

Preparation and Reactions of Optically Active Cyanohydrins Derived from 4- Chlorobenzaldehyde, Cyclohexanone and 2- Methylcyclohexanone using the (R) Hydroxynitrile lyase from *Prunus amygdalus*

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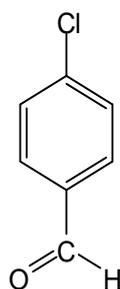
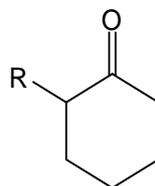
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CYANURATION of 4-chlorobenzaldehyde (1), cyclohexanone (2a) and 2-methylcyclohexanone (2b) yielded the racemic 2-hydroxy-2-(4-chlorophenyl)ethanenitrile (*R,S*)-3, cyclohexanone cyanohydrin 21a and (*R,S*)-2-methylcyclohexanone cyanohydrin (*R,S*)-21b. The same reaction can be completed by using acetone cyanohydrin (4) as a transcyanating agent. The optically active cyanohydrins (*R*)-3 and (*R*)-21b could be respectively obtained by hydrocyanation of 1 and 2b using (*R*)-hydroxynitrile lyase (*R*) PaHNL [EC 4.1.2.10] from almonds (*Prunus amygdalus*) as a chiral catalyst. Cyanohydrins 3 and 21 in their racemic and optically active forms undergo a number of transformations which involve either the hydroxyl group or the cyanide function. Moreover, derivatization of 3 and 21b with (*S*)-Naproxen chloride (*S*)-7 gave the respective diastereoisomers 8 and 22b. The optical activities of (*R*)-3 and 21b as well as their derivatives were recorded. The postulated structures of the new products were supported with compatible elementary and spectroscopic (IR, ¹H NMR, ¹³C NMR, MS and X-ray crystallography) analyses. The antitumor activity of some selected racemic new products and their respective optically active analogues were undertaken. The structure-activity relationship (SAR) was also discussed.

Keywords: Antitumor activity, Carbonyl compounds, Cyanohydrins, Enzymes and Stereochemistry.

Cyanohydrins are valuable key building blocks and expedient synthones for the one step synthesis of several classes of compounds such as α - hydroxycarboxylic acids⁽¹⁻⁶⁾ which display a vital role in organic syntheses⁽⁷⁻⁹⁾. Optically active cyanohydrins are also remarkable intermediates in organic synthesis and have received considerable amount of interest particularly in the last three decades^(1-3,10-12). A number of methods for the preparation of optically pure cyanohydrins have been developed using various chiral catalysts^(13,14) such as cyclic dipeptides^(15,16) as well as chiral complexes of titanium⁽¹⁷⁾, aluminum⁽¹⁸⁾

and boron⁽¹⁹⁾. The enantioselective preparation of cyanohydrins has also been performed enzymatically by means of hydroxynitrile lyases (oxynitrilases) from different plant sources^(3,20). This approach is rather precise, clean and cheap. It entails a high degree of stereoselectivity leading to optically pure chiral cyanohydrins^(1,21-24). The preparation of racemic and optically active cyanohydrins derived from 4-chlorobenzaldehyde (1), cyclohexanone (2a) and 2-methylcyclohexanone (2b) is the theme of the present study.

**1****2a**, R = H
b, R = CH₃

A comparative study on the antitumor activity of some newly prepared racemic products and their optically active analogues is also endeavored. This might be of a particular significance due to the well established correlation between the biological activity and stereochemical aspects^(6,25).

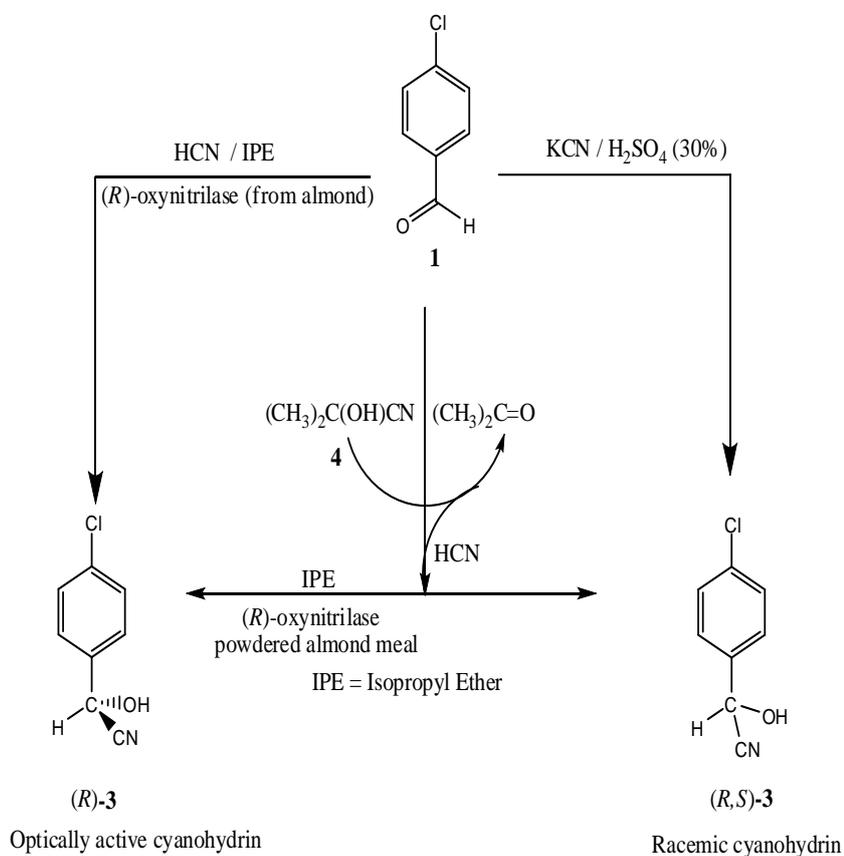
Results and Discussion

Hydrocyanation of 4-chlorobenzaldehyde (1)

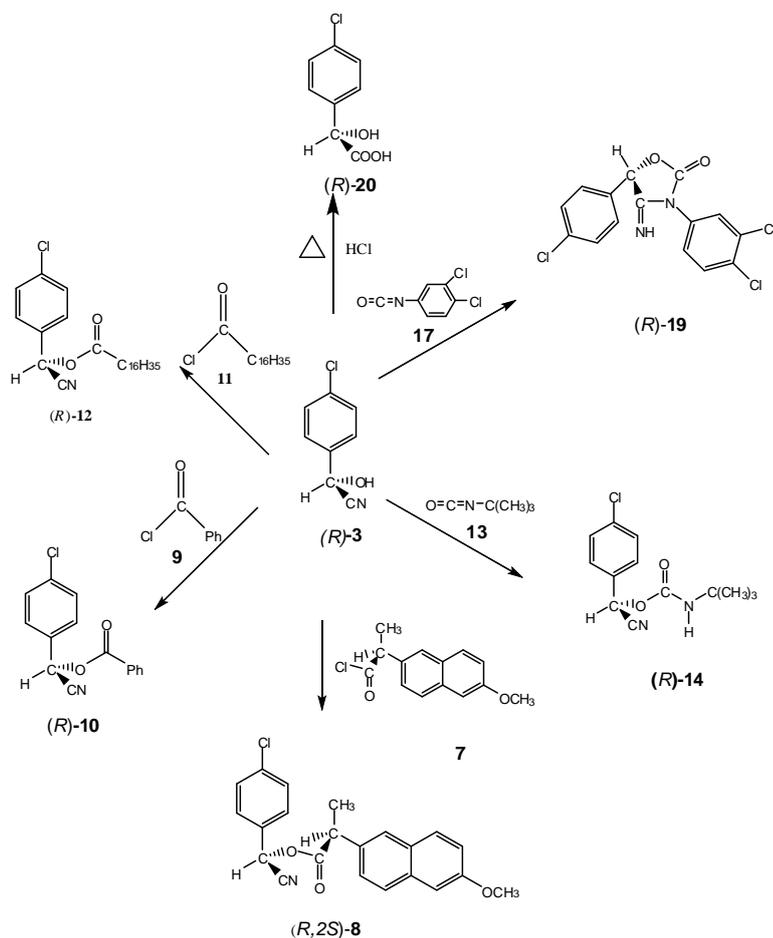
It has been now found that treatment of aldehyde 1 with aqueous potassium cyanide in presence of 30% aqueous sulphuric acid yields 2-hydroxyl-2-(4-chlorophenyl)ethane-nitrile (*R,S*) 3 in an 87 % yield. Formation of 3 can also be completed by using acetone cyanohydrin (4) as a transcyanating agent⁽²⁶⁾ (Scheme1).

The IR spectrum of (*R,S*) 3 (KBr, cm⁻¹) showed strong absorption bands at 3390 (O-H), 2254 (C≡N) and at 1595 (C=C aromatic). Its ¹H NMR spectrum (CDCl₃, δ ppm) showed signals at 4.69 (OH, D₂O exchangeable, bs) and at 5.48 (1H, HC-CN, s). The AB system due to the aromatic protons (4H) appeared as two doublets (each with J_{HH} = 8.4 Hz) at δ = 7.29 ppm and δ = 7.33 ppm. Similarly, (*R*)-2-hydroxyl-2-(4-chlorophenyl)ethane-nitrile (*R*)-3 could be obtained by cyanohydratation of 1 directly using (*R*)-oxynitrilase [EC 4.1.2.10]

from almonds which is a rich source of this enzyme,⁽²⁷⁾ or by using acetone cyanohydrin (4) as a transcyanating agent in the presence of powdered defatted almond meal as a catalyst. This meal provides an inexpensive catalyst; the use of which eliminates the need to purify and immobilize the enzyme⁽²⁶⁾. Compound (*R*)-3 was isolated as a yellow oil with $[\alpha]_{D,25} = +178$ and in an 98% yield. The (*R*) assignments for 3 and its derivatives are based on the Cahn–Ingold–Prelog (CIP) priority rule⁽²⁸⁾. Both of racemic (*R,S*)-3 and optically active (*R*)-3 forms of the cyanohydrin 3 undergo a number of transformations which involve either the hydroxyl group or the cyano-function in their molecules (Scheme 2).



Scheme 1

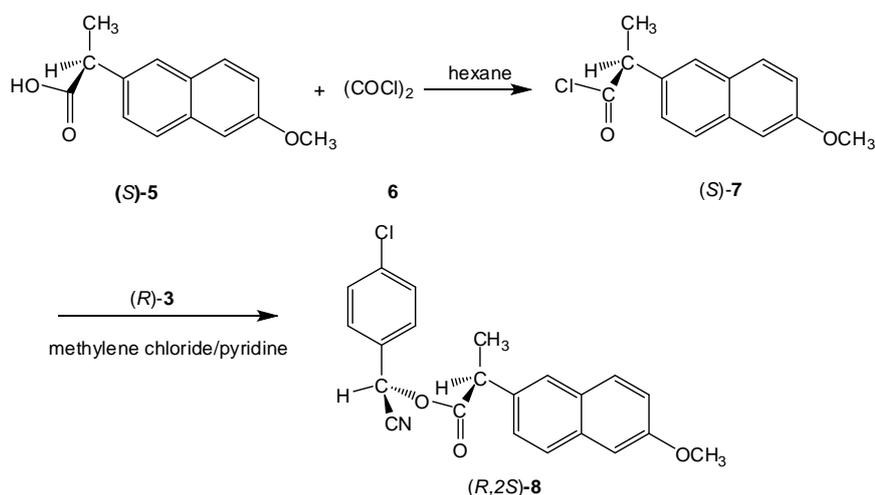


Scheme 2

Reactions of the hydroxyl group

The use of (*S*)-naproxen[®] (**5**) as a derivatizing agent to determine the optical purity of organic compounds⁽²⁹⁾ and as a chiral resolving agent for converting racemates to a mixture of diastereoisomers^(30,31) is very well known. Thus, it has been now found that derivatization of (*R*)-**3** with (*S*)-naproxen chloride (*S*)-**7** proceeds in CH_2Cl_2 in the presence of pyridine to give the respective diastereoisomer namely (2*S*)-((*R*)-(-)-(4-chlorophenyl)cyano) methyl-2-(6-methoxynaphthalene-2-yl)propanoate (*R,2S*)-**8** with a diastereomeric excess value (de) of 95% which reflects the enantiomeric excess and/or the optical purity of the starting cyanohydrin (*R*)-**3**. Naproxen[®] (**5**) could be obtained by extraction

from commercially available tablets⁽²⁹⁾ with chloroform. Treatment of (5) with oxalyl chloride (6) in hexane yields the acid chloride 7⁽²⁹⁾ (Scheme 3).



