

7. General procedure for the preparation of methyl (±)-4-arylmethyl-2-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates (6a–6e)

To a mixture of 3-arylmethylamino-4-hydroxybenzoates (**5a**–**5e**, 10 mmol), potassium carbonate (1.7 g, 12 mmol) and dimethylformamide (40 ml) was added under stirring α-bromo-γ-butyrolactone (2.0 g, 12 mmol) in small lots. The reaction mixture was stirred for 2 to 3 h under nitrogen at 25–28 °C till TLC showed completion of the reaction. The pH of the reaction mixture was adjusted to 1 with concentrated HCl and then heated at 80–85 °C for 20–30 min. On completion (analytical TLC), the reaction mixture was poured into ice-cold water (150 ml) and extracted with ethyl acetate (3× 50 ml). The combined organic phase was dried (Na_2SO_4), evaporated to dryness under reduced pressure and the crude solid thus obtained was subjected to column chromatography on silica gel using 30–32% ethyl acetate in petroleum ether (v/v) as eluent to afford 4-arylmethyl-2-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates **6a**–**6e** in 66–88%.

*7.1. Methyl (±)-2-(2-hydroxyethyl)-4-(3-nitrophenylmethyl)-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-6-carboxylate (**6a**)*

It was obtained as a white crystalline solid (2.54 g) in 66% yield, mp 123–125 °C. R_f: 0.30 (40% ethyl acetate in petroleum ether); UV (MeOH): 235 and 261 nm; IR (KBr): 3512, 1709 (CO), 1685 (CO), 1528, 1451, 1383, 1352, 1270, 1064, 980,

770 and 734 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.25 (2H, m, C-1″H), 2.80 (1H, br s, OH), 3.83 (3H, s, $COOCH_3$), 3.90 (2H, br s, C-2″), 4.99 (1H, t, J = 7.1 Hz, C-2H), 5.22 (1H, d, J = 16.3 Hz, N-$H_\alpha H_\beta$), 5.33 (1H, d, J = 16.3 Hz, N-$H_\alpha H_\beta$), 7.04 (1H, d, J = 8.3 Hz, C-8H), 7.53 (2H, m, C-5H and C-7H), and 7.65 and 8.11 (4H, 2m, 2H each, C-2′H, C-4′H, C-5′H and C-6′H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 34.92 (C-1″), 45.97 (N-CH_2), 52.53 ($COOCH_3$), 59.74 (C-2″), 76.36 (C-2), 117.67, 118.82, 123.18, 124.24, 126.34, 127.81, 129.39, 131.41, 134.21 (C-4a, C-5, C-7, C-8, C-1′, C-2′, C-4′, C-5′, C-6′), 139.20 (C-6), 149.47 and 150.09 (C-8a and C-3′), and 167.27 and 167.67 (2× CO); HRMS *m/z* calculated for $C_{19}H_{18}N_2O_7^+$ 386.1114, observed 386.1120.

*7.2. Methyl (±)-2-(2-hydroxyethyl)-4-(4-methoxyphenylmethyl)-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-6-carboxylate (**6b**)*

It was obtained as a white crystalline solid (2.56 g) in 69% yield, mp 105–107 °C. R_f: 0.30 (40% ethyl acetate in petroleum ether); IR (KBr): 3452 (OH), 1712 (CO), 1661 (CO), 1512, 1457, 1273, 1244, 1178, 1056, 977, 829 and 764 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.98 and 2.08 (2H, 2m, 1H each, C-1″H), 3.65 (2H, br s, C-2″H), 3.72 and 3.79 (6H, 2s, 3H each, OCH_3 and $COOCH_3$), 4.68 (1H, br s, OH), 4.96 (1H, d, J = 7.5 Hz, C-2H), 5.08 (1H, d, J = 16.2 Hz, N-$H_\alpha H_\beta$), 5.13 (1H, d, J = 16.2 Hz, N-$H_\alpha H_\beta$), 6.88 (2H, d, J = 7.2 Hz, C-3′H and C-5′H), 7.08 (1H, d, J = 7.9 Hz, C-8H), 7.20 (2H, d, J = 7.2 Hz, C-2′H and C-6′H) and 7.59 (2H, m, C-5H and C-7H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 33.81 (C-1″), 44.00 ($COOCH_3$), 52.10 (NCH_2), 55.19 and 56.48 (OCH_3 and C-2″), 74.06 (C-2), 114.34, 116.64, 117.20, 124.28, 125.53 and 127.94 (C-5, C-6, C-7, C-8, C-2′, C-3′, C-5′ and C-6′), 128.07 and 128.63 (C-4a and C-1′), 147.97 (C-8a), 158.83 (C-4′) and 165.76 (2× CO); HRMS *m/z* calculated for $C_{20}H_{21}NO_6H^+$ 372.1447, observed 372.1440.

