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Preparation and Reactions of Certain Racemic and Optically Active Cyanohydrins Derived from 2-Chlorobenzaldehyde, 4-Fluorobenzaldehyde, Benzo[*d*][1,3]-dioxole-5-carbaldehyde and 2,3-Dihydrobenzo[*b*][1,4]dioxine-6-carbaldehyde. Antimicrobial and *in vitro* Antitumor Evaluation of the Products

Hisham Abdallah A. Yosef [1*], A. M. Elmasry [2], Nabila M. Ibrahim [1], Eman H. I. Ismael [1] and M. R. H. Mahran [1]

[1]*Department of Organometallic and Organometalloid Chemistry, National Research Centre, El-Buhouth Street, P. O. 12622, Dokki, Giza, and* [2] *Department of Chemistry ,Zagazig University, Zagazig, Egypt.*

THE CHEMOENZYMATIC reaction of selected aldehydes, namely 2-chlorobenzaldehyde (1a), 4-fluorobenzaldehyde (1b), benzo[*d*][1,3]dioxole-5-carbaldehyde (1c) and/or 2,3-dihydrobenzo [*b*] [1,4] dioxine-6-carbaldehyde (1d) with hydrogen cyanide in presence of (*R*)-oxynitrilase (*R*)-Pa HNL [EC 4.1.2.10] from almonds, as a chiral catalyst, gave the optically active cyanohydrin enantiomers (*R*)-2a-c, respectively. Acetone cyanohydrin (3), was also used, as a transcyanating agent, to give the same products. The racemic cyanohydrins (*R,S*)-2a-d have been synthesized, as well, by treating compounds 1a-d with aqueous potassium cyanide solution in presence of a saturated solution of sodium metabisulphite ($Na_2S_2O_5$). The optical purity of cyanohydrins (*R*)-2a-c was determined through their derivatization with (*S*)-naproxen chloride (*S*)-5 to the respective diastereomers (*R,2S*)-6a-c which were obtained in diastereomeric excess (de) values up to 93 % (^{1}H NMR). Heating compounds (*R*)-2a,b and / or their racemic analogues (*R,S*)-2a-c with concentrated hydrochloric acid gave the respective α-hydroxycarboxylic acids 7a-c. Moreover, reduction of cyanohydrins (*R*,S)-2b,c under different conditions resulted in a hydrodecyanation giving the respective primary alcohols 8a,b. Structures and configurations of the new compounds were confirmed with compatible elementary microanalyses and spectroscopic (IR, ^{1}H NMR, ^{13}C NMR, MS and single crystal X-ray crystallography) measurements. The antimicrobial activity of derivatives 6a-d against four bacterial species *(Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa)* and two fungi (*Aspergillus flavus* and *Candida albicans*) were undertaken. Moreover, compounds (*R,2S*)-6b, (*R,2S*)(*S,2S*)-6b and (*R,2S*)−6c were screened for their *in virto* antitumor activity against three human solid cancer cell lines (HCT

* Corresponding author E-mail: hishamayosef@yahoo.com

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116, HepG2 and MCF-7). In general, the tested compounds were found inactive or showed weak activities in comparison with the standard drugs.

Keywords: Cyanohydrins, Chemoenzymatic synthesis, Reduction, Single crystal X-ray crystallography, Stereochemistry, Antimicrobial activity, Anticancer activity.

