Synthetic and biological activity evaluation studies on novel isoxazolidines


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Enantioselective deacetylation reactions on 5-acetoxy-methyl- and 5-acetoxy-3-aryl-2-phenylisoxazolidines using Candida rugosa lipase (CRL) are described. Varying degrees of enantioselectivity have been observed depending on the nature of the 3-aryl group. These compounds have also been evaluated for their antioxidant and antimycobacterial activities.

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Isoxazolidines are an important class of heterocyclic compounds having potential as intermediates for nitrogen and oxygen containing heterocycles. Isoxazolidines are masked amino acids, under appropriate conditions can give rise to hydroxynitro amino acids and β-amino alcohols which are important components in a wide-range of biologically active compounds such as neopoloxins, theonellamide and WS 47083 (ref. 3). Casuscelli et al. (ref. 4) reported a new and direct method for obtaining 3-amino-2(5H)-furanones from isoxazolidines, which are versatile synthons for β-lactams.

Isoxazoline derivatives are known to possess a variety of biological activities viz. antifungal, anti-inflammatory, antiviral, herbicidal, etc. Isoxazoline derivatives are also known to be selective β-adrenergic agonist of brown adipose tissue and thermogenesis in the rat. Recently there have been reports in the literature wherein isoxazolidines have been used to synthesize isoxazolidinyl nucleosides.

1,3-Dipolar cycloadditions (1,3-DC) have been extensively employed in the synthesis of a diverse array of heterocyclic compounds. The stereochemistry of the 1,3-DC reaction can be controlled by either choosing the appropriate chiral alkene, a chiral dipole or controlling the reaction by a chiral catalyst, which are quite often very expensive. Chiral intermediates can be prepared in few ways. Chiral auxiliary reagents are used to introduce chirality, auxiliaries are often expensive and cannot be recovered in many cases. One approach can rely on the synthesis with an enantioselectively enriched compound, which has been selected, from the large stock of enantiomerically pure compounds the so-called 'chiral pool'. It has its own limitation, as very small percentage of these compounds are available from the chiral pool at an affordable price. Keeping in view the problems with the alternative ways of getting enantiomeric compounds, the enzymatic resolution represents an unrenounceable method in contemporary organic synthesis. Lipases occupy a prominent place among biocatalysts, due to their ability to discriminate between enantiomeric groups of prochiral compounds and between the enantiomers of a racemate. They have been broadly used in the synthesis of optically enriched compounds. In recent years, extensive studies have been done in our group involving lipase catalyzed transformations on aryl alkyl ketones, hydroxymethylated phenolic compounds and chromones.
addition reactions of the allylic fluorides with nitrones by chemical methods using microwave irradiation methodology. Chiacchio et al.\textsuperscript{19} have described the diastere- and enantio-selective synthesis of isoxazolidinyl nucleosides by the 1,3-dipolar cycloaddition reaction of a N-glycosyl nitrite with vinyl acetate, these compounds have shown good anti AIDS activity.

In view of the importance of isoxazolidines in medicinal chemistry, we envisaged to synthesize 5-oxygenated 2,3-diarylisoxazolidines by utilizing [3+2] cycloaddition reaction of nitrones with allyl/vinyl acetate at 50-60°C in anhydrous solvents. We have recently reported the optical enrichment of (-)-5-acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine using Candida rugosa lipase with 99% ee\textsuperscript{20}. Encouraged by these results, we have synthesized a series of 5-acetoxy-3-aryl-2-phenylisoxazolidines 5a-e and 5-acyctoxy-3-aryl-2-phenylisoxazolidines 6a-e, some of these compounds exhibited interesting antioxidant and antitubercular activities. Further, these compounds have been resolved by carrying out lipase-catalyzed deacetylation of acetate group present at the C-5 or C-6 positions, as lipases are well known to carry out selective acetylation/deacetylation reactions.

Results and Discussion

The [3+2] cycloaddition reaction between \( \alpha, N \)-diarylnitrones 4a-e (refs 21, 22), separately with allyl acetate and vinyl acetate gave the isoxazolidines 5a-e and 6a-e, respectively in a highly stereoselective fashion leading to the formation of only one diastereomer in each case (Scheme I)\textsuperscript{23}. This was determined by the configuration of isoxazolidine using NOE application. Irradiation of the C-3 proton in isoxazolidines 5a-e resulted in enhancement of the signals for H\textsubscript{4}-4 and H-5. Likewise, when H-5 was irradiated, the signals corresponding to H\textsubscript{4}-4 and H-3 were enhanced. These results are possible only for the cis stereoisomer because there is no possible conformation for the trans isomer in which the H-3 and H-5 are close enough to induce enhancement in their respective signals. Our results are supported by the work of DeShong et al.\textsuperscript{24} who established the configuration of the 3-aryl-5-acetylisoxazolidines to be cis across C-3 and C-5 by using Nuclear Overhauser Effect Difference Spectroscopy. This was also confirmed by X-ray diffraction studies on the compounds 6a (ref. 25), 6c (ref. 25) and 7c (Figure 1). Resolution of the racemic isoxazolidines 5a-e, 6a and 6c-e was attempted by carrying out deacetylation reactions with Candida rugosa lipase (CRL) and porcine pancreatic lipase (PPL). The isoxazolidines were incubated in disopropyl ether/diisopropyl ether-THF mixture containing the lipase and n-butanol as acyl trap at 40-42°C. While there was no reaction with PPL, the reaction with CRL was facile. The progress of the reaction was monitored by TLC/HPLC and the reaction was quenched at 50% conversion by filtering off the enzyme. The reaction products were purified by column chromatography and were found to be optically active, thus indicating that the enzyme catalyzes the deacetylation reaction in an enantioselective manner (Schemes II and III). The enantiomeric excess values (ee) were determined by chiral shift NMR technique using \((S)-(+)-2,2,2$\text{-trifluoro-1-(9-anthryl)ethanol}\) (TFAE) and the compounds showed very moderate ee values ranging between 8-40 %.