*7.3. Methyl (±)-4-(3,4-dimethoxyphenylmethyl)-2-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-6-carboxylate (**6c**)*

It was obtained as a white crystalline solid (2.88 g) in 72% yield, mp 120–122 °C. R_f: 0.25 (40% ethyl acetate in petroleum ether); IR (KBr): 3332 (br, OH), 1715 (CO), 1677 (CO), 1520, 1445, 1289, 1268, 1247, 1159, 1111, 1047 and 766 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.98 and 2.07 (2H, 2m, 1H each, C-1″H), 3.65 (2H, br s, C-2″H), 3.74, 3.76 and 3.79 (9H, 3s, 3H each, 2× OCH_3 and $COOCH_3$), 4.66 (1H, br s, OH), 4.99 (1H, m, C-2H), 5.02 (1H, d, J = 15.7 Hz, N-$H_\alpha H_\beta$), 5.18 (1H, d, J = 15.7 Hz, N-$H_\alpha H_\beta$), 6.78 (1H, s, C-2′H), 6.86 (1H, d, J = 7.3 Hz, C-8H), 6.93 (1H, s, C-5H), 7.07 (1H, d, J = 7.7 Hz, C-6′H) and 7.61 (2H, br s, C-7H and C-5′H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 33.86 (C-1″), 44.24 ($COOCH_3$), 52.20 (NCH_2), 55.76 and 56.42 (C-2″ and 2× OCH_3), 74.07 (C-2), 111.14, 112.26, 116.78, 117.26, 118.98, 124.22, 125.59 and 128.64 (C-4a, C-5, C-6, C-7, C-8, C-1′, C-2′, C-5′ and C-6′), 147.91, 148.39 and 149.24 (C-3′, and C-4′ and C-8a), and 165.70 and 165.80 (2× CO); HRMS *m/z* calculated for $C_{21}H_{23}NO_7^+$ 401.1475, observed 401.1454.

*7.4. Methyl (±)-2-(2-hydroxyethyl)-4-(3,4,5-trimethoxyphenylmethyl)-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-6-carboxylate (**6d**)*

It was obtained as a white crystalline solid (2.93 g) in 68% yield, mp 96–98 °C. R_f: 0.25 (40% ethyl acetate in petroleum ether); IR (KBr): 3496 (OH), 1714 (CO), 1655 (CO), 1595, 1509, 1454, 1330, 1268, 1241, 1125, 1060, 1010, 982, 835 and 761 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.03 and 2.14 (2H, 2m, 1H each, C-1″H), 3.78 (14H, m, C-2″H, $COOCH_3$, 3× OCH_3), 4.40 (1H, br s, OH), 4.93 (1H, d, J = 15.6 Hz, N-$H_\alpha H_\beta$), 4.96 (1H, br s, C-2H), 5.22 (1H, d, J = 15.6 Hz, N-$H_\alpha H_\beta$), 6.54 (2H, s, C-2′H and C-6′H), 7.00 (1H, d, J = 8.2 Hz, C-8H), 7.63 (1H, d, J = 8.2 Hz, C-7H) and 7.67 (1H, s, C-5H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 32.32 (C-1″), 43.71 ($COOCH_3$), 50.62 (C-2″), 54.60 and 55.41 (3× OCH_3), 59.09 (NCH_2), 72.82 (C-2), 102.85, 115.32, 115.74, 122.94, 124.40, 127.11, 130.28 and 135.00 (C-4a, C-5, C-6, C-7, C-8, C-8a, C-1′, C-2′ and C-6′), 146.43 and 152.03 (C-3′, C-4′ and C-5′), and 164.44 and 164.56 (2× CO); HRMS *m/z* calculated for $C_{22}H_{25}NO_8Na^+$ 454.1478, observed 454.1456.