Scheme 3

Elementary and molecular weight determination (MS) for (*R,2S*)-8 corresponded to $C_{22}H_{18}ClNO_3$. Structural reasoning for (*R,2S*)-8 are:

(i) Its IR spectrum (KBr, cm^{-1}) disclosed the presence of strong absorption bands at 2935 (CH, aliphatic), 2238 ($C\equiv N$), 1744 ($C=O$, ester), 1624, 1604 ($C=C$, aromatic) and at 1247 ($C-O$, stretching).

(ii) Its 1H NMR spectrum (DMSO- d_6 , δ ppm) showed signals at 1.48 (3H, $C-CH_3$, d, $J_{HH} = 7.6$ Hz), 3.83 (3H, OCH_3 , s), 4.08 (1H, $C-CH$, q, $J_{HH} = 7.6$ Hz) and 7.26 (10H, aromatics) as a multiplet wherein emerge two doublets (each with $J_{HH} = 8.4$ Hz) due to protons of the 4-chlorophenyl moiety (4H) at $\delta = 7.32$ and 7.39 ppm.

(iii) The mass spectrum of (*R,2S*)-8 showed the molecular ion peak at m/z 379 (60 %, based on ^{35}Cl) and m/z 381 (20 % based on ^{37}Cl).

Derivatization of the hydroxyl groups through their reactions with acid chlorides gives the corresponding esters. Such protection of the hydroxyl group represents an important reaction in organic syntheses⁽³²⁾ as well as in gas chromatography analysis methods⁽³³⁾. Thus, benzylation of (*R*)-3 with benzoyl chloride (9) yielded (*R*)-(+)-(4-chlorophenyl)-cyanomethyl benzoate (*R*)-10 (Scheme 2). Similarly, treatment of (*R*)-3 with stearoyl chloride (11) in dry methylene chloride yielded (*R*)-(+)-(4-chlorophenyl)cyanomethyl stearate (*R*)-12 (Scheme 2).

Treatment of (*R*)-3 with *tert*-butylisocyanate (13) in THF in presence of a few drops of triethylamine (TEA) yielded a white crystalline substance formulated as (*R*)-(+)-(4-chlorophenyl)cyanomethyl *tert*-butylcarbamate (*R*)-(+)-14 (Scheme 2). An ORTEP overview of compound (*R*)-14 is represented in Fig. 1. The crystal structural data, selected bond lengths as well as bond angles of (*R*)-14 are represented in Tables 1, 2 and 3, respectively.

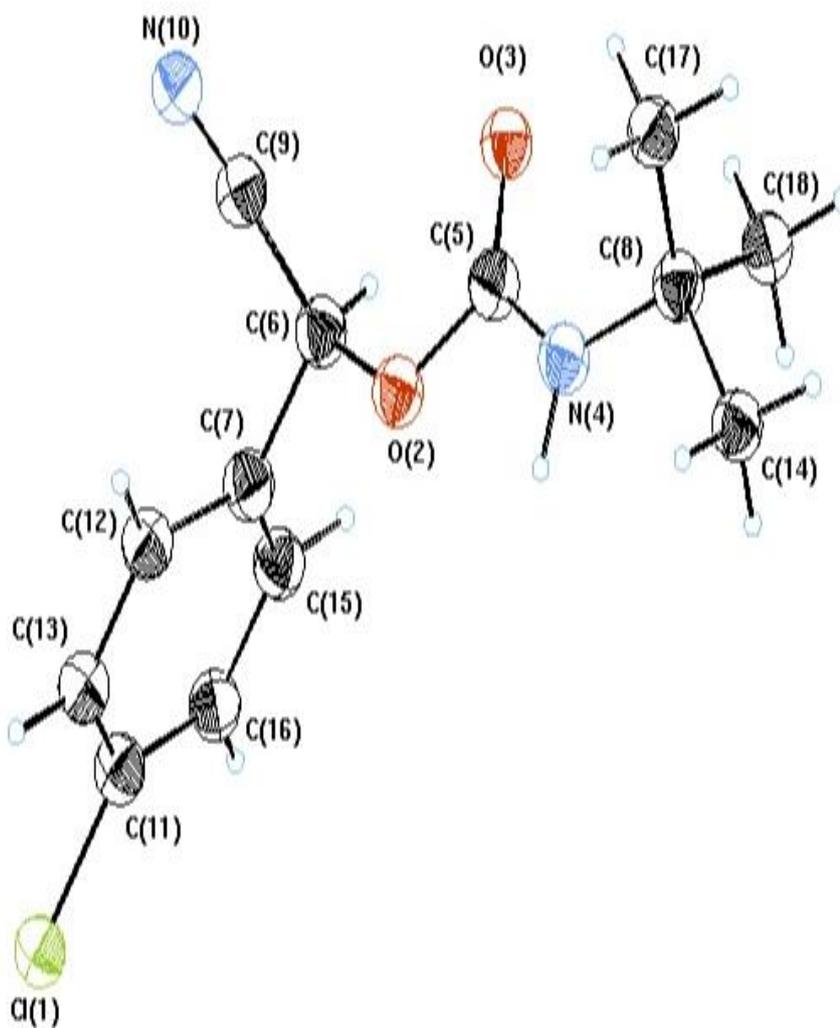


Fig. 1. An ORTEP overview of compound (*R*)-14.

TABLE 1. Crystal structure and data refinement parameters of compound (R)-14*.

Compound	(R)-14
Empirical Formula	C ₁₃ H ₁₅ ClN ₂ O ₂
Formula Weight	266.728
Crystal System / Space Group	Monoclinic / C2/c
a / Å	26.1653(8)
b / Å	11.3305(4)
c / Å	9.9275(4)
α / °	90.00
β / °	104.990(2)
γ / °	90.00
V / Å ³	2843.0(2)
Z	8
D _{calc} (g/cm ³)	1.246
μ (mm ⁻¹)	0.26
Colour / Shape	Colourless/Cube
Theta range for collection	2.910 – 27.485
Reflections collected	9858
Independent reflections	3622
Data / restraints / parameters	1763 / 0 / 163
Goodness of fit on F ²	1.989
Final R indices [I > 2σ(I)]	0.066
R indices (all data)	0.131
Largest difference peak / hole	0.48 / -0.52

*Temperature: 298 K, Wavelength: Mo Kα (0.71073 Å)

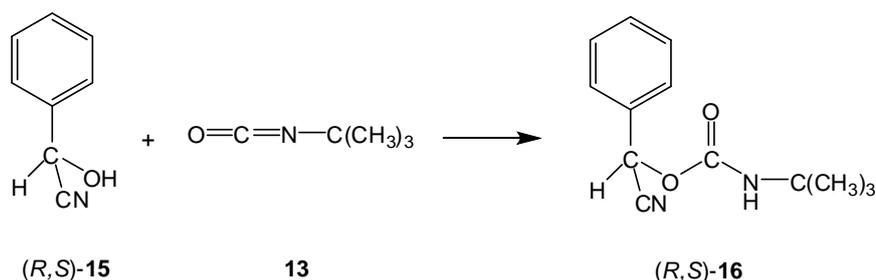
TABLE 2. Selected bond lengths (Å) of compound (R)-14.

Cl1 – C11	1.742 (3)	C7 – C15	1.366 (3)
O2 – C5	1.373 (2)	C8 – C14	1.510 (3)
O2 – C6	1.439 (2)	C8 – C17	1.479 (4)
O3 – C5	1.210 (2)	C8 – C18	1.534 (4)
N4 – C5	1.330 (3)	C9 – N10	1.135 (3)
N4 – C8	1.478 (3)	C11 – C13	1.369 (4)
C6 – C7	1.506 (3)	C11 – C16	1.373 (4)
C6 – C9	1.478 (3)	C12 – C13	1.381 (4)
C7 – C12	1.389 (3)	C15 – C16	1.380 (4)

TABLE 3. Selected bond angles (degree) of compound (*R*)-14.

C5 – O2 – C6	115.12 (14)	N4 – C8 – C17	111.2 (2)
C5 – N4 – C8	125.4 (2)	N4 – C8 – C18	109.4 (2)
O2 – C5 – O3	122.0 (2)	C14 – C8 – C17	109.8 (2)
O2 – C5 – N4	109.9 (2)	C14 – C8 – C18	106.3 (2)
O3 – C5 – N4	128.1 (2)	C17 – C8 – C18	112.9 (3)
O2 – C6 – C7	107.3 (2)	C6 – C9 – N10	177.3 (2)
O2 – C6 – C9	109.3 (2)	Cl 1 – C11 – C13	119.1 (2)
C7 – C6 – C9	112.3 (2)	Cl 1 – C11 – C16	120.0 (2)
C6 – C7 – C12	120.7 (2)	C13 – C11 – C16	120.8 (3)
C6 – C7 – C15	120.3 (2)	C7 – C12 – C13	120.4 (2)
C12 – C7 – C15	118.9 (2)	C11 – C13 – C12	119.4 (2)
N4 – C8 – C14	106.9 (2)	C7 – C15 – C16	121.1 (2)

Under similar conditions, the reaction of (*R,S*)-mandelonitrile⁽³⁴⁾ (*R,S*)-15 with isocyanate 13 yielded (*R,S*)-phenylcyanomethyl *tert*-butylcarbamate (*R,S*)-16.

**Scheme 4**

On the other hand, the reaction of (*R*)-3 with 3,4-dichlorophenyl isocyanate 17 yielded a yellow crystalline product formulated as (*5R*)-(+)-3-(3,4-dichlorophenyl)-5-(4-chlorophenyl)-4-iminoxazolidin-2-one (*R*)-19 (Scheme 5). Structural reasonings for (*R*)-19 are :

(i) Compatible elementary and molecular weight determination (MS) corresponded to $\text{C}_{15}\text{H}_9\text{Cl}_3\text{N}_2\text{O}_2$.