The \( ^1H \) and \( ^13C \) NMR spectra of the isoxazolidines 5a-e as well as those of the products (-)-7a-e and (+)-5a-e exhibited single set of peaks in their \( ^1H \) and \( ^13C \) NMR spectra indicating that the 5-hydroxy-methylisoxazolidines were single diastereomers. The unreacted, recovered acetates obtained from the enzymatic reactions on isoxazolidines 5a-e on complete deacetylation afforded the hydroxy compounds that had opposite sign of optical rotation, which suggested that the two hydroxy compounds were enantiomERICally enriched. It was further confirmed by enantiomeric excess values of the enzymatic products (Table I). All these reactions when performed under identical conditions but without addition of the enzyme did not yield any product. In the case of the 5-acetoxyisonoxazolidines 6a and 6c-e, the deacetylated product undergoes epimerization at C-5 as in the case of sugars (Scheme III) giving rise to a diastereomeric mixture as evident from the \( ^1H \) and \( ^13C \) NMR spectra of 8a and 8c-e, however the unreacted, recovered acetates were obtained in optically enriched form having moderate ee. The ratio of trans to cis isomer was determined from the ratio of integration of the C-3 proton of trans and cis isomers. Theoretically the 5-hydroxyisoxazolidines can be recycled giving rise to higher yields of optically enriched 5-acetoxyisoxazolidines as compared to classical enzymatic resolutions wherein a maximum of 50% conversion can be achieved. In case of compounds 6a and 6c-e, the substituent at C-3 and C-5 are cis to each other, but the enzymatic deacetylated products 8a and 8c-e
were mixtures of cis and trans isomers, with trans isomers being predominant because of thermodynamic reasons (Scheme III). The ratio of cis and trans isomers ranged between 1.8:1 to 2.7:1.

Biological activity evaluation

Antioxidant activity. Peroxidation of lipids of biomembranes is a complicated process involving formation and propagation of lipid radicals, oxygen uptake and rearrangement of double bonds in unsaturated lipids. Uncontrolled lipid peroxidation ultimately leads to deterioration of membrane lipids yielding a range of degradation products. Svingen et al.\textsuperscript{26} demonstrated that NADPH-dependent lipid peroxidation proceeds through the formation of lipid hydroperoxides, called initiation step followed by the
Figure 1—X-ray of 2-phenyl-3-(4-chlorophenyl)-5-hydroxymethylisoxazolidine (7c)

(i) CRL, DIPE, n-butanol, 40-42 °C, 26-52 hr
(ii) CRL, DIPE, n-butanol, 40-42 °C, 100-120 hr

<table>
<thead>
<tr>
<th>5 &amp; 7</th>
<th>R</th>
<th>R'</th>
</tr>
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<tbody>
<tr>
<td>a</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>b</td>
<td>OCH₃</td>
<td>H</td>
</tr>
<tr>
<td>c</td>
<td>Cl</td>
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<tr>
<td>d</td>
<td>H</td>
<td>Br</td>
</tr>
<tr>
<td>e</td>
<td>NO₂</td>
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Scheme II
propagation step which involves the breakdown of hydroperoxides yielding reactive radicals and oxidized products. In recent years, the role of free radicals and reactive oxygen species (ROS) in various human disease processes including cancer, rheumatoid arthritis, brain dysfunction, cataracts and malaria have become apparent.

We have examined the effect of isoxazolidines synthesized in this study on the initiation of lipid peroxidation in rat liver microsomes. NADPH-dependent liver microsomal lipid peroxidation was assayed by the method of Ernster and Nordenbrand. The results compiled in Table II illustrate the influence of 5-acetoxy-3-arylisoxazolidines and 5-acetoxyethyl-3-arylisoxazolidines on the enzymatic initiation of lipid peroxidation. It is interesting to note that the 5-acetoxyisoxazolidine 6e having a nitro group at para position of the C-3 phenyl was found to be most active, the analogue 5-acetoxyethylisoxazolidine 5e did not exhibit any significant activity. In general, the antioxidant activities of the isoxazolidines of the series 5 were better than that of the series 6 (Table II).

**Antibacterial activity.** Tuberculosis is one of the world’s most infectious diseases which kills one person every fifteen seconds across the globe. One of the important reasons of increase in the incidence of tuberculosis is the recent explosion of multi-drug resistant strains of *Mycobacterium tuberculosis*. Tuberculosis has not faced any new class of drugs with new mechanism of action for more than 30 years.

<table>
<thead>
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<th><strong>6 &amp; 8</strong></th>
<th>R</th>
<th>R’</th>
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<td>c</td>
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<tr>
<td>e</td>
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</table>

Scheme III
Table I—Enantioselective resolution of (±)-Sa-e and (±)-6a, 6c-e catalyzed by Candida rugosa lipase in diisopropylether containing n-BuOH as acyl trapper at 40-42°C

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction time (in hr)</th>
<th>Components of the reaction product mixture (mp; %Yield)</th>
<th>Optical rotation [α]D27 values</th>
<th>Optical rotation values [α]D27 of deacetylated products (+)-7a-e obtained by enzymatic deacetylation of the recovered (+)-5a-e</th>
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<tbody>
<tr>
<td>(±)-5a</td>
<td>29</td>
<td>(+)-5a (58°C; 67) and (-)-7a (semi-solid; 83)</td>
<td>5a: +45.5</td>
<td>(+)-7a: +41.2</td>
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<tr>
<td>(±)-5b</td>
<td>36</td>
<td>(+)-5b (77°C; 73) and (-)-7b (82°C; 87)</td>
<td>5b: +24.5</td>
<td>(+)-7b: +53.0</td>
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<tr>
<td>(±)-5c</td>
<td>48</td>
<td>(+)-5c (62-63°C; 77) and (-)-7c (94°C; 77)</td>
<td>5c: +16.6</td>
<td>(+)-7c: +30.6</td>
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<tr>
<td>(±)-5d</td>
<td>42</td>
<td>(+)-5d (oil; 60) and (-)-7d (semi-solid; 81)</td>
<td>5d: +33.6</td>
<td>(+)-7d: +28.4</td>
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<tr>
<td>(±)-5e</td>
<td>96</td>
<td>(+)-5e (110°C; 77) and (-)-7e (85-86°C; 70)</td>
<td>5e: +1.0</td>
<td>(+)-7e: +4.0</td>
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<tr>
<td>(±)-6a</td>
<td>26</td>
<td>(-)-6a (105-106°C; 76) and (+)-8a (97°C; 78)</td>
<td>6a: -145.7</td>
<td>-</td>
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<tr>
<td>(±)-6b</td>
<td>26</td>
<td>(-)-6b (98-100°C; 75) and (+)-8c (80-82°C; 79)</td>
<td>6c: -151.3</td>
<td>-</td>
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<tr>
<td>(±)-6c</td>
<td>30</td>
<td>(-)-6c (84-85°C; 72) and (+)-8d (120-122°C; 79)</td>
<td>6d: -12.0</td>
<td>-</td>
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<tr>
<td>(±)-6d</td>
<td>52</td>
<td>(-)-6d (18°C; 71) and (+)-8e (semi-solid; 84)</td>
<td>6e: -78.0</td>
<td>-</td>
</tr>
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</table>

a) All these reactions, when performed under identical conditions, but without adding Candida rugosa lipase, did not yield any product.

b) Yields are calculated by assuming corresponding single enantiomer as 100% in the starting (±)-isoxazolidines 5a-e and 6a, 6c-e.