*7.5. Methyl (±)-4-(4-ethylphenylmethyl)-2-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-6-carboxylate (**6e**)*

It was obtained as a white crystalline solid (3.25 g) in 88% yield, mp 115–116 °C. R_f: 0.30 (40% ethyl acetate in petroleum ether); IR (KBr): 3506 (OH), 1706 (CO), 1665 (CO), 1510, 1455, 1397, 1297, 1270, 1060, 979 and 767 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.19 (3H, t, J = 7.4 Hz, CH_2CH_3), 2.00 and 2.13 (2H, 2m, 1H each, C-1″H), 2.58 (2H, q, J = 7.4 Hz, CH_2CH_3), 3.71 (2H, br s, C-2″H), 3.80 (3H, s, $COOCH_3$), 4.60 (1H, br s, OH), 4.95 (1H, m, C-2H), 5.10 (1H, d, J = 16.0 Hz, N-$H_\alpha H_\beta$), 5.18 (1H, d, J = 16.0 Hz, N-$H_\alpha H_\beta$), 7.06 (1H, d, J = 8.2 Hz, C-8H), 7.19 (4H, m, C-2′H, C-3′H, C-5′H and C-6′H), 7.57 (1H, s, C-5H) and 7.62 (1H, d, J = 8.2 Hz, C-7H); ^{13}C NMR (75.5 MHz, DMSO): δ 16.05 (CH_2CH_3), 28.72 (CH_2CH_3), 34.29 (C-1″), 44.94 ($COOCH_3$), 52.55 (NCH_2), 57.05 (C-2″), 74.57 (C-2), 117.08, 117.68, 124.84, 126.08, 127.18, 128.81 and 129.20 (C-5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′ and C-6′), 133.72 (C-4a), 143.69 (C-1′), 148.44 (C-8a) and 166.28 (2× CO); HRMS *m/z* calculated for $C_{21}H_{23}NO_5H^+$ 370.1654, observed 370.1621.

8. General procedure of acetylation of methyl (±)-4-arylmethyl-2-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates 6a–6e: synthesis of methyl (±)-2-(2-acetoxyethyl)-4-arylmethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates (7a–7e)

To a solution of (±)-1,4-benzoxazines (**6a**–**6e**, 5 mmol) in acetic anhydride (5 equiv.), was added catalytic amount of *N*,*N*-dimethylaminopyridine and the reaction mixture stirred at 25–28 °C for 1–1.5 h. On completion (analytical TLC), the

reaction mixture was poured into ice-cold water (100 ml) and solid separated was filtered. The crude product thus obtained was subjected to column chromatography on silica gel using 15–20% ethyl acetate in petroleum ether (v/v) as eluent to afford 2-(2-acetoxyethyl)-4-arylmethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates **7a**–**7e** in 83–95% yields.

*8.1. Methyl (±)-2-(2-acetoxyethyl)-4-(3-nitrophenylmethy)-3-oxo-3,4-dihydro-2H-1, 4-benzoxazine-6-carboxylates (**7a**)*