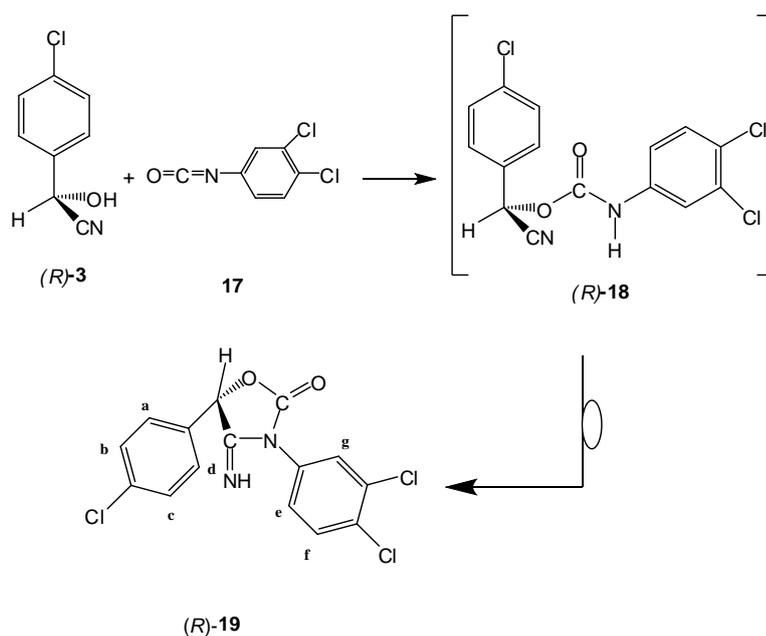
(ii) Its IR spectrum (KBr, cm^{-1}) showed strong absorption bands at 3293 (N–H), 3095 (C–H, aromatic), 1770 (C = O, lactone) and at 1594 (C=C, aromatic). The spectrum revealed the absence of (CN) group absorption around 2200 cm^{-1} . On the other hand, it showed a strong band at 1683 cm^{-1} due to the exocyclic C=N group absorption.

(iii) The ^1H NMR spectrum (CDCl_3 , δ ppm) revealed the presence of signals at 5.83 ppm (1H, CH-O,s), 7.35 – 7.60 (7H, aromatic protons a-g) as a multiplet

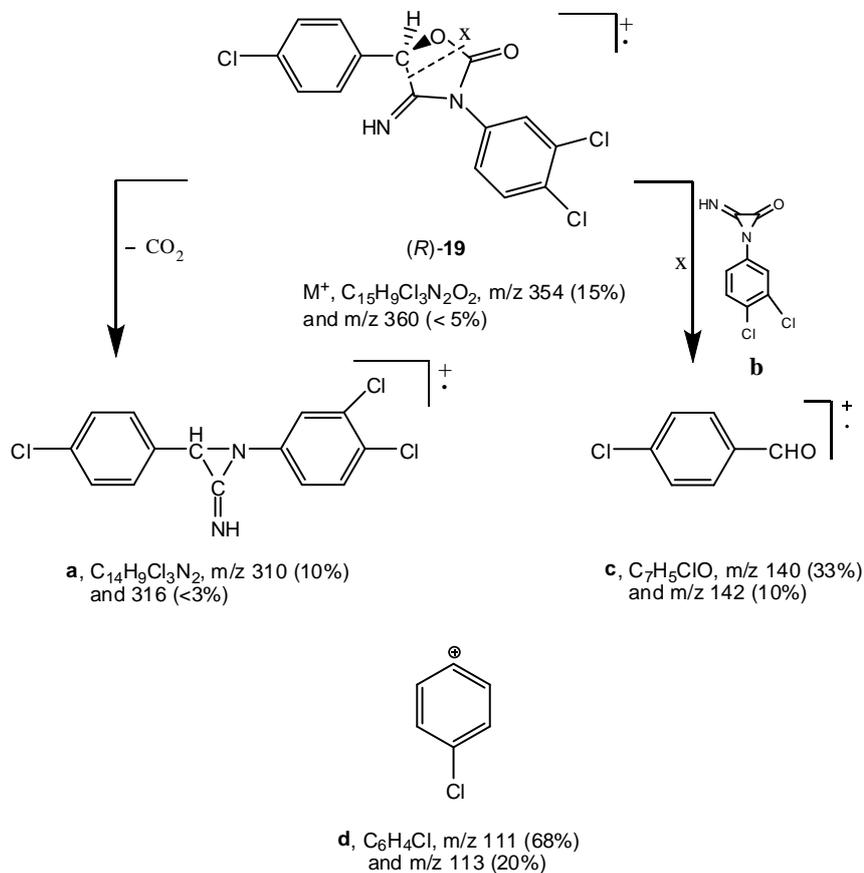
wherein emerge two doublets (each with $J_{\text{HH}} = 7.65 \text{ Hz}$) at 7.42 and 7.47 ppm due to protons a,d and b,c of the 4-chlorophenyl moiety (4H), respectively.

(iv) The mass spectrum of compound (*R*)-19 (Scheme 6) showed the molecular ion peak at m/z 354 (15% based on ^{35}Cl) and 360 (< 5% based on ^{37}Cl). Loss of carbon dioxide molecule from M^+ can afford the radical cation a at m/z 310 (10% based on ^{35}Cl) and 316 (<3% based on ^{37}Cl). The fragmentation of M^+ at axis x with loss of a molecule like b can give the radical cation c at m/z 140 (33% based on ^{35}Cl) and at 142 (10% based on ^{37}Cl). The spectrum also showed ion peaks at m/z 111 (68 %) and 113 (20 %) for the 4-chloro- phenyl cation d.

Apparently, the reaction of cyanohydrin (*R*)-3 with the isocyanate reagent 17 proceeds to give the respective carbamic acid ester (*R*)-18 which undergoes then an intramolecular rearrangement to yield the final product (*R*)-19 (Scheme 5).



Scheme 5



Scheme 6

Reactions of the cyanide function

Upon heating (*R*)-3 with concentrated hydrochloric acid, it yielded (*R*)-(-)-1-(4-chloro-phenyl)-1-hydroxyacetic acid (*R*)-20. Compatible elementary and molecular weight determination (MS) for (*R*)-20 corresponded to $C_8H_7ClO_3$.

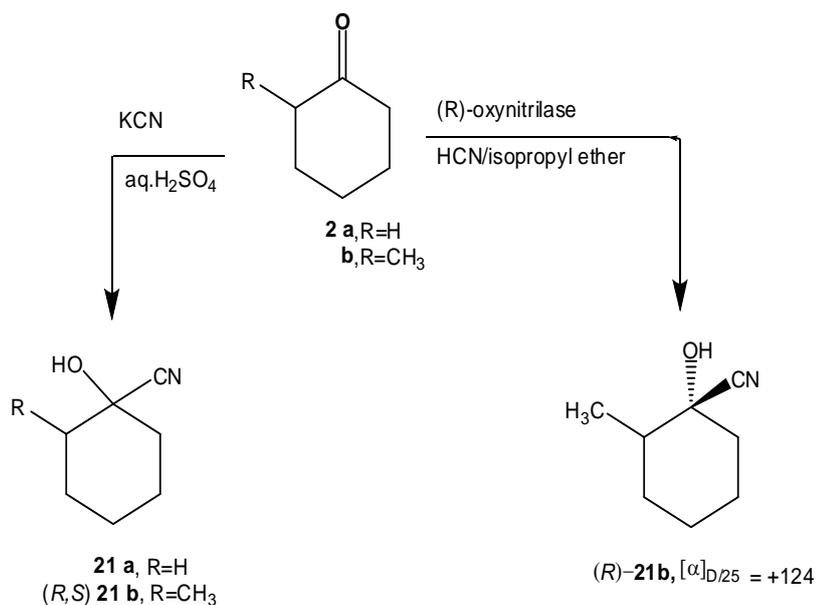
Structural reasonings for (*R*)-20 are :

a- Its IR spectrum (KBr, cm^{-1}) disclosed the presence of absorption bands at 3374 (O–H), 1671 (C=O, acid), 1591 (C=C, aromatic) and 1284 (C–O , stretching).

b- Its 1H NMR spectrum (DMSO- d_6 , δ ppm) showed signals at 4.82 (1H, CH methine, d, $J_{HH} = 3.8$ Hz; simplified to a singlet upon deuteration, 6.10 (1H, OH, D_2O exchangeable) and 7.42 (1H, OH, D_2O exchangeable). The AB system due to the 4-chlorophenyl nucleus (4H) gave two doublets (each with $J_{HH} = 8.4$ Hz) at 7.35 and 7.40 ppm.

Hydrocyanation of ketones 2a,b

The non-enzyme and enzyme catalyzed cyanuration of cyclohexanone 2a and 2-methyl cyclohexanone 2b afforded the respective racemic and optically active cyanohydrins (*R,S*)-21a and (*R*)-21b (Scheme 8).

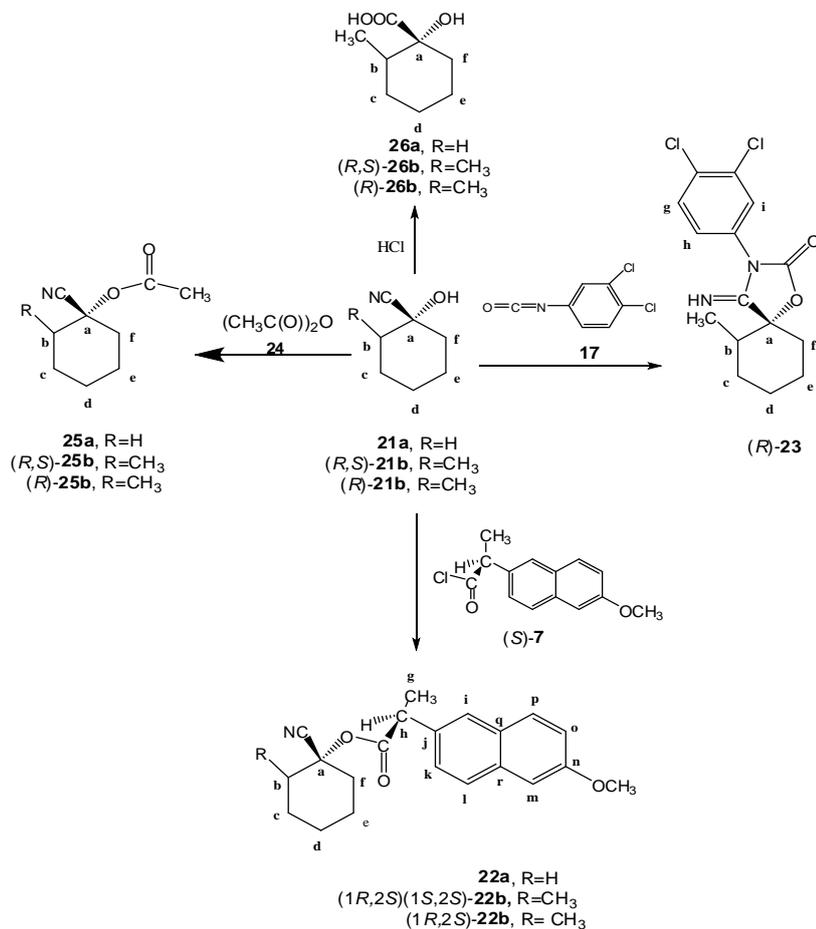
**Scheme 8**

The racemic (*R,S*)-21a,b and optically active (*R*)-21b forms undergo a number of transformations which involve either the hydroxyl group or the cyano-function in their molecules (Scheme 9). Thus, derivatization of (*R*)-21b with (*S*)-Naproxen chloride (*S*)-7 proceeded in CH_2Cl_2 in the presence of pyridine to give the respective diastereoisomer, namely, (*2S*)-((*1R*)-1-cyano-2-methylcyclohex-1-yl)-2-(6-methoxynaphthalen-2-yl)-propanoate (*1R,2S*)-22b. Elementary and molecular weight determination (MS) for (*1R,2S*)-22b corresponded to $\text{C}_{22}\text{H}_{25}\text{NO}_3$. Its structural reasonings are:

(i) The IR spectrum (KBr, cm^{-1}) disclosed the presence of strong absorption bands at 3057 (C–H, aromatic), 2936, 2862 (C–H, aliphatic), 2237 ($\text{C}\equiv\text{N}$); 1744 (C=O, ester), 1631, 1606 (C=C, aromatic) and 1222 (C–O, ester).