Table II—Antioxidant and antimycobacterial activities of isoxazolidines

<table>
<thead>
<tr>
<th>Compd</th>
<th>% of inhibition of NADPH-dependent liver microsomal lipid peroxidation</th>
<th>% of inhibition of Mycobacterium tuberculosis at MIC level of 6.25 μg/mL</th>
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<tr>
<td>5a</td>
<td>62.8</td>
<td>-</td>
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<tr>
<td>5b</td>
<td>40.9</td>
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<tr>
<td>5c</td>
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<td>5d</td>
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<td>5e</td>
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<tr>
<td>6a</td>
<td>-</td>
<td>59</td>
</tr>
<tr>
<td>6b</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>6c</td>
<td>12.8</td>
<td>83</td>
</tr>
<tr>
<td>6d</td>
<td>53.8</td>
<td>91</td>
</tr>
<tr>
<td>6e</td>
<td>90.8</td>
<td>99</td>
</tr>
</tbody>
</table>

*Compound 5e has not been tested for anti-tubercular activity.

Encouraged by the antimicrobial activities of different types of heterocyclic compounds, we tested the antimycobacterial activity of isoxazolidines 5a, 5b, 5d, 5e and 6a-e against Mycobacterium tuberculosis H37Rv. Isoxazolidines of the series 5 did not show any antitubercular activity, however, 6a-e showed some activity (Table II). Interestingly compound 6e was found to have moderate antimycobacterial activity.

Conclusions

To conclude, the present study has shown interesting and potentially useful enantioselectivity during CRL catalyzed deacetylation of acetoxy isoxazolidines in organic solvents. As it is difficult to synthesize such compounds in optically pure form by purely chemical methods, the biocatalytic approach reported here should find utility in the synthesis of new optically enriched bioactive isoxazolidines. Further, some of these compounds have exhibited considerable antioxidant as well as antitubercular activities, interestingly the compound 6e showed very high antioxidant activity as well as antimycobacterial activity (> 90% inhibition) in each case.

Experimental Section

Reactions were monitored either by TLC on Merck silica gel 60F254 aluminium plates by visualizing the
isoxazolidines 5a-e and 6a-e were fully characterized on the basis of their spectral data. The structures of the known isoxazolidines 6a and 6e were confirmed on the basis of the comparison of their mp’s and spectral data with those reported in the literature.

(±)-5-Acetoxymethyl-2,3-diphenylisoxazoline 5a: It crystallized from benzene as a white solid (2.55 g) in 72% yield; Rf 0.36 (ethyl acetate-petroleum ether, 1:9); IR (KBr, cm⁻¹): 2362, 1731 (COCH₃), 1597, 1488, 1450, 1368, 1258, 1230, 1048, 913, 766; ¹H NMR (300 MHz, CDCl₃): δ 2.03 (3H, s, COCH₃), 2.24 (1H, ddd, J=14.7, 9.4, 7.5 Hz, C-4H₆), 2.92 (1H, ddd, J=14.7, 9.9, 8.7 Hz, C-4H₄), 4.25 (1H, dd, J=18.0, 6.0 Hz, C-6H), 4.33 (1H, dd, J=18.0, 4.5 Hz, C-6H), 4.45-4.54 (1H, m, C-5H), 4.79 (1H, dd, J=9.9, 7.5 Hz, C-3H), 6.91-7.03 (3H, m, C-2'H and C-4'H and C-6'H), 7.19-7.39 (5H, m, C-3'H, C-5'H, C-3''H, C-4''H and C-5''H), 7.45-7.50 (2H, m, C-2''H and C-6''H); ¹³C NMR (75.5 MHz, CDCl₃): δ 20.70 (COCH₃), 41.72 (C-4), 64.27 (C-6), 70.00 (C-5), 75.93 (C-3), 114.86 (C-2' and C-6'), 121.99 (C-4'), 126.30 (C-3' and C-5'), 127.36 (C-4''), 128.75 (C-3'' and C-5''), 128.84 (C-2'' and C-6''), 142.06 (C-1''), 151.39 (C-1'), 170.80 (CO); MS: m/z (EI) 297(M⁺, 100%), 297 (3), 237 (3), 219 (6), 194 (11), 182 (92), 180 (38), 147 (8), 129 (60), 104 (25), 91 (55), 77 (40), 43 (30); HRMS (m/z): Calcd for C₁₉H₂₁N0₄: 297.1365. Found: 297.1394.

(±)-5-Acetoxymethyl-3-(4-methoxyphenyl)-2-phenylisoxazoline 5b: It crystallized from benzene as a white solid (2.87 g) in 73% yield; Rf 0.40 (ethyl acetate-petroleum ether, 1:4); IR (KBr, cm⁻¹): 2365, 1734 (COCH₃), 1598, 1513, 1491, 1478, 1383, 1285, 1256, 1183, 1105, 1030, 979, 839; ¹H NMR (300 MHz, CDCl₃): δ 2.06 (3H, s, COCH₃), 2.20 (1H, ddd, J=14.7, 7.9, 6.6 Hz, C-4H₆), 2.88 (1H, ddd, J=14.7, 8.2, 7.3 Hz, C-4H₄), 3.81 (3H, s, OCH₃), 4.25 (1H, dd, J=12.3, 7.2 Hz, C-6H), 4.27 (1H, dd, J=12.3, 3.6 Hz, C-6H), 4.46-4.54 (1H, m, C-5H), 4.71 (1H, dd, J=8.2, 6.6 Hz, C-3H), 6.88-7.01 (5H, m, C-2'H, C-3'H, C-4'H, C-5'H and C-6'H), 7.23 (2H, d, J=8.7 Hz, C-3''H and C-5''H), 7.37 (2H, d, J=8.7 Hz, C-2''H and C-6''H); ¹³C NMR (75.5 MHz, CDCl₃): δ 20.73 (COCH₃), 41.97 (C-4), 55.32 (OCH₃), 64.53 (C-6), 69.71 (C-5), 75.91 (C-3), 114.26 (C-2' and C-6'), 115.21 (C-3' and C-5''), 122.12 (C-4'), 127.62 (C-3' and C-5'), 128.87 (C-2'' and C-6''), 134.03 (C-1''), 151.50 (C-1'), 159.09 (C-2'' and C-6''), 170.88 (CO); MS: m/z (EI) 327(M⁺, 43%), 267 (14), 210 (56), 184 (22), 177 (14), 167 (39), 159 (100), 134 (23), 121 (25), 104 (27), 91 (51), 77 (60); HRMS (m/z): Calcd for C₁₉H₂₃NO₄: 327.1471. Found: 327.1507.