It was obtained as a white crystalline solid (1.84 g) in 86% yield, 87–89 °C. R_f: 0.50 (40% ethyl acetate in petroleum ether); IR (KBr): 1732 (2 × CO), 1686 (CO), 1528, 1447, 1389, 1356, 1286, 1250, 1110, 1053, 765 and 728 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.06 (3H, s, $OCOCH_3$), 2.26 and 2.44 (2H, 2m, 1H each, C-1″H), 3.85 (3H, s, $COOCH_3$), 4.34 (2H, br s, C-2″H), 4.87 (1H, m, C-2H), 5.27 (2H, s, NCH_2), 7.07 (1H, d, J = 8.3 Hz, C-8H), 7.54 (2H, m, C-5H and C-7H), 7.62 (1H, m, C-5′H), 7.72 (1H, d, J = 8.3 Hz, C-6′H) and 8.14 (2H, m, C-2′H and C-4′H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 22.21 ($OCOCH_3$), 31.18 (C-1″), 45.98 (NCH_2), 53.62 ($COOCH_3$), 61.09 (C-2″), 75.64 (C-2), 117.70, 118.94, 123.21, 124.29, 126.54, 127.83, 129.49, 131.45 and 134.22 (C-5, C-6, C-7, C-8, C-1′, C-2′, C-4′, C-5′ and C-6′), 139.20 (C-4a), 149.26 and 150.11 (C-8a and C-3′), and 166.82, 167.23 and 172.17 (3× CO); HRMS *m/z* calculated for $C_{21}H_{20}N_2O_8$ 428.1220, observed 428.1234.

*8.2. Methyl (±)-2-(2-acetoxyethyl)-4-(4-methoxyphenylmethyl)-3-oxo-3,4-dihydro-2H-1, 4-benzoxazine-6-carboxylate (**7b**)*

It was obtained as a white crystalline solid (1.96 g) in 95% yield, mp 74–75 °C. R_f: 0.45 (40% ethyl acetate in petroleum ether); IR (KBr): 1728 (CO), 1710 (CO), 1687 (CO), 1512, 1455, 1392, 1272, 1241, 1113, 1046, 978 and 767 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.00 (3H, s, $OCOCH_3$), 2.18 and 2.29 (2H, 2m, 1H each, C-1″H), 3.72 and 3.78 (6H, 2s, 3H each, $COOCH_3$ and OCH_3), 4.25 (2H, br s, C-2″H), 4.97 (1H, br s, C-2H), 5.12 (2H, s, NCH_2), 6.88 (2H, d, J = 6.6 Hz, C-3′H and C-5′H), 7.10 (1H, d, J = 7.8 Hz, C-8H), 7.20 (2H, d, J = 6.6 Hz, C-2′H and C-6′H) and 7.59 (2H, br s, C-5H and C-7H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 20.80 ($OCOCH_3$), 29.82 (C-1″), 44.19 (NCH_2), 52.05 ($COOCH_3$), 55.13 (OCH_3), 59.73 (C-2″), 74.04 (C-2), 114.28, 116.75, 117.09, 124.54, 125.61, 127.70, 128.08 and 128.49 (C-4a, C-5, C-6, C-7, C-8, C-1′, C-2′, C-3′, C-5′ and C-6′), 147.86 (C-8a), 158.87 (C-4′), and 164.95, 165.61 and 170.24 (3× CO); HRMS *m/z* calculated for $C_{22}H_{23}NO_7H^+$ 414.1553, observed 414.1552.

*8.3. Methyl (±)-2-(2-acetoxyethyl)-4-(3,4-dimethoxyphenylmethyl)-3-oxo-3,4-dihydro-2H-1, 4-benzoxazine-6-carboxylate (**7c**)*