Racemic and optically active cyanohydrins belong to ubiquitous family and they have proven to be valuable building blocks for many synthetic purposes [1-9]. They are essentially prepared by hydrocyanation of the appropriate aldehyde or ketone [1-3, 5, 6]. In particular, optically active cyanohydrins are expedient starting materials for the direct synthesis of different classes of biologically interesting principles such as α–hydroxy carboxylic acids [1, 4, 5, 10, 11] and 2-amino alcohols [10, 12-14]. The enantioselective preparation of optically pure cyanohydrins is catalytically developed by making use of cyclic dipeptides [9], nanocrystalline magnesium oxide [15] and various chiral complexes of titanium [16], aluminum[17] and boron [18]. Besides, it can be enzymatically catalyzed by means of hydroxynitrile lyases (oxynitrilases) extracted from plant sources [2,4,19]. This approach is rather decisive, clean and cheap [5,20-23]. The increasing interest in synthesizing optically pure compounds is due to the well established correlation between the biological activity and stereochemical aspects [24-27]. Thus, the present work aims at preparing optically active cyanohydrins as well as their racemic analogous derived from 2-chlorobenzaldehyde (1a), 4-fluorobenzaldehyde (1b), benzo[*d*][1,3] dioxole-5-carbaldehyde (piperonal, heliotropine) (1c) and 2,3-dihydrobenzo[*b*][1,4] dioxine-6-carbaldehyde (1d) as well as studying their acid hydrolysis, reduction and derivatization with (*S*)-naproxen chloride (*S*)-5. The antimicrobial and antitumor activities of the new products have been also evaluated.

Results and Discussion

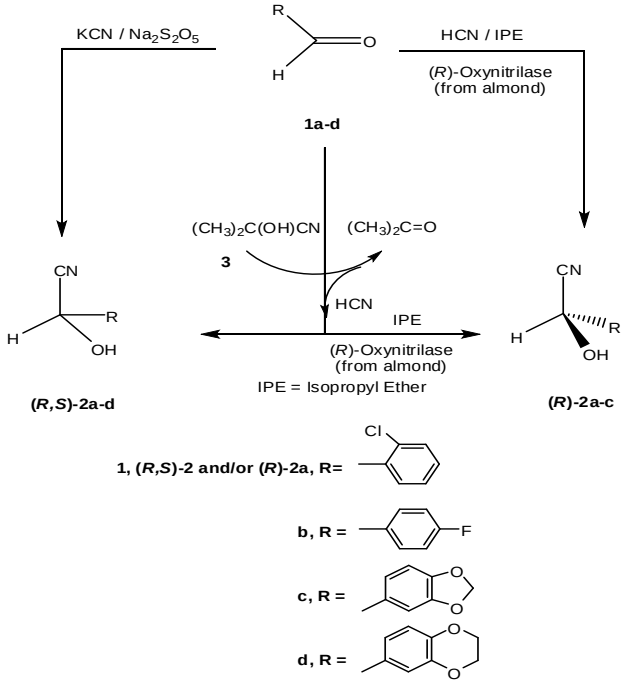
Chemistry

Synthesis of the optically active cyanohydrins 2a-d

Treatment of aldehydes 1a-d with aqueous KCN in presence of saturated aqueous sodium metabisulphite ($Na_2S_2O_5$) solution afforded the respective racemic cyanohydrins (*R*,*S*)-2a-d in yield values up to 85 %. The same reaction could also be completed by using acetone cyanohydrin (3) as a transcyanating reagent [28,29] (Scheme 1). Structures of compounds (*R*,*S*)-2a-d were confirmed with compatible elementary microanalyses and / or spectroscopic measurements (*cf.* experimental).

On the other hand, the optically active (*R*)-enantiomers of compounds 2a-c could be obtained by the treatment of aldehydes 1a-c with HCN in presence of (*R*)-oxynitrilase [EC 4.1.2.10] as a chiral catalyst. Almonds provide a rich source of this enzyme [2,19-23]. Thus, for example, compound (*R*)-2b was obtained with enantiomeric excess value (ee %) of 91 % (^{1}H NMR) and a specific rotation value $[\alpha]_{D/25}$ of + 38.4 (*c* 0.01666, acetone). Its IR spectrum (neat, ν_{max}, cm^{-1}) showed two bands at 3417 and 2253 cm^{-1} which are attributed to the O—H and

C≡N groups [30], respectively. The spectrum disclosed also the presence of strong absorption bands at 3090 (C—H, aromatic), 2920 (C—H, aliphatic) and at 1606 (C=C, aromatic).