General method of preparation of α-aryl, N-phenylnitrones 4a-e: To a solution of phenyl-hydroxylamine (2, 40 mmol, 4.36g) in ethanol (20 mL), appropriate aromatic aldehyde (3a-e, 40 mmol) was added and the reaction mixture was stirred at 25-28°C for 5-24 hr during which time yellow/white solid precipitated out. The reaction mixture was cooled in an ice-bath to complete the precipitation, crude solid filtered and recrystallized from ethanol to afford the nitrones 4a-e in 56 to 85% yields. All the nitrones 4a-e were identified on the basis of their spectral data and by comparison of their mp’s and spectral data with those reported in the literature.

General method of preparation of isoxazolidines 5a-e and 6a-e: The mixture of α-aryl-N-phenylnitrene (4a-e, 12 mmol) and allyl acetate or vinyl acetate (12 mmol) in anhydrous benzene (20 mL) was stirred at 60°C for 10-15 hr. On completion of reaction, solvent was removed under vacuum and the product isolated either by crystallization from benzene or by column chromatography to afford the pure isoxazolidines in 66-77% yields. All the ten isoxazolidines 5a-e and 6a-e were fully characterized.
(±)-5-Acetoxymethyl-3-(4-chlorophenyl)-2-phenylisoxazolidine 5c: It crystallized from benzene as a white solid (3.07 g) in 77% yield; Rf. 0.47 (ethyl acetate-petroleum ether, 1:4); IR (KBr, cm⁻¹): 3262, 1729 (COCH₃), 1596, 1514, 1490, 1348, 1250, 1110, 1033, 854, 777; ¹H NMR (300 MHz, CDCl₃): δ 2.03 (3H, s, COCH₃), 2.18 (1H, dd, J=12.6, 18.1, 6.3 Hz, C-4H₄), 2.90 (1H, dd, J=12.6, 8.1, 7.2 Hz, C-4H₄), 4.22 (1H, dd, J=12.6, 6.6 Hz, C-6H₄), 4.26 (1H, dd, J=12.0, 3.3 Hz, C-6H₄), 4.46-4.55 (1H, m, C-5H₄), 4.77 (1H, dd, J=8.1, 6.3 Hz, C-3H₄), 6.92-7.00 (3H, m, C-2H₄, C-4H₄ and C-6H₄), 7.22-7.27 (2H, m, C-3’H and C-5’H), 7.35 (2H, d, J=8.4 Hz, C-3’H and C-5’H), 7.41 (2H, d, J=8.4 Hz, C-2’H and C-6’H); ¹³C NMR (75.5 MHz, CDCl₃): δ 20.66 (COCH₃), 41.60 (C-4), 64.18 (C-6), 66.39 (C-5), 76.05 (C-3), 114.98 (C-2’ and C-5’), 122.32, 122.85 (C-3’ and C-5’), 128.99 (C-3’ and C-5’), 129.02 (C-2’ and C-6’), 133.24 (C-4’), 140.76 (C-1’), 151.19 (C-1’), 170.80 (CO); MS: m/z (EI) 331 (M⁺, 62%), 271 (5), 216 (85), 181 (10), 163 (35), 129 (25), 104 (20), 91 (100), 77 (31); HRMS (m/z): Calcd for C₁₉H₁₅BrNO₃: 331.0975. Found: 331.0999.

(±)-5-Acetoxymethyl-3-(3-bromophenyl)-2-phenylisoxazolidine 5d: It was purified by flash column chromatography on silica gel using ethyl acetate-petroleum ether (1:4) as eluent to afford the pure compound as an oil (2.97 g) in 66% yield; Rf. 0.47 (ethyl acetate-petroleum ether, 1:4); IR (KBr, cm⁻¹): 3301, 1741 (COCH₃), 1596, 1514, 1490, 1348, 1250, 1110, 1033, 854, 777; ¹H NMR (300 MHz, CDCl₃): δ 2.03 (3H, s, COCH₃), 2.18 (1H, dd, J=12.6, 18.1, 6.3 Hz, C-4H₄), 2.90 (1H, dd, J=12.6, 8.1, 7.2 Hz, C-4H₄), 4.22 (1H, dd, J=12.6, 6.6 Hz, C-6H₄), 4.26 (1H, dd, J=12.0, 3.3 Hz, C-6H₄), 4.46-4.55 (1H, m, C-5H₄), 4.77 (1H, dd, J=8.1, 6.3 Hz, C-3H₄), 6.92-7.00 (3H, m, C-2H₄, C-4H₄ and C-6H₄), 7.22-7.27 (2H, m, C-3’H and C-5’H), 7.35 (2H, d, J=8.4 Hz, C-3’H and C-5’H), 7.41 (2H, d, J=8.4 Hz, C-2’H and C-6’H); ¹³C NMR (75.5 MHz, CDCl₃): δ 20.66 (COCH₃), 41.60 (C-4), 64.18 (C-6), 66.39 (C-5), 76.05 (C-3), 114.98 (C-2’ and C-5’), 122.32, 122.85 (C-3’ and C-5’), 128.99 (C-3’ and C-5’), 129.02 (C-2’ and C-6’), 133.24 (C-4’), 140.76 (C-1’), 151.19 (C-1’), 170.80 (CO); MS: m/z (EI) 331 (M⁺, 62%), 271 (5), 216 (85), 181 (10), 163 (35), 129 (25), 104 (20), 91 (100), 77 (31); HRMS (m/z): Calcd for C₁₉H₁₅BrNO₃: 331.0975. Found: 331.0999.

(±)-Acetoxy-3-(4-methoxyphenyl)-2-phenylisoxazolidine 6b: It crystallized from benzene as a white solid (2.81g) in 75% yield; Rf 0.37 (ethyl acetate-petroleum ether, 1:4); IR (KBr, cm⁻¹): 3261, 1736 (COCH₃), 1600, 1513, 1492, 1234, 1174, 1037, 990, 971, 841; ¹H NMR (300 MHz, CDCl₃): δ 2.13 (3H, s, COCH₃), 2.52 (1H, ddd, J=13.8, 6.9, 2.1 Hz, C-4H₄), 3.19 (1H, ddd, J=13.8, 9.3, 6.3 Hz, C-4H₄), 3.81 (3H, s, OCH₃), 4.33 (1H, dd, J=9.3, 6.9 Hz, C-3H₄), 6.57 (1H, dd, J=6.3, 2.1 Hz, C-5H₄), 6.92 (2H, d, J=8.7 Hz, C-3’H and C-5’H), 6.93-7.01 (3H, m, C-2’H, C-4’H and C-6’H), 7.14-7.20 (2H, m, C-3’H and C-5’H), 7.39 (2H, d, J=8.7 Hz, C-2’H and C-6’H); ¹³C NMR (75.5 MHz, CDCl₃): δ 21.32 (COCH₃), 46.28 (C-4), 55.30 (OCH₃), 69.30 (C-3), 94.57 (C-5), 114.36 (C-3’ and C-5’), 117.43 (C-2’ and C-6’), 123.53 (C-3’ and C-5’), 128.60 (C-2’ and C-6’), 132.07 (C-1’), 149.62 (C-1’), 159.46 (C-4’), 170.49 (CO); MS: m/z (EI) 313 (M⁺, 26%), 254 (9), 224 (4), 210 (11), 163 (100), 135 (60), 121 (3), 105 (8), 91 (15), 77 (29).