(ii) Its ^1H NMR spectrum (CDCl_3 , δ ppm) showed signals at 0.81 (3H, Cb- CH_3 , d, $J_{\text{HH}} = 6.9$ Hz), 1.56 ($\text{CH}_3\text{-CH-C}=\text{O}$, d, $J_{\text{HH}} = 6.9$ Hz), 3.84 ($\text{CH}_3\text{-CH-C}=\text{O}$, q, $J_{\text{HH}} = 6.9$ Hz) and 1.21 – 2.12 (8H, cyclohexyl protons c-f, m), 2.75 (1H, Cb-H, m), 3.91 (3H, OCH_3 , s) and 7.14 – 7.78 (6H, aromatic protons i,j-m,o,p, m).

(ii) The mass spectrum of (*1R,2S*)-22b showed the molecular ion peak at m/z 351 (10%).



Scheme 9

Treatment of *(R)*-**21b** with 3,4-dichlorophenyl isocyanate (**17**) yielded *(5R)*-3-(3,4-dichlorophenyl)-5-(2-methylcyclohex-1-yl)-4-iminoxazolidin-2-one *(R)*-**23**. Compatible elementary and molecular weight determination (MS) for *(R)*-**23**, corresponded to C₁₅ H₁₆ Cl₂N₂O₂. Its IR spectrum (KBr, cm⁻¹) showed strong absorption bands at 3422 (N–H), 3077 (C–H, aromatic), 2927, 2856 (C–H, aliphatic), 1727 (C = O, ester), 1677 (C=N, exocyclic) and 1590 (C=C, aromatic). Its ¹H NMR spectrum (DMSO-d₆, δppm) showed signals at 0.96 (3H, Cb-CH₃, d, J_{HH} = 6.9 Hz); 1.21 – 2.21 (8H, cyclohexyl protons c-f, m), 2.49 (Cb-H, m) and at 7.34, 7.40, 7.61 (3H, aromatic g,i,h protons) the NH proton gave a D₂O-exchangeable broad singlet at 6.09 ppm.

Acetylation of 21a, (*R,S*)-21b and (*R*)-21b with acetic anhydride 24 produced the respective esters 25a, (*R,S*)-25b and (*R*)-25b.

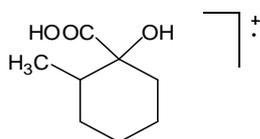
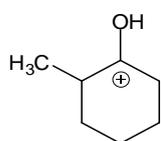
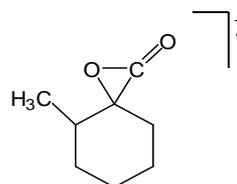
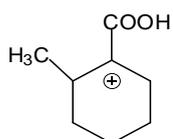
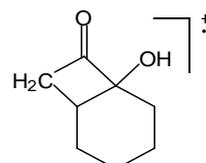
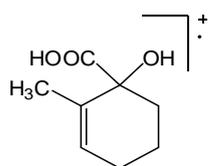
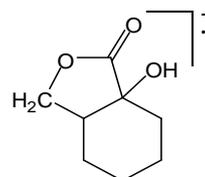
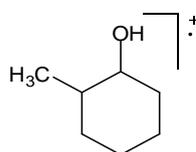
Upon heating 21a, (*R,S*)-21b and (*R*)-21b with concentrated hydrochloric acid, they yielded the respective hydroxycarboxylic acids 26a, (*R,S*)-26b and (*R*)-26b. For example, compatible elementary and molecular weight determination (MS) for (*R*)-(+)-1-hydroxy-2-methylcyclohexane carboxylic acid (*R*)-26b corresponded to C₈ H₁₄ O₃, MS *m/z*: 158 (20%). Its IR spectrum (KBr, cm⁻¹) showed strong absorption bands at 3458 and 3340 (O–H), 2949 and 2884 (C–H, aliphatic and alicyclic), 1705 (C=O) and at 1248 (C–O, acid). Its ¹H NMR spectrum (DMSO-d₆, δ ppm) showed signals at 6.25 (1H, OH, D₂O-exchangeable), 6.80 (1H, COOH, D₂O-exchangeable) 0.77(3H, Cb-CH₃, d, J_{HH} = 6.9 Hz), 0.89 – 1.89 (8H, cyclohexyl *c-f* protons, m) and 2.01 (1H, Cb-CH, m).

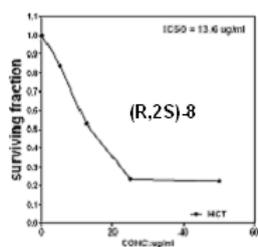
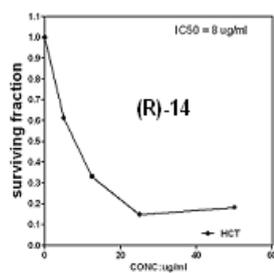
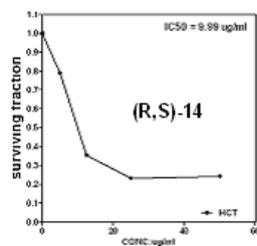
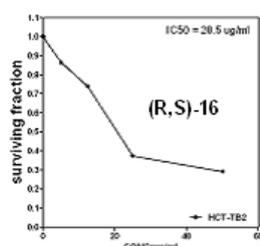
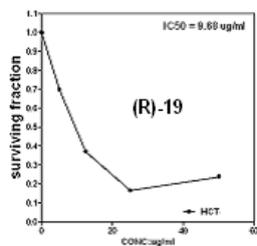
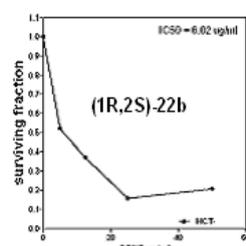
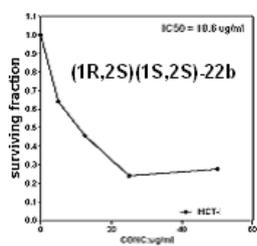
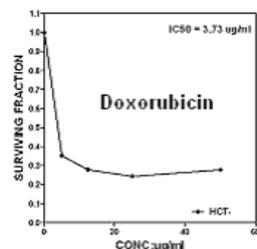
The mass spectrum of compound (*R*)-26b (Scheme 10) showed the molecular ion peak at *m/z* 158 (base peak). Loss of water molecule from M⁺ can afford the radical cation a (or b) at *m/z* 140. The molecular ion peak can also lose one hydrogen molecule to give radical cation e (or f) at *m/z* 156. Loss of COOH radical from M⁺ yields cation c at *m/z* 113. Meanwhile, loss of OH radical from M⁺ yields cation d at *m/z* 141. Cation g at *m/z* 114 can result *via* expulsion of CO₂ molecule from the molecular ion peak. This is evidenced by presence of a prominent ion at *m/z* 44 (86%) due to radical cation of CO₂ which is also frequent in the mass spectra of several carboxylic acid derivatives⁽⁶⁾.

Biological Results

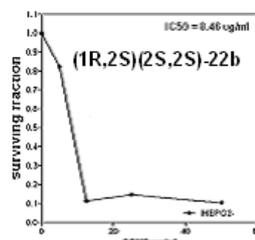
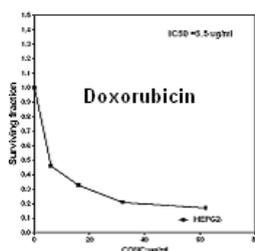
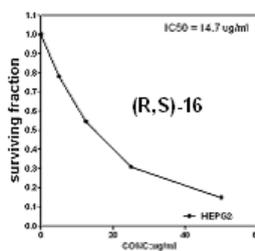
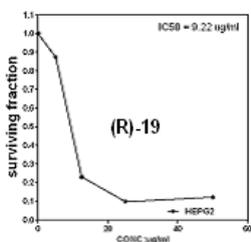
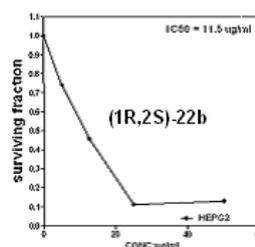
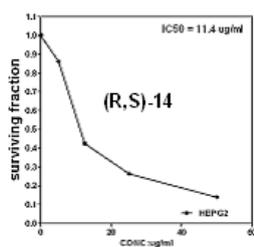
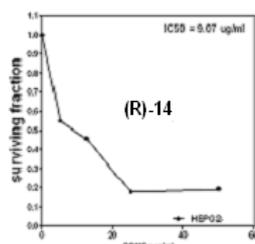
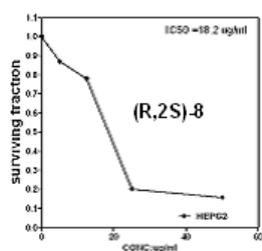
The anticancer activity of selected newly synthesized compounds was investigated. The evaluations for *in vitro* cytotoxicity of the investigated compounds were carried out against a panel of three human solid tumor cell lines which are HCT116 (colon carcinoma cell line), HEPG2 (liver carcinoma cell line) and MCF (breast carcinoma cell line). The sulphorhodamine-B method⁽³⁵⁾ was used for the assay of the cytotoxic activity and Doxorubicin⁽³⁶⁾ drug was used as a standard. The Doxorubicin cytotoxicity appears to be due to its ability to intercalate with DNA, interact with plasma membrane and to take part in oxidation-reduction reactions⁽³⁷⁾.

The investigation showed that there is a structure-activity relationship (SAR). For example, the screening of the investigated compounds against HCT-116 cell line showed that the optically active compound (*R*)-14 (Chart 2) is more active than its racemic form (*R,S*)-14 (Chart 3). Both (*R*)-14 and (*R,S*)-14 are more active than compound (*R,S*)-16 which show the effect of presence of a chlorine atom in this structure. Similarly, compound 22b in its optically active form (Chart 6) is more active than its racemic analogue (*1R,2S*)(*1S,2S*)-22b (Chart 7). Based on these results, there is a marked difference in the activity of a given optically active compound in comparison to that of its racemic form which reflects the influence of the optical purity on the biological activity.