Scheme 1. Synthesis of cyanohydrins 2a-d.

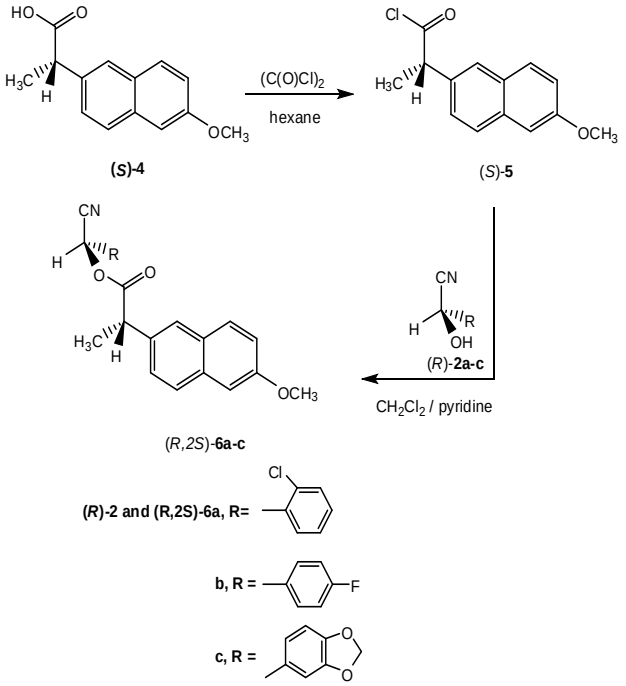
The ^{1}H NMR spectrum ($CDCl_3$, δ ppm) of (*R*)-2b showed signals at 4.09 (s, 1H, O*H*, D_2O exchangeable) and at 5.47 (s, 1H, O-C*H*-CN). The AA`BB` system due to protons of the 1,4-disubstituted benzene ring [30] (4H) appeared as two doublets at δ 7.06 (2H, *ortho* to the F atom) and at δ 7.42 (2H, *meta* to the F atom) each with J_{HH} = 7.6 Hz. Similarly, the (*R*)-2c enantiomer, namely (*R*)-2-(benzo[d][1,3]dioxol-5-yl)-2-hydroxyacetonitrile, was isolated as yellow oil in a yield value of 80 %. It was obtained with enantiomeric excess value (ee) of 93 % and has recorded a specific rotation $[\alpha]_{D/25}$ = + 26.4 (c 0.01666, acetone). The elemental microanalysis for compound (*R*)-2c corresponded to a molecular formula of $C_9H_7NO_3$ (177.16). The IR spectrum (neat) of (*R*)-2c revealed absorption bands at 3415 (O–H), 2904 (C–H, aliphatic) and 2247 (C≡N). Its ^{1}H NMR spectrum showed a D_2O exchangeable signal at δ 4.02 ppm attributed to the –O*H* group proton. The spectrum showed two singlet signals at 5.35 (1H) and at 5.94 (2H) due to the saturated methine NC-C*H*- and methylene O-CH_2-O protons, respectively. The aromatic protons (3H) appeared as a doublet (J_{HH} = 8.6 Hz, 1H) and a multiplet (2H) in the δ 6.89 - 6.91 ppm region.

In general, both racemic and optically active cyanohydrins undergo a number of transformations which involve the hydroxyl and/or cyanide functions in their molecules [1,2,4,5,12].

Determination of the enantiomeric excess (ee %) values of the optically active cyanohydrins (R)-2a-c.