(±)-Acetoxy-3-(4-chlorophenyl)-2-phenylisoxazolidine 6c: It crystallized from benzene as a white solid (2.83 g) in 75% yield; Rf 0.51 (ethyl acetate-petroleum ether, 1:4); IR (KBr, cm⁻¹): 3469, 3261, 1745 (COCH₃), 1593, 1488, 1236, 1077, 994, 761; ¹H NMR (300 MHz, CDCl₃): δ 2.10 (3H, s, COCH₃), 2.47 (1H, ddd, J=15.5, 6.0, 1.7 Hz, C-4H₄), 3.20 (1H, ddd, J=15.5, 9.6, 6.3 Hz, C-4H₄), 4.41 (1H, dd, J=9.6, 6.0 Hz, C-3H₄), 6.58 (1H, dd, J=6.3, 1.7 Hz, C-
5H), 6.93-7.03 (3H, m, C-2'H, C-4'H and C-6'H), 7.17-7.23 (2H, m, C-3'H and C-5'H), 7.37 (2H, d, J=8.1 Hz, C-3''H and C-5''H), 7.44 (2H, d, J=8.1 Hz, C-2''H and C-6''H); 13C NMR (75.5 MHz, CDCl3): δ 21.31 (COCH3), 45.83 (C-4'), 68.56 (C-3), 94.45 (C-5), 117.10 (C-2' and C-6'), 127.58 (C-4'), 128.65 (C-3', C-5', C-2'' and C-6''), 129.23 (C-3'' and C-5''), 133.64 (C-4''), 139.01 (C-1''), 149.31 (C-1''), 170.38 (CO); MS: m/z (EI) 319.5 [M+2], 236, 174, 154, 137, 102, 81, 70, 69, 68, 58, 57, 43, 31, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1.

(±)-5-Acetoxy-3-(3-bromophenyl)-2-phenylisoxazolidine 6d: It was crystallized from benzene as a white crystalline solid (3.11 g) in 72% yield; Rf 0.44 (ethyl acetate-petroleum ether, 1:4); IR (KBr, cm⁻¹): 3232, 1746 (COCH3), 1594, 1487, 1340, 1259, 1228, 1180, 1082, 1040, 991; 1H NMR (300 MHz, CDCl3): δ 2.01 (3H, s, COCH3), 2.18 (1H, ddd, J=13.5, 3.7, 1.2 Hz, C-4'Ha), 3.18 (1H, ddd, J=13.5, 9.6, 6.3 Hz, C-4'Hb), 3.75 (1H, q, J=7.2 Hz, C-5'H), 6.93-7.02 (3H, m, C-2'H, C-4'H and C-6'H), 7.18-7.25 (3H, m, C-3'H, C-5'H and C-6'H), 7.41-7.46 (2H, m, C-4''H and C-5''H), 7.72-7.77 (1H, m, C-2''H); 13C NMR (75.5 MHz, CDCl3): δ 21.24 (COCH3), 45.60 (C-4'), 68.38 (C-3), 94.57 (C-5'), 116.85 (C-2' and C-6'), 123.08 (C-3''), 129.05 (C2''), 128.76 (C-3' and C-5'), 130.34, 130.48, 131.06 (C-4'' and C-5''), 143.28 (C-1''), 149.54 (C-1''), 170.29 (CO); MS: m/z (EI) 362 [M⁺, 27%], 319 (24), 302 (16), 276 (16), 260 (67), 236 (10), 213 (12), 193 (20), 183 (42), 163 (12), 104 (78), 91 (35), 77 (100); HRMS (m/z): Caled for C18H14BrO3: 361.0314. Found: 361.0339.

General procedure for enzymatic deacetylation of isoxazolines 5a-e, 6a and 6c-e: To a solution of (±)-acetoxyethyl-acetoxyisoxazoline 5a-e or 6a, 6c-e (1.6 mmol) in anhydrous diisopropyl ether (20 mL) or a mixture of diisopropyl ether and THF (in case of isoxazoline 5e and 6e) containing n-butanol (3 mol eq.), the enzyme Candida rugosa lipase (200 mg) was added. The suspension was stirred at 40-42°C and progress of the reaction was monitored periodically by TLC/HPLC. When 50% conversion of the starting material to the product was reached (26-52hr), enzyme was filtered off and the solvent evaporated in vacuo to afford the crude product which was purified by column chromatography using a gradient mixture of petroleum ether-ethyl acetate to yield the optically enriched enzymatically deacetylated hydroxymethyl-hydroxyisoxazolines (-)-7a-e and (+)-(8a, 8c-e), unreacted acetoxyethyl-acetoxyisoxazolines (+)-5a-e and (-)-6a, (-)-6c-e in 67-87% yield. All the enzymatic reactions reached the 50% conversion level in 26-52 hr, except the compound 5e which took 96 hr, thus showing that it is not a good substrate for the enzymatic resolution. It was also supported by the optical rotation values, which are too low for the reaction product of (±)-5e (see Table I). The enzymatically deacetylated (-)-5-hydroxymethylisoxazolines (-)-7a-e, (+)-(8a, 8c-e) and recovered, unreacted (+)-5-acetoxyisoxazolines 5a-e and (-)-5-acetoxyisoxazolines 6a, 6c-e were found identical with the corresponding data of chemically synthesized racemic 5-acetoxyisoxazolines (±)-5a-e and 5-acetoxyisoxazolines (±)-6a and (±)-6c-e, respectively.