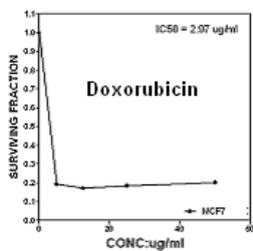
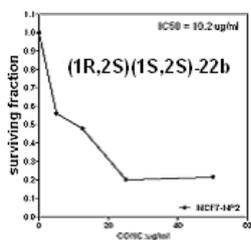
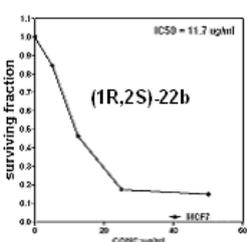
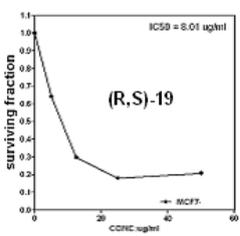
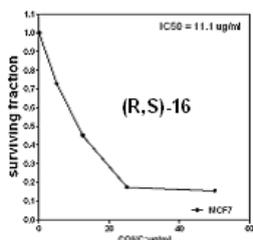
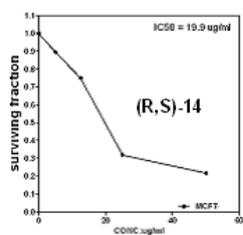
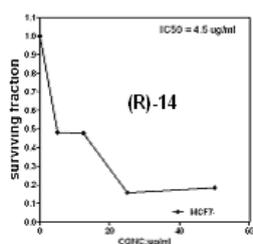
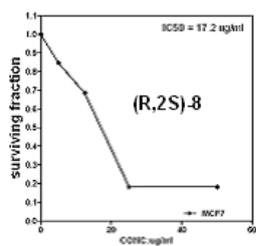
**(R)-26b**, M^+ , $C_8H_{14}O_3$, m/z 158 (100%, base peak)**c**, $C_{17}H_{13}O$, m/z 113 (85%)**a**, $C_8H_{12}O_2$, m/z 140 (87%)**d**, $C_8H_{13}O_2$, m/z 141 (27%)**b**, $C_8H_{12}O_2$, m/z 140 (87%)**e** $C_8H_{12}O_3$, m/z 156 (8%)**f****g**, $C_7H_{14}O$, m/z 114 (19%)**Scheme 10**

HCT-116**(Chart 1)****(Chart 2)****(Chart 3)****(Chart 4)****(Chart 5)****(Chart 6)****(Chart 7)****(Chart 8)**

HEPG-2



MCF-7



Experimental

Reactions with air-sensitive reagents were carried out in flame-dried glassware under dry argon atmosphere. The liquid reagents were added using the syringe technique. Solvents were rigorously dried and purified according to usual procedures. The reacting aldehydes and ketones were purified directly before use by distillation and/or crystallization. The almond meal and the isolated cyanohydrins were stored at -15 °C. (*R*)- Oxynitrilase enzyme (HNL) [EC4.1.2.10] was extracted⁽³⁸⁾, assayed and its activity was measured⁽³⁹⁾ according to the given procedures. The reactions were monitored (TLC) and the purity of the isolated products were controlled by using silica gel with fluorescent indicator F₂₅₄ coated on aluminum sheets of layer thickness 0.2 mm [Fluka]. The products were isolated and purified by preparative thin layer chromatography: on glass plates (20 × 20 cm) coated with silica gel 60 with fluorescent indicator F₂₅₄.

pH-Values were determined by Precisa Digital pH-Meter pH 900 with Ag/AgCl electrode. The activity of enzyme was measured by Shimadzu UV-2401 PC UB-VIS Recording Spectrophotometer. Optical Rotations were performed in: Carl Zeiss 212503 Polarometer, at The Chemical Industries Development Co. (CID) and / or ATAGO Polax-2L, USA, Code = OC50R₇ and A. K RÜSS, Opt. Ronic, Germany at the National Research Centre. $[\alpha]_{D/25} = \alpha / c \cdot d$, Path length (d) = 10 cm, Concentration (c) = 10 mg/ml α is the measured angle of rotation and $[\alpha]$ is the specific rotation expressed in (°·L) (Kg·dm). Melting points are uncorrected and recorded on Stuart SMP1 apparatus. Infrared Spectra were performed either neat or in KBr discs using: JASCO FT/IR-300E Fourier Transform Infrared Spectrophotometer, National Research Centre and / or Bruker Vector 22 Spectrophotometer, Microanalysis Centre, Cairo University. The spectra were reported in cm⁻¹.

¹H NMR spectra were recorded on Varian Gemini 200 equipment operating at 200 MHz, Microanalysis Centre, Cairo University and/or JOEL JNM-EX 270 (at 270 MHz) and JOEL 500 AS (at 500 MHz) equipments, National Research Centre. The spectra were obtained from deuterated chloroform (CDCl₃) and/or deuterated dimethylsulphoxide (DMSO-d₆) and the chemical shifts were reported in δ ppm units downfield from tetramethylsilane (TMS). ¹³C NMR spectra were recorded on JOEL 500 AS (at 125 MHz) equipment, National Research Centre. The Mass Spectra were recorded on Finnigan SSQ 7000 Spectrometer at 70 eV (Electron Impact). The enantiomeric excess ee % was obtained after derivatization of cyanohydrins with Naproxen chloride to the corresponding diastereoisomers whose ratios were determined with ¹H NMR by quantitative analysis of the spectral data through comparable evaluation of the integral level for both diastereoisomers. X-Ray diffraction: The intensity data was performed with a Kappa-CCD Enraf Nonius FR 590 Single Crystal Diffractometer. The structure was solved by direct methods using the SIR92 program⁽⁴⁰⁾ and

refined using maXus⁽⁴¹⁾. The molecular graphics were made with ORTEP⁽⁴²⁾. Crystallographic data (CIF) for the structure reported in this article have been deposited in the Cambridge Crystallographic Data Centre (CCDC) as supplementary publication No. CCDC 801828. Copies of the data can be obtained, free of charge, upon application to the CCDC, 12 Union Road, Cambridge CB 12EZ, UK. (FAX: + 44(1223)336-033; E-mail:deposit@ccdc.cam.ac.uk).

Synthesis of the racemic cyanohydrins

General procedure

In a three necked flask equipped with a mechanical stirrer and a dropping funnel, a saturated solution of sodium metabisulphite (150 g in 200 ml water) was added dropwise to a mixture of the appropriate carbonyl compound (aldehyde and/or ketone) (0.25 mole) and potassium cyanide solution (0.3 mole in 50 ml of distilled water). During the initial stages of addition, the reaction mixture was cooled by adding crushed ice in several portions through the third neck. After completion of addition (about 30 min), the reaction mixture was stirred for further 12 hr at room temperature then extracted with diethyl ether (3 × 100 ml). The combined organic extracts were washed with water (2 × 50 ml) and dried over anhydrous sodium sulphate. After removal of the solid materials, the ether filtrate was evaporated well *in vacuo* to leave the corresponding racemic cyanohydrin.

(R,S)-2-(4-chlorophenyl)-2-hydroxyethanenitrile (R,S)-3

Yellow oil, yield 87%. IR (neat, cm⁻¹): 3390 (O–H); 3095 (C–H, aromatic); 2906 (C–H aliphatic, asymmetric), 2808 (C–H aliphatic, symmetric); 2254 (C≡N); 1595 (C=C, aromatic); 1091 (C–Cl, aromatic) and 1039 (C–O, alcohol). ¹H-NMR (CDCl₃, δ ppm): 4.69 (OH, D₂O exchangeable); 5.48 (NC–CH, s); 7.29 (2H, aromatic protons *meta* to the Cl atom, AB system, d, J_{HH}=8.4 Hz) and 7.33 (2H, aromatic protons *ortho* to the Cl atom, AB system, d, J_{HH}=8.4 Hz).

(R,S)-2-phenyl-2-hydroxy ethanenitrile (R,S)-15⁽³⁴⁾

Yellow oil, yield 88%. IR (neat, cm⁻¹): 3382 (O–H); 3091 (C–H, aromatic); 2922 (C–H aliphatic, asymmetric), 2818 (C–H aliphatic, symmetric); 2255 (C≡N); 1598 (C=C, aromatic) and 1044 (C–O, alcohol). ¹H NMR (CDCl₃): 4.72 (OH, D₂O exchangeable) 5.46 (1H, NC–CH, s); 7.42 (1H, *para* aromatic proton, t, J_{HH}=3.1 Hz); 7.44 (2H, *ortho* aromatic protons, t, J_{HH}=6.1 Hz); 7.52 (2H, *meta* aromatic protons, dt, J_{HH}=6.1 Hz, J_{HH}=3.1 Hz).

Cyclohexanone cyanohydrin (21a)

Colourless oil, yield 78 %. IR (neat, cm⁻¹): 3429 (O–H); 2932 (asymmetric C–H aliphatic); 2871 (symmetric C–H aliphatic); 2235 (C≡N); 1074 (C–O, alcohol). ¹H NMR (CDCl₃): 1.42 – 1.54 (6H, C3, C4, C5 protons, m); 1.81 (2H, C2, C6 axial protons, dt, J_{HHgem}= 12.8 Hz, J_{HHvic}= 6.9 Hz); 2.05 (2H, C2, C6 equatorial protons, dt, J_{HHgem}=13.1 Hz, J_{HHvic}= 6.9Hz) and 5.59 (1H, OH, D₂O exchangeable).

(R,S)-2-Methylcyclohexanone cyanohydrin (*R,S*)-21b

Colourless oil, yield 83 %. IR (neat, cm^{-1}): 3423 (O–H); 2936 (C–H aliphatic asymmetric); 2863 (C–H aliphatic, symmetric); 2237 (C \equiv N); 1074 (C–O, alcohol). ^1H NMR(CDCl_3): 0.72 (3H, C2- CH_3 , d, $J_{\text{HH}} = 6.9$ Hz); 1.27 – 1.53 (6H, C3, C4, C5 protons, m); 1.78 (1H, C6 axial proton, dt, $J_{\text{HHgem}} = 12.8$ Hz, $J_{\text{HHvic}} = 6.8$ Hz); 2.03 (1H, C6 equatorial proton, dt, $J_{\text{HHgem}} = 12.8$ Hz, $J_{\text{HHvic}} = 6.9$ Hz); 2.71 (1H, C2-H, m) and 5.52 (1H, OH, D_2O exchangeable).

Synthesis of the racemic cyanohydrins using acetone cyanohydrin as a transcyanating agent ⁽²⁶⁾

To a stirred solution of the appropriate carbonyl compound (0.1 mole) in diisopropyl ether (20 ml), acetone cyanohydrin (4) was added dropwise followed by sodium hydroxide (15 ml of 1M solution) at room temperature. The reaction mixture was stirred for 6 hr then followed-up as described in the previous general procedure to give the cyanohydrins (*R,S*)-3, 21a and (*R,S*)-21b in yields of 78, 68 and 57 %, respectively.

*Synthesis of the optically active cyanohydrins**Step 1: Preparation of hydrogen cyanide solution* ⁽⁴³⁾

Orthophosphoric acid (0.2 mole, 13.7 ml) was added dropwise with stirring to a solution of potassium cyanide (0.2 mole, 13 g) in distilled water (30 ml) and diisopropyl ether (50 ml) over a period of 5 min at 0°C. After stirring the reaction mixture for further 15 min, the cooling bath was removed and the ethereal layer was separated off.

Step 2: Addition of hydrogen cyanide solution to the carbonyl compounds 1 and 2a,b

General procedure: The ethereal solution of hydrogen cyanide was added dropwise at 0°C under stirring with a mechanical stirrer to a mixture of the crude enzyme extract and the carbonyl compound 1 and /or 2b in diisopropyl ether (30 ml). After completion of addition (30 min), the reaction mixture was stirred for further 24 hr. The cooling bath was removed and the reaction mixture was stirred vigorously with a saturated solution of sodium chloride (200 ml) and diisopropyl ether (100 ml) for 30 min. The ethereal layer was separated, washed with distilled water (2 \times 50 ml) and dried over anhydrous sodium sulphate. After removal of the solid material, the ethereal solution was evaporated well *in vacuo* to give the respective optically active cyanohydrin (*R*)-3 or (*R*)-21b.