Utilization of (*S*)-naproxen chloride (5) as a derivatizing agent for determining the optical purity of organic compounds [31] and as a chiral resolving agent for converting racemates to a mixture of diastereomers is well established [32]. We have now found that derivatization of (*R*)-2a-c with (*S*)-5 in CH_2Cl_2 in the presence of pyridine yields the respective diastereomers (*R,2S*)-6a-c (Scheme 2) with diastereomeric excess (de) values up to 93 % as calculated by quantitative analysis of the ^{1}H NMR spectra [32]. Comparable evaluation of the integral levels gives the diastereomeric excess ratios of (*R,2S*)-6a-c over the other diastereomers (*S,2S*)-6a-c, respectively. These values reflect the enantiomeric excess (*i.e.* the optical purity) of the starting cyanohydrins (*R*)-2a-c. For example, *(2S)-((R)*-cyano(4-fluorophenyl)methyl)-2-(6-methoxynaphthalen-2-yl)propanoate (*R,2S*)-6b was obtained as colourless crystals with a diastereomeric excess (de) value of 91 % and recorded $[\alpha]_{D/25}$ = + 48.0 (*c* 0.0025, acetone). Elementary and molecular weight determination (MS) of (*R,2S*)-6b corresponded to the molecular formula $C_{22}H_{18}FNO_3$ (MS : m/z 363, $M^{\bullet+}$, 5 %). Its IR spectrum (KBr, cm^{-1}) showed strong absorption bands at 2977, 2940 (C—H, aliphatic), 2260 (C≡N), 1744 (C=O, ester), 1603 (C=C, aromatic) and 1224, 1019 (C–O, stretching). The ^{1}H NMR spectrum of (*R,2S*)-6b ($CDCl_3$, δ ppm) showed signals at 1.61 (d, J_{HH} = 6.7 Hz, 3H, CH–C*H*$_3$), 3.88 - 3.90 (m, 4H, C*H*−CH$_3$ and O-C*H*$_3$), 6.40 (s, 1H, O–C*H*) and 6.94 -7.62 (m, 10 H, aromatics). Moreover, the absolute structural configurations of (*R,2S*)-6b and (*R,2S*)-6c were confirmed by X-ray crystallographic analyses. Their ORTEP overviews are outlined in Fig.1 and 2, respectively. Moreover, the X-ray crystal structural data, selected bond lengths, bond angles, torsion angles are represented, respectively, in Tables 1, 2, 3 and 4 for compounds (*R, 2S*)-6b,c.

Compounds (*R,2S*)(*S,2S*)-6a, b, d were similarly isolated as 50 : 50 mixtures of (*R,2S*) and (*S,2S*) diastereomers by reacting (*S*)-5 with the racemic cyanohydrins (*R,S*)-2a,b,d, respectively (*cf.* experimental). Naproxen® (*S*)-4 can be obtained by extraction with chloroform from commercially available tablets [31]. Treatment of compound 4 with oxalyl chloride in hexane yields the acid chloride (*S*)-5 [31] (Scheme 2).



Scheme 2. Derivatization of the optically active cyanohydrins (*R*)-2a-c with (*S*)-naproxen chloride (*S*)-5 to the respective diastereomers (*R,2S*)-6a-c.

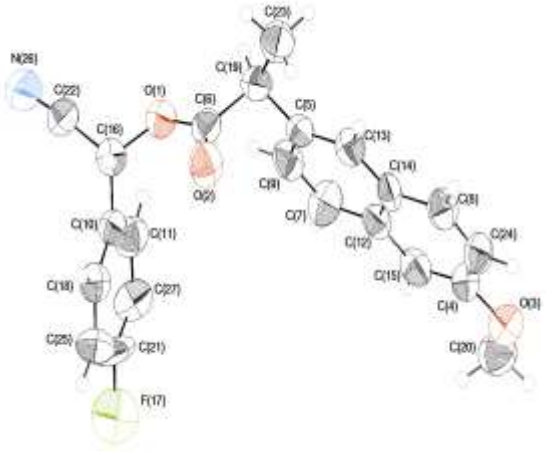


Fig. 1. ORTEP overview of compound (*R,2S*)-6b.