(-)-5-Hydroxymethyl-2,3-diphenylisoxazolidine 7a: It was purified by flash column chromatography on silica gel using ethyl acetate-petroleum ether (1:9) as an eluent to afford the pure compound as a semi-solid (170 mg) in 83% yield. Rf 0.23 (ethyl acetate-petroleum ether, 1:4); [α]D²⁷⁻⁻⁻⁰.43 (c 0.80, CHCl3); IR (KBr, cm⁻¹): 3379 (OH), 1713, 1597, 1487, 1452, 1364, 1259, 1177, 1029, 756, 697; 1H NMR (400 MHz, CDCl3): δ 2.31 (1H, ddd, J=12.4, 8.8, 7.2 Hz, C-4'Ha), 2.82 (1H, ddd, J=12.4, 8.8, 6.8 Hz, C-4'Hb), 3.74 (1H, ddd, J=14.4 and 4.4, C-6'H), 3.85 (1H, ddd, J=14.4, 4.4 Hz, C-6'H), 4.39-4.45 (1H, m, H-5), 4.72 (1H, dd, J=8.8, 7.2 Hz, C-3'H), 6.92-7.01 (3H, m, C-2'H, C-4'H and C-6'H), 7.19-7.28 (3H, m, C-3'H, C-4''H and C-5''H), 7.32-7.37 (2H, m, C-3'H and C-5'H), 7.45-7.47 (2H, m, C-2''H and C-6''H); 13C NMR (100 MHz, CDCl3): δ 41.25 (C-4'), 66.80 (C-6), 71.32 (C-5'), 78.75 (C-3'), 115.49 (C-2' and C-6'), 122.16 (C-4'), 126.67 (C-3' and C-5'), 127.52 (C-4''), 128.80 (C-3' and C-5''), 129.05 (C-2'' and C-6''), 142.00 (C-1''), 151.50 (C-1'); MS: m/z (EI) 255 (M⁺, 10%), 233 (3), 194 (3), 182 (30), 167 (4), 139 (6), 125 (10), 111 (21), 97 (41), 91 (19), 83 (47), 71 (54), 57 (100); HRMS (m/z): Caled for C18H14NO2: 255.1259. Found: 255.1291.

(-)-5-Hydroxyethyl-3-(4-methoxyphenyl)-2-phenylisoxazolidine 7b: It was purified by flash column chromatography on silica gel using ethyl acetate-petroleum ether (1:4) as eluent to afford the pure compound as a solid (197 mg) in 87% yield; Rf 0.23
(ethyl acetate-petroleum ether, 1:4); [α]27D -53.3 (c 0.15, CHCl3); IR (Nujol, cm⁻¹): 3393 (OH), 1597, 1511, 1488, 1463, 1376, 1301, 1246, 1175, 1107, 1056, 1031, 859, 829, 765; ¹H NMR (300 MHz, CDCl3): δ 2.23 (1H, brs, OH), 2.32 (1H, ddd, J=12.8, 7.8, 5.1 Hz, C-4'H), 2.82 (1H, ddd, J=12.8, 8.0, 7.5 Hz, C-4'H), 3.71-3.86 (5H, m, OCH3 and C-6'H), 4.42-4.44 (1H, m, C-5'H), 4.65 (1H, dd, J=8.0, 5.1 Hz, C-3'H), 6.90 (2H, d, J=8.5 Hz, C-3'H and C-5'S'H), 6.92-7.01 (3H, m, C-2'H, C-4'H and C-6'H), 7.19-7.25 (2H, m, C-3'H and C-5'H), 7.36 (2H, d, J=8.5 Hz, C-2'H and C-6'H); ¹³C NMR (75.5 MHz, CDCl3): δ 40.62 (C-4), 54.86 (OCH3), 63.07 (C-6), 70.00 (C-5), 79.24 (C-3), 113.71 (C-2' and C-6'), 114.96 (C-3' and C-5'), 121.79 (C-4'), 127.24 (C-3' and C-5'), 128.37 (C-2' and C-6'), 133.36 (C-1'), 151.03 (C-1'), 158.49 (C-4'); MS: m/z (EI) 285 (M⁺, 88%), 252 (6), 224 (9), 212 (34), 177 (65), 159 (90), 144 (28), 135 (70), 121 (46), 104 (23), 91 (100), 77 (46), 65 (22).

(-)-3-(4-Chlorophenyl)-5-hydroxymethyl-2-phenylisoxazolidine 7c: It was purified by flash column chromatography on silica gel using ethyl acetate-petroleum ether (1:4) as eluent to afford the pure compound as a white solid (177 mg) in 77% yield; Rf 0.23 (ethyl acetate-petroleum ether, 1:4); [α]27D -30.2 (c 0.15, CHCl3); IR (Nujol, cm⁻¹): 3217 (OH), 2922, 2362, 1592, 1483, 1284, 1047, 1014, 845, 820, 752; ¹H NMR (300 MHz, CDCl3): δ 2.00 (1H, brs, OH), 2.33 (1H, ddd, J=12.4, 7.7 and 6.8, C-4'H), 2.86 (1H, ddd, J=12.8, 8.2, 7.2 Hz, C-4'Ha), 3.75 (1H, dd, J=12.2, 5.5 Hz, C-6'H), 3.89 (1H, dd, J=12.2, 2.9 Hz, C-6'H), 4.40-4.49 (1H, m, C-5'H), 4.73 (1H, dd, J=8.2, 6.8 Hz, C-3'H), 6.94-7.01 (3H, m, C-2'H, C-4'H and C-6'H), 7.20-7.29 (2H, m, C-3'H and C-5'H), 7.34 (2H, d, J=8.6 Hz, C-3'H and C-5'S'H), 7.40 (2H, d, J=8.6 Hz, C-2'H and C-6'H); ¹³C NMR (75.5 MHz, CDCl3): δ 40.59 (C-4), 63.22 (C-6), 70.32 (C-5), 78.89 (C-3), 115.35 (C-2' and C-6'), 122.66 (C-4'), 127.93 (C-3' and C-5'), 128.95 (C-2', C-3' and C-5'), 133.29 (C-2'), 140.76 (C-1'), 151.19 (C-1'); MS: m/z (EI) 291 [(M⁺)+, 20%), 289 (M⁺, 60), 273 (3), 216 (22), 181 (19), 163 (14), 125 (23), 104 (18), 91 (100), 77 (43); HRMS (m/z): Caled for C₁₆H₁₆BrNO₂: 333.0394. Found: 333.0390.