(R)-2-(4-chlorophenyl)-2-hydroxyethanenitrile (*R*)-3

Enantiomeric excess (ee) 94%, $[\alpha]_{\text{D}}^{25} = +178$. Yellow oil, yield 98 %. IR (neat, cm^{-1}): 3390 (O–H); 3095 (C–H, aromatic); 2906 (C–H aliphatic); 2254 (C \equiv N), 1595 (C=C aromatic); 1091 (C–Cl, aromatic) and 1039 (C–O alcohol). ^1H -NMR (CDCl_3): 4.44 (1H, OH, D_2O -exchangeable); 5.46 (1H, NC–C–H, s); 7.34 (2H, aromatic protons *m* to the Cl atom, AB system, d, $J_{\text{HH}} = 8.3$ Hz) and 7.37 (2H, aromatic protons *o* to the Cl atom, AB system, d, $J_{\text{HH}} = 8.3$ Hz).

(R)-2-methylcyclohexanone cyanohydrin (*R*)-21b

Colourless oil, yield 88 %. IR (neat, cm^{-1}): 3441 (O–H); 2926 (C–H aliphatic, asymmetric); 2860 (C–H aliphatic, symmetric); 2237 (C≡N); 1069 (C–O, alcohol). ^1H NMR(CDCl_3): 0.82 (3H, C2- CH_3 , d, $J_{\text{HH}} = 6.9$ Hz); 1.33 – 1.54 (6H, C3, C4, C5 protons, m); 1.78 (1H, C6-*H* axial, dt, $J_{\text{HHgem}} = 12.8$ Hz, $J_{\text{HHvic}} = 6.8$ Hz); 2.04 (1H, C6-*H* equatorial, dt, $J_{\text{HHgem}} = 12.8$ Hz, $J_{\text{HHvic}} = 6.9$ Hz); 2.72 (1H, C2-*H*, m) and 5.59 (1H, O-*H*, D_2O exchangeable).

Preparation of optically active cyanohydrins using acetone cyanohydrin as a transcyanating agent

Acetone cyanohydrin (4) (0.015 mole, 1.2 ml) was added to a mixture of the carbonyl compound 1 or 2b and the crude enzyme extract in diisopropyl ether (5 ml) at room temperature. After stirring for 6 hours, the reaction was followed up (TLC) and the products were separated as described in the previous general procedure to obtain cyanohydrin (*R*)-3 or (*R,S*)-21b in yields of 82 % and 67 %, respectively.

*Determination of the enantiomeric excess through derivatization with naproxen chloride**Step 1: Preparation of naproxen chloride (S)-7: (29)*

Naproxen[®] (*S*)-5 (obtained from commercially available tablets after extraction with chloroform) (0.03 mole, 7g) was refluxed with freshly distilled oxalyl chloride 6 (0.03 mole, 3.8 g, 2.6 ml) in dry hexane for 2 hr under dry argon atmosphere. The volatile materials were removed *in vacuo* to leave naproxen chloride as a pale yellow residue.

Step 2: Addition of naproxen chloride

A solution of naproxen chloride (*S*)-7 (from step 1), in 10 ml of dry methylene chloride, was added dropwise to a mixture of the appropriate cyanohydrin (0.01 mole) and pyridine (0.01 mole, 0.8 g, 0.85 ml) in 10 ml of dry methylene chloride with stirring at 0°C under dry argon atmosphere. The cooling bath was removed and the reaction mixture was stirred for further 3 hr at room temperature. An additional volume of methylene chloride (30 ml) was added, then the reaction mixture was washed with a saturated solution of sodium carbonate (3 × 20 ml), distilled water (3 × 20 ml) and dried over anhydrous sodium sulphate. The solid material was filtered off and the volatile materials were removed under reduced pressure. The solid product, so obtained, was collected and chromatographed on silica gel through eluting with petroleum ether 60-80 °C/ acetone (9 : 1).

(2S)-((R)-4-Chlorophenyl)(cyano)methyl-2-(6-methoxynaphthalen-2-yl) propanoate (R,2S)-(8)

de 95 %, $[\alpha]_{\text{D}25} = -104$. Colourless crystals, m. p. 130 – 132 °C, yield 60 %. IR [KBr , cm^{-1}): 3059 (C–H, aromatic); 2935 (C–H aliphatic, asymmetric); 2858 (C–H aliphatic, symmetric); 2238 (C≡N); 1744 (C=O, ester); 1624, 1604 (C=C, aromatic) and 1247 (C–O, ester). ^1H -NMR (DMSO-d_6 , δ ppm): 1.48 (3H, C-

CH_3 , d, $J_{HH} = 7.6$ Hz); 3.83 (3H, O- CH_3 , s); 4.08 (1H, CH- CH_3 , q, $J_{HH} = 7.6$ Hz); 6.72 (1H, O-CH, s); 7.26-7.74 (10H, aromatics, m). MS m/z (%): 379 (60 %). Analyses calculated (%) for $C_{22}H_{18}ClNO_3$ (379.84): C, 69.57; H, 4.78; Cl, 9.33; N, 3.69. Found (%): C, 69.62; H, 4.77; Cl, 9.22; N, 3.60. C, 69.57; H, 4.78; Cl, 9.33; N, 3.69.

(2*S*)-(1-Cyanocyclohex-1-yl)-2-(6-methoxynaphthalen-2-yl)propanoate (2*S*)-22*a*

IR (KBr, cm^{-1}): 3035 (C-H aromatic); 2972 (C-H aliphatic, asymmetric); 2936 (C-H aliphatic, symmetric); 1737 (C=O, ester); 1628, 1603 (C=C, aromatic) and 1230 (C-O, ester). 1H NMR (DMSO- d_6 , δ ppm): 1.19-1.40 (6H, Cc, Cd and Ce cyclohexyl protons, m); 1.47 (3H, CH- CH_3 , d, $J_{HH} = 6.9$ Hz); 1.80 (2H, Cb and Cf axial protons, dt, $J_{HH} = 6.9$ Hz, $J_{HH} = 13.0$ Hz); 2.02 (2H, Cb and Cf equatorial protons, dt, $J_{HH} = 6.9$ Hz, $J_{HH} = 13.0$ Hz); 3.83 (3H, O- CH_3 , s); 3.99 (1H, CH- CH_3 , q, $J_{HH} = 6.9$ Hz); 7.13 (1H, Ck-H, d, $J_{HH} = 9.2$ Hz); 7.27 (1H, Cm-H, s); 7.37 (1H, Co-H, d, $J_{HH} = 8.4$ Hz); 7.72 (1H, Ci-H, s) and 7.78 (2H, Cl-H, Cp-H, m). MS m/z (%): 337 (25 %). Analyses calculated (%) for $C_{21}H_{23}NO_3$ (337.42): C, 74.75; H, 6.87; N, 4.15. Found (%): C, 74.79; H, 6.86; N, 4.14.

(1*R*,2*S*)(2*S*)-((1*R*,1*S*)-(1-Cyano-2-methylcyclohex-1-yl)-2-(6-methoxynaphthalen-2-yl)-propanoate (1*R*,2*S*)(1*S*,2*S*)-22*b*

Brown crystals, m. p. 72-74; yield 90%. IR (KBr, cm^{-1}): 3048 (C-H, aromatic); 2938 (C-H, aliphatic asymmetric); 2862 (C-H aliphatic, symmetric); 2100 (C \equiv N); 1747 (C=O, ester); 1604 (C=C, aromatic); 1452 (C-H aliphatic, asymmetric deformation); 1482 (C-H aliphatic, symmetric deformation); 1227 (C-O, ester); 1174 (C-O, aromatic); 1084 (C-O, ester). 1H NMR (DMSO- d_6 , δ ppm): 0.67 (3H, Cb- CH_3 , d, $J_{HH} = 6.9$ Hz); 1.18- 1.53 (6H, Cc, Cd, Ce cyclohexyl protons, m); 1.48 (3H, CH- CH_3 , d, $J_{HH} = 6.9$ Hz); 1.69 (1H, axial Cf-H, dt, $J_{HHgem} = 12.3$ Hz, $J_{HHvic} = 7.6$ Hz); 1.82 (1H, equatorial Cf-H, m); 2.75 (1H, Cb-H of both isomers, m); 3.83 (3H, O- CH_3 , s); 3.97 (1H, CH- CH_3 , q, $J_{HH} = 6.9$ Hz); 7.14 (1H, Ck-H, dd, $J_{HH} = 9.2$ Hz, $J_{HH} = 3.1$ Hz); 7.25 (1H, Cm-H, s); 7.36 (1H, Co-H, dd, $J_{HH} = 8.4$ Hz, $J_{HH} = 1.5$ Hz); 7.64 (1H, Ci-H, s) and 7.78 (2H, Cl-H, Cp-H, dd, $J_{HH} = 8.4$ Hz, $J_{HH} = 2.4$ Hz). MS m/z (%): 351 (10 %). Analyses calculated (%) for $C_{22}H_{25}NO_3$ (351.45): C, 75.19; H, 7.17; N, 3.99. Found (%): C, 75.15; H, 7.17; N, 3.89.

(2*S*)-((1*R*)-1-Cyano-2-methylcyclohex-1-yl)-2-(6-methoxynaphthalen-2-yl)-propanoate (1*R*,2*S*)-22*b*

de : 90% . $[\alpha]_{D^{25}} = +61$. Brown crystal, yield 80 %, m.p. 74-76 °C. IR (KBr, cm^{-1}): 3057 (C-H aromatic); 2936 (C-H aliphatic, asymmetric); 2862 (C-H aliphatic, symmetric); 2237 (C \equiv N); 1744 (C=O ester); 1631, 1606 (C=C aromatic); 1455 (C-H deformation, asymmetric); 1387 (C-H deformation, symmetric); 1222 (C-O ester) and 1028 (C-O ether). 1H NMR (CDCl $_3$, δ ppm): 0.81 (3H, Cb- CH_3 , d, $J_{HH} = 6.9$ Hz); 1.21- 1.65 (6H, Cc, Cd, Ce cyclohexyl protons, m); 1.56 (3H, CH- CH_3 , d, $J_{HH} = 6.9$ Hz); 1.81 (1H, axial Cf-H, dt, $J_{HH} = 7.6$ Hz, $J_{HH} = 12.3$ Hz); 2.12 (1H, equatorial Cf-H, m); 2.75 (1H, Cb-H, m); 3.84 (1H, CH- CH_3 , q, $J_{HH} = 6.9$ Hz); 3.91 (3H, O- CH_3 , s); 7.14 (1H, Ck-H, dd,

$J_{\text{HH}}=9.2$ Hz, $J_{\text{HH}}=3.1$ Hz); 7.25 (1H, Cm-H, s); 7.36 (1H, Co-H, dd, $J_{\text{HH}}=8.4$ Hz, $J_{\text{HH}}=1.5$ Hz); 7.64 (1H, Ci-H, s) and 7.78 (2H, Cl-H, Cp-H, dd, $J_{\text{HH}}=8.4$ Hz, $J_{\text{HH}}=2.4$ Hz). ^{13}C NMR (CDCl_3): 16.08 (Cg); 18.29 (Cb- CH_3); 22.99 (Ce); 24.48 (Cd); 31.04 (Cc); 34.37 (Cf); 40.09 (Cb); 45.85 (Ch); 55.39 (O- CH_3); 78.21 (Ca); 105.69, 116.43, 119.17, 125.99, 126.09, 127.43, 129.36, 135.05 (C \equiv N and aromatic carbons); 157.78 (Cn) and 172.49 (C=O). MS m/z (%): 351 (10 %). Analyses calculated (%) for $\text{C}_{22}\text{H}_{25}\text{NO}_3$ (351.45): C, 75.19; H, 7.17; N, 3.99. Found (%): C, 75.16; H, 7.17; N, 3.91.