It was obtained as a white crystalline solid (1.83 g) in 83% yield, mp 88–90 °C. R_f: 0.40 (40% ethyl acetate in petroleum ether); IR (KBr): 1737 (CO), 1713 (CO), 1686 (CO), 1608, 1523, 1450, 1388, 1269, 1237, 1136, 1025 and 767 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (3H, s, $OCOCH_3$), 2.21 and 2.38 (2H, 2m, 1H each, C-1″H), 3.86 (9H, br s, 2× OCH_3 and $COOCH_3$), 4.32 (2H, s, C-2″H), 4.81 (1H, br s, C-2H), 5.07 (1H, d, J = 15.2 Hz, N-$H_\alpha H_\beta$), 5.15 (1H, d, J = 15.2 Hz, N-$H_\alpha H_\beta$), 6.84 (3H, m, C-2′H, C-5′H and C-6′H), 7.01 (1H, d, J = 7.5 Hz, C-8H), 7.68 (1H, d, J = 7.5 Hz, C-7H) and 7.75 (1H, s, C-5H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 23.21 ($OCOCH_3$), 32.31 (C-1″), 47.37 (NCH_2), 54.51 ($COOCH_3$), 58.24 (2× OCH_3), 62.17 (C-2″), 76.59 (C-2), 113.13, 113.85, 119.41, 119.54, 121.91, 127.21, 128.36, 130.50, 130.80 (C-4a, C-5, C-6, C-7, C-8, C-1′, C-2′, C-5′ and C-6′), 150.16, 150.99 and 151.71 (C-8a, C-3′ and C-4′), and 167.60, 168.46 and 173.16 (3× CO); HRMS *m/z* calculated for $C_{23}H_{25}NO_8Na^+$ 466.1478, observed 466.1493.

*8.4. Methyl (±)-2-(2-acetoxyethyl)-4-(3,4,5-trimethoxyphenylmethyl)-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-6-carboxylate (**7d**)*

It was obtained as a white crystalline solid (2.15 g) in 91% yield, mp 84–86 °C. R_f: 0.40 (40% ethyl acetate in petroleum ether); IR (KBr): 1739 (CO), 1709 (CO), 1683 (CO), 1595, 1509, 1453, 1423, 1389, 1330, 1267, 1236, 1129, 1073, 1041, 1009, 981, 887, 830 and 761 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.01 (3H, s, $OCOCH_3$), 2.23 and 2.32 (2H, 2m, 1H each, C-1″H), 3.71, 3.78 and 3.82 (12H, 3s, 3H, 6H and 3H, respectively, $COOCH_3$ and 3× OCH_3), 4.28 (2H, br s, C-2″H), 4.91 (1H, br s, C-2H), 5.07 (1H, d, J = 15.2 Hz, N-$H_\alpha H_\beta$), 5.16 (1H, d, J = 15.2 Hz, N-$H_\alpha H_\beta$), 6.58 (2H, s, C-2′H and C-6′H), 7.06 (1H, d, J = 8.0 Hz, C-8H), 7.64 (1H, d, J = 8.0 Hz, C-7H) and 7.71 (1H, s, C-5H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.79 ($OCOCH_3$), 29.80 (C-1″), 45.00 (NCH_2), 52.05 ($COOCH_3$), 55.98, 59.68 and 60.29 (C-2″ and 3× OCH_3), 74.07 (C-2), 104.45, 117.11, 117.33, 124.50, 125.75, 128.50 and 131.66 (C-4a, C-5, C-6, C-7, C-8, C-1′, C-2′ and C-6′), 136.89 (C-8a), 147.75 (C-4′), 153.40 (C-3′ and C-5′), and 165.04, 165.61 and 170.24 (3× CO); HRMS *m/z* calculated for $C_{24}H_{27}NO_9Na^+$ 496.1584, observed 496.1609.

*8.5. Methyl (±)-2-(2-acetoxyethyl)-4-(4-ethylphenylmethyl)-3-oxo-3,4-dihydro-2H-1, 4-benzoxazine-6-carboxylate (**7e**)*

It was obtained as a white crystalline solid (1.95 g) in 95% yield, mp 70–72 °C. R_f: 0.45 (40% ethyl acetate in petroleum ether); IR (KBr): 1732 (CO), 1709 (CO), 1683 (CO), 1608, 1510, 1455, 1392, 1269, 1243, 1113, 1048, 978 and 766 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.20 (3H, t, J = 7.6 Hz, CH_2CH_3), 2.05 (3H, s, $OCOCH_3$), 2.23 and 2.42 (2H, 2m, 1H each, C-1″H), 2.61 (2H, q, J = 7.6 Hz, CH_2CH_3), 3.85 (3H, s, $COOCH_3$), 4.33 (2H, t, J = 6.2 Hz, C-2″H), 4.82 (1H, t, J = 4.9 Hz, C-2H), 5.14 (2H, br s, NCH_2), 7.02 (1H, d, J = 8.8 Hz, C-8H), 7.18 (4H, m, C-2′H, C-3′H, C-5′H and C-6′H), 7.67 (2H, br s, C-5H and C-7H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 16.06 (CH_2CH_3), 21.35 ($OCOCH_3$), 28.70 (CH_2CH_3), 30.34 (C-1″), 45.03 (NCH_2), 52.60 ($COOCH_3$), 60.28 (C-2″), 74.57 (C-2), 117.23, 117.66, 125.07, 126.15, 127.23, 128.82 and 129.16 (C-