(-)-5-Hydroxymethyl-3-(4-nitrophenyl)-2-phenylisoxazolidine 7e: It was purified by flash column chromatography using ethyl acetate-petroleum ether (1:4) as eluent to afford the pure compound as a white solid (169 mg) in 70% yield; Rf 0.13 (ethyl acetate-petroleum ether; 1:4); [α]27D -1.2 (c 1.93, CHCl3); IR (Nujol, cm⁻¹): 3300 (OH), 2360, 1592, 1461, 1377, 1343, 1016, 855, 754, 668; ¹H NMR (250 MHz, CDCl3): δ 2.04 (1H, brs, OH), 2.33 (1H, ddd, J=12.5, 7.8, 6.3 Hz, C-4'H), 2.92 (1H, ddd, J=12.5, 8.3, 7.8 Hz, C-4'Ha), 3.73 (1H, ddd, J=12.3, 5.3 Hz, C-6'H), 3.91 (1H, ddd, J=12.3, 2.5 Hz, C-6'H), 4.44-4.51 (1H, m, C-5'H), 4.91 (1H, dd, J=8.3, 6.3 Hz, C-3'H), 6.97-7.01 (3H, m, C-2'H, C-4'H and C-6'H), 7.24-7.29 (2H, m, C-3'H and C-5'H), 7.68 (2H, d, J=8.6 Hz, C-2'H and C-6'H), 8.21 (2H, d, J=8.6 Hz, C-3'H and C-5'S'H); ¹³C NMR (75.5 MHz, CDCl3): δ 40.46 (C-4), 62.84 (C-6), 69.96 (C-5), 79.00 (C-3), 114.88 (C-2' and C-6'), 122.66 (C-4'), 124.12 (C-3' and C-5'), 127.32 (C-2' and C-6'), 129.16 (C-3' and C-5'), 147.37 (C-1'), 149.81 (C-4'), 150.89 (C-1'); MS: m/z (EI) 300 (M⁺, 7%), 299 (37), 269 (2), 243 (5), 227 (23), 195 (3), 181 (8), 146 (3), 136 (8), 109 (10), 91 (100), 77 (36).

(+)-5-Hydroxy-2,3-diphenylisoxazolidine (diaspective mixture, 8a): It was purified by flash column chromatography on silica gel using ethyl acetate-petroleum ether (1:4) as eluent to afford an equilibrium mixture of cis and trans compounds (2:7:1) as a white solid in 78% yield (150 mg); Rf 0.15 (ethyl acetate-petroleum ether; 1:4); [α]27D -27.1 (c 1.24, CHCl3); IR (Nujol, cm⁻¹): 3225 (OH), 1900, 1600, 1593, 1455, 1377, 1055, 870, 840, 756; ¹H NMR (400 MHz, CDCl3): δ 2.27 (1H, ddd, J=12.4, 7.2, 5.6 Hz, C-4'Ha), 2.81 (1H, ddd, J=12.4, 8.8, 7.2 Hz, C-4'Hb), 3.72 (1H, dd, J=12.8, 6.8 Hz, C-6'H), 3.86 (1H, dd, J=12.8, 4.0 Hz, C-6'H), 4.44-4.47 (1H, m, C-5'H), 4.72 (1H, dd, J=8.8, 5.6 Hz, C-3'H), 6.99-6.92 (3H, m, C-2'H, C-4'H and C-6'H), 7.19-7.26 (3H, m, C-3'H, C-5'S'H and C-6'S'H), 7.37-7.41 (2H, m, C-4'H and C-5'S'H), 7.63-7.65 (1H, m, C-2'H).
0.24 (ethyl acetate–petroleum ether, 1:4); [α]$_D$$^2$  +134.4 (c 0.83, CHCl$_3$); IR (Nujol, cm$^{-1}$): 3268 (OH), 1682, 1598, 1493, 1454, 1377, 1280, 1095, 1072, 1046, 752; $^1$H NMR (400 MHz, CDCl$_3$) of cis isomer: δ 2.37-2.48 (1H, m, C-4H), 7.32-7.47 (4H, m, C-3'H and C-5'H), 7.15-7.31 (2H, m, C-2'H and C-6'H), 7.46-7.52 (2H, m, C-2'H and C-6''H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) of trans isomer: δ 47.62 (C-4), 66.91 (C-3), 97.24 (C-5), 114.54 (C-2' and C-6'), 121.73 (C-3' and C-5'), 128.75 (C-2'' and C-6''), 129.18 (C-3'' and C-5''), 133.44 (C-4''), 140.09 (C-1''), 153.05 (C-1''); MS: m/z (EI) 277 [M+2]$,^2$ (20%), 275 (M', 56), 258 (6), 230 (8), 216 (10), 193 (6), 167 (48), 149 (6), 139 (83), 125 (8), 109 (41), 91 (100), 77 (85), 64 (12).