2- Reaction of cyanhydrins (R,S)-3 and (R)-3 with benzoyl chloride (9) and stearoyl chloride (11)

A solution of the acid chloride 9 and/or 11 (0.02 mole) in 10 ml of dry methylene chloride was added dropwise by a syringe to a mixture of the cyanohydrin (0.01 mole, 1.83 g) and pyridine (0.01 mole, 0.85 ml) in 10 ml dry methylene chloride with stirring at 0°C under dry argon atmosphere. The cooling bath was removed and the reaction mixture was stirred for further 5 hr at room temperature. An additional volume of methylene chloride (30 ml) was added, then the reaction mixture was washed with a saturated solution of sodium carbonate (3 × 20 ml), distilled water (3 × 20 ml) and dried over anhydrous sodium sulphate. The solid material was filtered off and the filtrate was evaporated under reduced pressure where the residual substance were crystallized from petroleum ether 60 – 80 °C.

(R)-(+)-(4-chlorophenyl)cyanomethyl benzoate (R)-(+)-10

$[\alpha]_{\text{D}/25} = +102$. Colourless crystals, m. p. 50 – 52 °C, yield 65%. IR (KBr, cm^{-1}): 3068 (C-H, aromatic); 2959 (C-H, aliphatic); 2333 (C \equiv N); 1719 (C=O, ester); 1258 (C-O, ester) and 1088 (C-Cl, aromatic). ^1H NMR (CDCl_3): 6.44 (1H, CH methine, s); 7.45 (2H, aromatic protons *ortho* to the Cl atom, AB system, d, $J_{\text{HH}}=8.4$ Hz); 7.47 (2H, aromatic protons *meta* to carbonyl, dt, $J_{\text{HH}}=7.6$ Hz, $J_{\text{HH}}=6.8$ Hz); 7.52 (2H, aromatic protons *meta* to the Cl atom, d, AB system, $J_{\text{HH}}=8.4$ Hz); 7.62 (1H, aromatic proton *para* to carbonyl, t, $J_{\text{HH}}=7.6$ Hz); 8.05 (2H, aromatic protons *ortho* to carbonyl, t, $J_{\text{HH}}=6.8$ Hz). MS m/z (%): 271 (5%, M^+) and 273 (2%, M^++2). Analyses calculated (%) for $\text{C}_{15}\text{H}_{10}\text{ClNO}_2$ (271.70): C, 66.31; H, 3.71; Cl, 13.05; N, 5.16. Found (%): C, 66.36; H, 3.65; Cl, 13.15; N, 5.03.

(R,S)- (4-chlorophenyl)(cyano)methyl stearate (R,S)-12

Colourless crystals, m.p. = 42-44 °C, yield 70%. IR (KBr, cm^{-1}): 2918 (C-H aliphatic, asymmetric); 2850 (C-H aliphatic, symmetric); 1750 (C=O, ester); 1597 (C=C, aromatic); 1466 (C-H aliphatic, asymmetric deformation); 1379 (C-H aliphatic, symmetric deformation); 1154 (C-O, ester) and 1096 (C-Cl, aromatic). ^1H NMR ($\text{DMSO}-d_6$): 0.87 (3H, CH_3 , t, $J_{\text{HH}}=6.1$ Hz); 1.25 (28H, $(\text{CH}_2)_{13}$, m); 1.63 (2H, C(O)- CH_2 - CH_2 , t, $J_{\text{HH}}=7.7$ Hz); 2.39 (2H, C(O)- CH_2 , t, $J_{\text{HH}}=7.7$ Hz); 6.39 (1H, NC-CH, s); 7.42 (2H, aromatic protons *meta* to the Cl atom, d, AB system, $J_{\text{HH}}=8.4$ Hz) and 7.45 (2H, aromatic protons *ortho* to the Cl atom, d, AB system, $J_{\text{HH}}=8.4$ Hz). MS m/z (%): 433 (8 %) [M^+], 435 (2%)

[M⁺+2]. Analyses calculated (%) for C₂₆H₄₀ClNO₂ (434.06): C, 71.95; H, 9.29; Cl, 8.17; N, 3.23. Found (%): C, 71.90; H, 9.34; Cl, 8.11; N, 3.19.

(*R*)-(+)(4-chlorophenyl)(cyano)methyl stearate (*R*)-(+)-12
[α]_{D25}=+162. Colourless crystals, m.p. 42-44 °C, yield 75%.

Reaction of cyanohydrins (*R,S*)-3, (*R*)-3, (*R,S*)-15 and (*R*)-21b with isocyanates 13 and 17

To a stirred mixture of the appropriate racemic and/or optically active cyanohydrin (0.01 mole) and triethylamine (10ul) in dry toluene (10 ml) was added a solution of the appropriate isocyanate reagent (0.012 mole) in toluene (5 ml) at 0°C under an inert gas atmosphere. After stirring the reaction mixture for further 48 hr at room temperature, the volatile materials were removed under reduced pressure where the residual substance was collected, washed with light petroleum, dried and recrystallized from petroleum ether 80-100 °C.

(*R,S*)-(4-chlorophenyl)(cyano)methyl tert-butylcarbamate (*R,S*)-14
Colourless crystals, m. p. 80 – 82 °C, yield 95%.

(*R*)-(4-chlorophenyl)(cyano)methyl tert-butylcarbamate (*R*)-(+)-14
[α]_{D25}=+55.8. Colourless crystals, m.p. 80-82 °C, yield 80 %. IR (KBr, cm⁻¹): 3345 (N–H); 3045 (C–H aromatic); 2976 (C–H aliphatic, asymmetric); 2935 (C–H aliphatic, symmetric); 2043 (C≡N); 1711 (C=O ester); 1598 (C=C aromatic); 1455 (C–H deformation, asymmetric); 1391 (C–H deformation, symmetric); 1270 (C–O ester); 1069 (CH–O). ¹H NMR (CDCl₃, δ ppm): 1.32 (9H, C(CH₃)₃, s); 4.77 (1H, NH, D₂O exchangeable); 6.35 (1H, NC–CH, s); 7.40 (2H, aromatic protons meta to the Cl atom, AB system, d, J_{HH}=8.4 Hz) and 7.44 (2H, aromatic protons ortho to the Cl atom, AB system, d, J_{HH}=8.4 Hz). ¹³C NMR (CDCl₃): 28.78 ((CH₃)₃); 51.39 (C–(CH₃)₃); 62.31 (CH–O); 116.55 (C≡N); 129.12 (2C, carbons ortho to the Cl atom); 129.50 (2C, carbons meta to the Cl atom); 131.19 (C–C–CN); 136.36 (C–Cl); 151.83 (C=O). MS m/z (%): 266 (M⁺, 8 %); 268 (M⁺+2, 2%). Analysis calculated (%) for C₁₃H₁₅ClN₂O₂ (266.73): C, 58.54; H, 5.67; Cl 13.29; N, 10.50. Found (%): C, 58.49; H, 5.77; Cl, 13.20; N, 10.39.

(*R,S*)-(phenyl)(cyano)methyl tert-butylcarbamate (*R,S*)-16

Colourless crystals, m. p. 54 °C, yield 95%. IR (KBr, cm⁻¹): 3361 (N–H); 3032 (C–H aromatic); 2977 (C–H aliphatic, asymmetric); 2938 (C–H aliphatic, symmetric); 2215 (C≡N); 1742 (C=O ester); 1527 (C=C, aromatic); 1458 (C–H deformation, asymmetric); 1396 (C–H deformation, symmetric); 1263 (C–O ester); 1073 (CH–O). ¹H NMR (CDCl₃): 1.29 (9H, C(CH₃)₃, s); 4.75 (1H, NH, s, D₂O exchangeable); 6.37 (1H, NC–CH, s); 7.43 (1H, para proton, t, J_{HH}=3.1 Hz); 7.44 (2H, ortho protons, t, J_{HH}=6.2 Hz); 7.50 (2H, meta protons, J_{HH}=6.2 Hz, J_{HH}=3.1 Hz). MS m/z (%): 232 (10 %). Analysis calculated (%) for C₁₃H₁₆N₂O₂ (232.28): C, 67.22; H, 6.94; N, 12.06. Found (%): C, 67.17; H, 7.04; N 12.09.

(5R)- (3-(3,4-dichlorophenyl) -5-(4-chlorophenyl) -4- iminoxazolidin-2-one (R)-(+)-19

$[\alpha]_{D_{25}} = +129$. Yellowish crystal, m.p. 126 – 130 °C, yield 65 %. IR (KBr, cm^{-1}): 3293 (N–H); 3095 (C–H, aromatic); 2924 (C–H, aliphatic); 1770 (C=O, lactone); 1683 (C=N, exocyclic); 1594 (C=C, aromatic) and 1130, 1090, 1045 (C–Cl, aromatic). ^1H NMR (CDCl_3): 5.83 (1H, NC–CH, s); 6.01 (1H, NH, bs); 7.35 (1H, Cg–H, s); 7.39 (1H, Ce–H, d, $J_{\text{HH}}=7.6$ Hz); 7.42 (2H, Ca and Cd protons, d, $J_{\text{HH}}=7.6$ Hz); 7.47 (2H, Cb and Cc protons, d, $J_{\text{HH}}=7.6$ Hz) and 7.60 (1H, Cf–H, d, $J_{\text{HH}}=7.6$ Hz). MS m/z (%): 354 (8 %). Analyses calculated for $\text{C}_{15}\text{H}_9\text{Cl}_3\text{N}_2\text{O}_2$ (355.61): C, 50.66; H, 2.55; Cl, 29.91; N, 7.88. Found (%): C, 50.71; H, 2.49; Cl 29.79; N, 7.77.

(5R)-3-(3,4-dichlorophenyl) -5-(2-methylcyclohex-1-yl) -4- iminoxazolidin-2-one (R)-23

Yellowish crystal, yield 54 %. IR (KBr, cm^{-1}): 3422 (N–H); 3077 (C–H, aromatic); 2927 (C–H aliphatic, asymmetric); 2856 (C–H aliphatic, symmetric); 1727 (C=O, lactone); 1677 (C=N, exocyclic); 1590 (C=C, aromatic); 1471 (C–H aliphatic, asymmetric deformation); 1377 (C–H aliphatic, symmetric deformation) and 1087, 1033 (C–Cl, aromatic). Its ^1H NMR spectrum (DMSO-d_6 , δ ppm): 0.96 (3H, Cb–CH₃, d, $J_{\text{HH}} = 6.9$ Hz); 1.21–2.21 (8H, cyclohexyl protons c–f, m), 2.49 (Cb–H, m); 6.09 (1H, NH, D₂O-exchange- able, bs) and 7.34 (1H, Ci–H, s) 7.40 (1H, Ch–H, d, $J_{\text{HH}} = 7.6$ Hz); 7.61 (1H, Cg–H, d, $J_{\text{HH}} = 7.6$ Hz). Analyses calculated (%) for $\text{C}_{15}\text{H}_{16}\text{Cl}_2\text{N}_2\text{O}_2$ (327.21): C, 55.06; H 4.93; Cl, 21.67; N, 8.56. Found (%): 55.14; H, 4.88; Cl, 21.72, N, 8.49.