5, C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′ and C-6′), 133.63 (C-4a), 143.72 (C-1′), 148.40 (C-8a), and 165.51, 166.11 and 170.75 (3× CO); HRMS *m/z* calculated for $C_{23}H_{25}NO_6H^+$ 412.1760, observed 412.1714.

9. General procedure of Novozyme®-435 catalyzed deacetylation of methyl (±)-2-(2-acetoxyethyl)-4-arylmethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates (7a–7e)

To a solution of (±)-2-(2-acetoxyethyl)-4-arylmethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates (**7a–7e**, 3 mmol) in anhydrous THF (30 ml) containing *n*-butanol (3–4 equiv.) was added Novozyme®-435 (600 mg). The suspension was stirred at 45 °C for 3–4.5 h in an incubator shaker and the progress of the reaction monitored periodically by TLC. After about 45–50% conversion of the starting material into the product, reaction quenched by filtering off the enzyme and the solvent evaporated to dryness under reduced pressure. The crude product thus obtained was subjected to column chromatography on silica gel using 25–35% ethyl acetate-petroleum ether (v/v) as eluent to afford optically enriched enzymatically deacetylated (+)-1,4-benzoxazines **6a–6e** and unreacted acetates, (−)-1,4-benzoxazines **7a–7e** in 64–90 and 66–90% yields, respectively. The enzymatically deacetylated (+)-1,4-benzoxazines **6a–6e** and unreacted acetates, (−)-1,4-benzoxazines **7a–7e** were identified on the basis of their spectral data, which were found identical with the spectral data of the corresponding racemic compounds, i.e. (±)-**6a–6e** and (±)-**7a–7e**, respectively, as reported above.

10. General procedure of chemical acetylation of enzymatically deacetylated (+)-1,4-benzoxazines 6a–6e

To a solution of (+)-1,4-benzoxazines (**6a–6e**, 0.5 mmol) in acetic anhydride (5 equiv.), was added catalytic amount of *N*,*N*-dimethylaminopyridine and the reaction mixture stirred at 25–28 °C for 1 to 1.5 h. On completion (analytical TLC), the reaction mixture was poured into ice-cold water (20 ml) and solid separated was filtered. The crude product thus obtained was subjected to column chromatography on silica gel using 15–20% ethyl acetate in petroleum ether (v/v) as eluent to afford (+)-2-(2-acetoxyethyl)-4-arylmethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylates **7a–7e** in 90–95% yields. These chemically acetylated (+)-1,4-benzoxazines **7a–7e** were identified on the basis of their spectral data, which were found identical with the spectral data of the corresponding racemic compounds, i.e. (±)-**7a–7e** as reported above.

11. General procedure of chemical deacetylation of unreacted, recovered acetates (−)-7a–7e

To a solution of unreacted, recovered (−)-1,4-benzoxazines (**7a–7e**, 0.5 mmol) in methanol was added saturated methanolic ammonia (10 ml) and the reaction stirred for 2–2.5 h at 25–28 °C until TLC showed complete deacetylation. The solvent was evaporated under reduced pressure, and the crude solid thus obtained was co-evaporated with toluene and washed twice with chloroform–petrol mixture to afford pure (−)-1,4-benzoxazines **6a–6e** in 78–85% yields. These chemically deacetylated (−)-1,4-benzoxazines **6a–6e** were identified on the basis of their spectral data, which were found identical with the spectral data of the corresponding racemic compounds, i.e. (±)-**6a–6e** as reported above.

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