(+)-3-(3-Bromophenyl)-5-hydroxy-2-phenylisoxazolidine (diastereomeric mixture, 8d): It was purified by flash column chromatography using ethyl acetate–petroleum ether (1:4) as eluent to afford the equilibrium mixture of cis and trans compounds (1:8:1) as a white solid in 79% yield (202 mg); Rf 0.27 (ethyl acetate–petroleum ether, 1:4); [α]$_D$$^2$ +70.0 (c 0.60, CHCl$_3$); IR (Nujol, cm$^{-1}$): 3283 (OH), 1595, 1568, 1492, 1463, 1377, 1344, 1261, 1198, 1159, 1066, 1049, 941; $^1$H NMR (300 MHz, CDCl$_3$) of cis isomer: δ 2.34-2.47 (1H, m, C-4H), 3.07 (1H, d, J=13.2, 9.3, 5.7 Hz, C-4H), 4.44 (1H, d, J=9.6, 6.0 Hz, C-3H), 5.75-5.78 (1H, m, C-5H), 6.89-6.93 (3H, m, C-2'H, C-4'H and C-6'H), 7.18-7.24 (3H, m, C-3'H, C-5'H and C-6''H), 7.41-7.48 (2H, m, C-4''H and C-5''H), 7.70-7.71 (1H, m, C-2''H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) of cis isomer: δ 26.91 (C-4), 47.57 (C-3), 96.08 (C-5), 116.67 (C-2' and C-6'), 123.03 (C-4''), 123.20 (C-1''), 125.72 (C-6''), 129.54 (C-3'' and C-5''), 130.12, 130.92 and 131.23 (C-2'', C-4'' and C-5''), 143.28 (C-3''), 149.83 (C-1''); $^1$H NMR (300 MHz, CDCl$_3$) of trans isomer: δ 2.34-2.47 (1H, m, C-4H), 2.85 (1H, d, J=12.3, 7.2 Hz, 4-Ha), 4.85 (1H, dd, J=9.9, 7.2 Hz, C-3H), 5.80 (1H, d, J=3.9 Hz, C-5H), 6.89-6.93 (3H, m, C-2'H, C-4'H and C-6'H), 7.18-7.24 (3H, m, C-3'H, C-5'H and C-6''H), 7.41-7.48 (2H, m, C-4''H and C-5''H), 7.70-7.71 (1H, m, C-2''H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) of trans isomer: δ 29.70 (C-4), 46.85 (C-3), 97.23 (C-5), 114.22 (C-2' and C-6'), 121.57 (C-4'), 123.07 (C-1'), 125.09 (C-6'), 128.73 (C-3' and C-5'), 130.51, 130.60, 130.78 (C-2'', C-4'' and C-5''), 143.98 (C-3''), 152.98 (C-1''); MS: m/z (EI) 322 [M+2]$^2$ (6%), 320 (M', 5), 203 (5), 285 (21), 260 (18), 211 (14), 193 (6), 183 (38), 146 (8), 132 (12), 118 (8), 104 (74), 91 (86), 77 (100), 65 (12); HRMS (m/z): Caled for C$_{13}$H$_{14}$BrN$_2$: 319.0238. Found: 319.0239.
(+)-5-Hydroxy-3-(4-nitrophenyl)-2-phenylisoxazolidine (diastereomeric mixture, 8e): It was purified by flash column chromatography using ethyl acetate-petroleum ether (1:4) as eluent to afford the equilibrium mixture of cis and trans compounds (2.3:1) as a semi-solid in 84% yield (173 mg); Rf 0.11 (ethyl acetate-petroleum ether, 1:4); [α]D +131 (c 0.2, CHCl3); IR (Nujol, cm−1): 3207 (OH), 1739, 1684, 1602, 1513, 1461, 1377, 1190, 1062; ¹H NMR (300 MHz, CDCl3) of cis isomer: δ 2.34-2.51 (1H, m, C-4'H), 3.10 (1H, dd, J=13.3, 9.6, 5.8 Hz, C-4'H), 4.58 (1H, dd, J=9.6, 5.2 Hz, C-3'H), 5.80-5.81 (1H, m, C-5'H), 6.84-6.95 (3H, m, C-2'H, C-4'H and C-6'H), 7.18-7.30 (2H, m, C-3'H and C-5'H), 7.46 (2H, d, J=8.7 Hz, C-2''H and C-6''H), 8.24 (2H, d, J=8.7 Hz, C-3''H and C-5''H); ¹³C NMR (75.5 MHz, CDCl3) of cis isomer: δ 46.43 (C-4), 69.30 (C-3), 96.09 (C-5), 116.48 (C-2' and C-6'), 123.39 (C-4'), 124.15 (C-3' and C-5'), 128.05, 128.87 (C-3', C-4', C-2'' and C-6''), 148.87 (C-1'), 149.16 (C-4'), 149.76 (C-1'); ¹H NMR (300 MHz, CDCl3) of trans isomer: δ 2.34-2.51 (1H, m, C-4'H), 2.91 (1H, dd, J=12.4, 7.2 Hz, C-4'H), 4.98 (1H, dd, J=9.6, 7.3 Hz, C-3'H), 5.81 (1H, d, J=3.8 Hz, C-5'H), 6.84-6.95 (3H, m, C-2'H, C-4'H and C-6'H), 7.18-7.30 (2H, m, C-3'H and C-5'H), 7.68 (2H, d, J=8.7 Hz, C-2''H and C-6''H), 8.24 (2H, d, J=8.7 Hz, C-3''H and C-5''H); ¹³C NMR (75.5 MHz, CDCl3) of trans isomer: δ 47.39 (C-4), 68.59 (C-3), 97.21 (C-5), 114.23 (C-2' and C-6'), 121.87 (C-4'), 124.27 (C-3' and C-5'), 127.36, 129.06 (C-3', C-4', C-2'' and C-6''), 147.53 (C-1'), 149.16 (C-4'), 152.67 (C-1'); MS: m/z (El) 286 (M+, 100%), 270 (17), 253 (10), 241 (14), 227 (23), 205 (6), 179 (30), 160 (10), 150 (31), 131 (10), 109 (65), 91 (68), 77 (74).

General procedure for the enzymatic deacetylation of optically enriched unreacted, recovered (+)-5-acetoxyethylisoxazolidines 5a-e. To a solution of the (+)-5-acetoxyethylisoxazolidine (5a-e, 100 mg) in anhydrous disopropyl ether (5 mL) containing n-butanol (3-4 equiv.) was added Candida rugosa lipase (70 mg) and the suspension was stirred at 40-42°C in an incubator, and progress of the reaction was monitored by TLC. On completion (100-120 hr), the enzyme was filtered off and solvent removed under reduced pressure to afford the (+)-5-hydroxyethylisoxazolidines 7a-e in quantitative yields, whose spectral data completely matched with those of the corresponding (-)-7a-e reported earlier.

Antioxidant Activity

NADPH-catalysed liver microsomal lipid peroxidation. Detailed assay procedure has been described in our earlier communication. In short, rat liver microsomes (1 mg protein) were preincubated with Tris-HCl (0.025M, pH 7.5) and test compound (100 mM in DMSO) was added at 37°C for 10 min, followed by the addition of ADP (3 mM) and FeCl₃ (0.15 mM). The reaction for inhibition of enzymatic lipid peroxidation was started by the addition of NADPH (0.5 mM) and incubation of the reaction mixture continued for 10 min. The products of lipid peroxidation were quantified by the estimation of thiobarbituric acid reactive substances (TBARS) thus formed as described in earlier communication. The inhibitory potential of various isoxazolidines were assessed by determining the inhibitory concentration for 50% inhibition of lipid peroxidation.

In vitro evaluation of antimycobacterial activity. Preliminary screening against Mycobacterium tuberculosis H₃₇Rv (ATCC 27294) was conducted at a concentration of 6.25 μg/mL in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA)31. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system.

X-ray crystallography. The crystallographic measurements on compounds 7c were made using a Siemens SMART area-detector diffractometer. Graphite monochromated Mo-Kα radiation was used in all cases. The structure was solved using SHELXTL-PLUS and refined with SHELXL-96 (ref. 33).

2-Phenyl-3-(4-chlorophenyl)-5-hydroxyethylisoxazolidine 7c. C₁₆H₁₃ClNO₂, M=289.76, Orthorhombic, a=11.5778(2), b=34.1598(5), c=7.55730(10) Å, V=2988.88(8) Å³, T=180(2) K, space group Ima2, Z=8, D₅ = 1.328 Mg/m³, μ = 0.261 mm⁻¹, F (000) = 1256. Crystal size 0.48 × 0.45 × 0.44mm; 0 range for data collection 1.86-23.29, limiting indices 1 ≤ h ≤ 14, -14 ≤ k ≤ 14, -10 ≤ l ≤ 10; reflections collected 9151; independent reflections 3481 [Rint = 0.0171]; refinement method full-matrix least-squares on F²; data/restraints/parameters 3481/3/202; goodness-of-fit on F² 1.069; R (F) [I > 2σ (I)] = 0.0267; wR =0.0697; largest diff. peak and hole 0.165 and -0.188 eÅ⁻³.

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References