Acetylation of cyanohdrins 21a, (R,S)-21b and (R)-21b:

A solution of the cyanohydrin (0.02 mole), pyridine (0.04 mole, 3.2 ml) and acetic anhydride (24) (0.04 mole, 5ml) in methylene chloride (100 ml) was magnetically stirred for 12 hr at room temperature. The organic layer was separated, washed with 5% sulphuric acid (2 × 25 ml), distilled water (2 × 25 ml), saturated sodium bicarbonate solution (2 × 25 ml) then dried over anhydrous sodium sulphate. Removal of the volatile materials under reduced pressure followed by chromatography on silica gel and eluting with petroleum ether 40-60/acetone °C (98:2) afforded the pure acetylated cyanohydrin.

1-Cyancyclohex-1-yl acetate (25a)

Colourless crystals, m. p. 44–46 °C, yield 80%. IR (KBr, cm^{-1}): 2946 (C–H aliphatic, asymmetric); 2865 (C–H aliphatic, symmetric); 2248 (C≡N); 1752 (C=O, ester); 1452 (C–H aliphatic, asymmetric deformation); 1376 (C–H aliphatic, symmetric deformation) and 1234 (C–O, ester). ^1H (DMSO-d_6): 1.28 – 1.64 (6H, Cc–Ce protons); 1.83 (2H, Cb, Cf axial protons, dt, $J_{\text{HHgem}} = 12.2$ Hz,

$J_{\text{HHvic}}=9.9$ Hz); 2.07 (3H, CH_3 , s) and 2.14 (2H, Cb, Cf equatorial protons, dt, $J_{\text{HHgem}}=13.7$ Hz, $J_{\text{HHvic}}=7.7$ Hz). MS m/z (%): 167 (70%, M^+). Analyses calculated (%) for $\text{C}_9\text{H}_{13}\text{NO}_2$ (167.21): C, 64.65; H, 7.84; N, 8.38. Found (%): C, 64.71; H, 7.78; N, 8.31.

(R,S)-(2-methylcyclohex-1-yl)(cyano)methyl acetate (R,S)-25b

Colourless crystals, Yield: 85 %; m. p. 54 °C. IR (KBr, cm^{-1}): 2942 (C–H aliphatic, asymmetric); 2871 (C–H aliphatic, symmetric); 2243 ($\text{C}\equiv\text{N}$); 1748 (C=O ester); 1456 (C–H aliphatic, asymmetric deformation); 1373 (C–H aliphatic, symmetric deformation); 1219 (C–O, ester); 1032 (NCC–O). ^1H NMR (DMSO- d_6 , δ ppm): 1.00 (3H, Cb- CH_3 , d, $J_{\text{HH}}=6.5$ Hz); 1.22- 1.59 (6H, Cc-Ce cyclohexyl protons, m); 1.73 (1H, Cf axial proton, dt, $J_{\text{HH}}=10.0$ Hz, $J_{\text{HH}}=7.6$ Hz); 1.91 (1H, Cf equatorial proton, m); 2.06 (3H, O=C- CH_3 , s); 2.51 (1H, Cb-H, m). MS m/z (%): 181 (3 %). Analysis calculated (%) for $\text{C}_{10}\text{H}_{15}\text{NO}_2$ (181.23): C, 66.27; H, 8.34; N, 7.73. Found (%): C, 66.20; H, 8.38; N, 7.69.

(R)-(+)-(2-methylcyclohex-1-yl)(cyano)methyl acetate (R)-(+)-25b

$[\alpha]_{\text{D}/25}=+87$, Yield: 80 %; m. p. 54 °C.

Preparation of the corresponding α -hydroxycarboxylic acids from cyanohydrins (R)-3, (R,S)-3, 21a, (R,S)-21b and (R)-21b

General procedure

A solution of the appropriate cyanohydrin (0.03 mole) in concentrated hydrochloric acid (50 ml) was stirred for 16 hr at room temperature, then refluxed for 5 hr. The reaction mixture was poured onto distilled water, then extracted with methylene chloride (3×25 ml). The combined organic extracts were dried over anhydrous sodium sulphate, filtered and evaporated *in vacuo* to yield the respective α -hydroxycarboxylic acid.

(R,S)-1-(4-chlorophenyl)-1-hydroxyacetic acid (R,S)-20

Colourless crystals, m. p. 114 – 116 °C, yield 95%.

(R)-(-)-1-(4-chlorophenyl)-1-hydroxyacetic acid (R)-(-)-

$[\alpha]_{\text{D}/25}=-75.4$. Colourless crystals, m. p. 114 – 116 °C, yield 90%. IR (KBr, cm^{-1}): 3374 (O–H); 3185 (C–H, aromatic); 2924 (C–H aliphatic, asymmetric); 2852 (C–H aliphatic, symmetric); 1671 (C=O, acid); 1591 (C=C, aromatic); 1284 (C–O acid); 1100 (C–O alcohol) and 1069 (C–Cl, aromatic). ^1H NMR (DMSO- d_6): 4.82 (1H, CH methine, d, $J_{\text{HH}}=3.8$ Hz); 6.10 (1H, OH, d, D_2O -exchangeable, d, $J_{\text{HH}}=3.8$ Hz); 7.35 (2H, CH meta to the Cl atom, AB system, $J_{\text{HH}}=8.4$ Hz); 7.40 (2H, CH ortho to the Cl atom, d, $J_{\text{HH}}=8.4$ Hz) and 7.42 (1H, OH acid, D_2O exchangeable). MS m/z (%): 186 (M^+ , 3 %) and 188 (M^++2 , <1 %). Analyses calculated (%) for $\text{C}_9\text{H}_9\text{ClO}_3$ (200.62): C, 53.88; H, 4.52; Cl, 17.67. Found (%): C, 53.93; H, 4.47; Cl, 17.60.

1-hydroxycyclohexanecarboxylic acid (26a)

Colourless crystals, m.p. 116-118 °C, yield 80%. IR (KBr, cm⁻¹): 3403 (free O-H); 3282 (hydrogen bonded O-H); 2941 (C-H aliphatic, asymmetric); 2855 (C-H aliphatic, symmetric); 1682 (C=O, acid); 1447 (C-H aliphatic, asymmetric deformation); 1395 (C-H aliphatic, symmetric deformation); 1160 (C-O acid) and 994 (C-O alcohol). ¹H NMR (DMSO-d₆): 1.08 -1.39 (6H, Cc-Ce protons, m); 1.50 (2H, Cb,Cf axial protons, dt, J_{HHvic}=6.9 Hz, J_{HHgem}=12.3 Hz); 1.57 (2H, Cb,Cf equatorial protons, dt, J_{HHvic}= 6.9 Hz, J_{HHgem}=12.3Hz); 6.93 and 7.07 (2H, D₂O exchangeable protons of hydroxyl and carboxyl groups). MS m/z (%): 144 (35%). Analyses calculated (%) for C₇H₁₂O₃ (144.17): C, 58.32; H, 8.39. Found (%): 58.37; H, 8.31.

(R,S)-1-hydroxy-2-methylcyclohexanecarboxylic acid (R,S)-26b

Colourless crystals, yield 95 %, m. p. 98-100°C.

(R)-(+)-1-hydroxy-2-methylcyclohexanecarboxylic acid (R)-(+)-26b

[α]_{D25}=+26. Colourless crystals, m. p. 98 – 100 °C, yield 90 %. IR (KBr, cm⁻¹): 3458 (O-H, acid); 3340 (O-H, alcohol); 2949 (C-H aliphatic, asymmetric); 2884 (C-H aliphatic, symmetric); 1705 (C=O acid); 1452 (C-H aliphatic, asymmetric deformation); 1350 (C-H, symmetric deformation); 1248 (C-O, acid) and 1082 (C-O, alcohol). ¹H NMR (DMSO-d₆): 0.77 (3H, CH₃, d, J_{HH}=6.9 Hz); 0.89 – 1.67 (6H, Cc-Ce protons, m); 1.77 (1H, Cf axial proton, dt, J_{HHgem}=10.7 Hz, J_{HHvic}=4.6Hz); 1.89 (1H, Cf equatorial proton, dt, J_{HH}=10.7Hz, J_{HH}=3.8 Hz), 2.01 (1H, Cb-H, m) and 6.25 and 6.80 (D₂O exchangeable protons). MS m/z (%): 158 (20%). Analyses calculated (%) for C₈H₁₄O₃ (158.20): C, 60.74; H, 8.92. Found (%): C, 60.81; H, 8.88.

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**تحضير و تفاعلات سيانو هيدرينات نشطه ضوئياً مشتقه من ٤-
كلوروبنزالدهيد و حلقي هكسانون و ٢-ميثيل حلقي هكسانون باستخدام
إنزيم (R)- هيدروكسي نيتريلاز المستخرج من نبات اللوز**

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عند معالجه مركبات ٤- كلوروبنزالدهيد (1) وحلقي هكسانون (2a) و ٢-ميثيل حلقي هكسانون (2b) بمحلول مائي من سيانيد البوتاسيوم في وجود محلول مشبع من مركب ميتا بايكيريتيت الصوديوم فإنه يتكون السيانو هيدرينات المخلطه المقابله ٢-٤- (كلورو فينيل) ٢-هيدروكسي إيثن نيتريل 3-(R,S) و ١- هيدروكسي حلقي هكسان كاربونيتريل (21a) و ١-هيدروكسي-٢-ميثيل حلقي هكسان كاربونيتريل 21b-(R,S) ، على التوالي. كما يمكن تحضير المركبات 3-(R,S) و 21a و 21b-(R,S) بتفاعل المركبات 1 و 2a,b مع مركب الأستون سيانو هيدرين (4). كما أمكن تحضير السيانو هيدرينات النشطه ضوئياً 3-(R) و 21b-(R) بمعالجه المركبين 1 و 2b ، على التوالي ، بمحلول هيدروجين سيانيد في وجود إنزيم (R)- هيدروكسي نيتريلاز (R)-PaHNL [EC 4.1.2.10] المستخرج من ثمار نبات اللوز. و تتفاعل السيانو هيد رينات 3 و 21 في صورتها المخلطه أو صورتها النشطه ضوئياً من خلال مجموعه الهيدروكسيل أو مجموعه السيانيد الموجوده في جزيئاتها. علاوه على ذلك ، فإن تفاعل مركبي 3 و 21b مع مركب (S)-نابروكسين كلوريد يعطى الدياستيريوميرات المقابله 8 و 22b ، على التوالي. كما تم قياس درجه النشاط الضوئي للمركبين 3-(R) و 21b-(R) و مشتقاتهما. وقد تأيدت التركيبات البنائيه للمركبات الجديده بواسطه التحاليل العنصريه الدقيقه والتحاليل الطيفيه مثل طيف الأشعه تحت الحمراء و طيف الرنين النووي المغناطيسي لنواه ذرتي الهيدروجين و الكربون و طيف الكتله وكذلك طيف حيود الأشعه السينيه. كما تم دراسه النشاط المضاد للأورام لبعض المركبات الجديده المخلطه ونظائرها ذات النشاط الضوئي و كذلك مناقشه العلاقه بين التركيب الكيمياءى والنشاط البيولوجى.