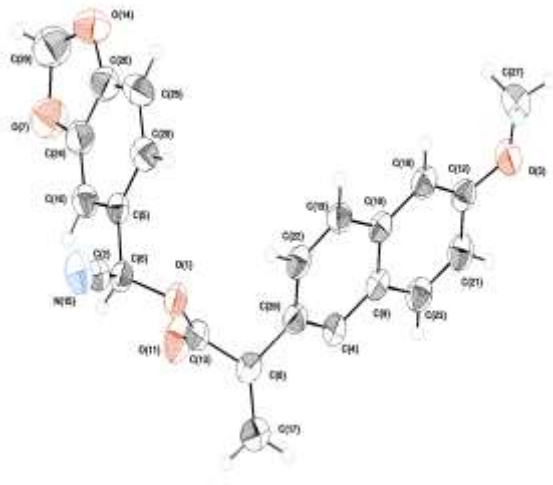


Fig. 2. ORTEP overview of compound (*R,2S*)−6c.

TABLE 1. Crystal structure and data refinement of compounds (*R,2S*)-6b and (*R,2S*)-6c.

	Compounds	
	(*R,2S*)-**6b**	(*R,2S*)-**6c**
Empirical Formula	$C_{22}H_{18}FNO_3$	$C_{23}H_{19}NO_5$
Formula Weight	363.388	389.407
Crystal System / Space Group	Monoclinic / $P2_1$	Monoclinic / $P2_1$
a / Å	9.6598 (4)	9.1266 (3)
b / Å	5.7545 (3)	5.7372 (2)
c / Å	16.7556 (9)	18.5929 (7)
α / º	90.00	90.00
β / º	93.481 (2)	101.034 (2)
γ / º	90.00	90.00
V / $Å^3$	929.68 (8)	955.55 (6)
Z	2	2
D_{calc} (g/cm^3)	1.298	1.353
μ (mm^{-1})	0.093	0.096
Colour / Shape	Colourless / Needles	Colourless / Needles
Wavelength	Mo *Ka* (0.71073 Å).	Mo *Ka* (0.71073 Å)
Temperature	298 K	298 K
Theta range for collection / º	2.910—30.034	0.00 − 34.08
Reflections collected	4274	6472
Independent reflections	4268	6465
Data / restraints / parameters	4268 / 1 / 239	1124 / 0 / 262
Goodness of fit on F^2	0.731	0.803
Final R indices [$I > 2\sigma$ (I)]	0.0585	0.031
R indices (all data)	0.2625	0.180
Largest difference peak / hole	0.220 / -0.288	0.131 / -0.161

TABLE 2. Selected bond lengths (Å) of compounds (*R,2S*)-6b and (*R,2S*)-6c.

(*R,2S*)-6b		(*R,2S*)-6c	
O1—C6	1.351 (7)	O1—C6	1.448 (3)
O1—C16	1.435 (7)	O1—C13	1.358 (4)
O2—C6	1.205 (7)	C2—C6	1.473 (5)
O3—C4	1.362 (7)	C2—N15	1.147 (5)
O3—C20	1.422 (8)	O3—C12	1.368 (4)
C4—C15	1.353 (8)	O3—C27	1.424 (4)
C4—C24	1.405 (8)	C5—C6	1.513 (4)
C5—C19	1.528 (7)	C5—C28	1.384 (5)
C6—C19	1.509 (8)	O7—C24	1.373 (4)
C8—C24	1.358 (7)	O7—C29	1.419 (5)
C8—C14	1.427 (7)	C8—C17	1.515 (5)
C10—C18	1.373 (8)	C9—C10	1.415 (4)
C10—C11	1.383 (8)	C10—C18	1.424 (4)
C10—C16	1.516 (8)	C10—C19	1.421 (4)
C11—C27	1.367 (9)	O11—C13	1.184 (4)
C12—C15	1.411 (7)	C12—C18	1.361 (4)
C13—C14	1.407 (7)	O14—C26	1.379 (5)
C16—C22	1.466 (9)	O14—C29	1.425 (6)
F17—C21	1.397 (7)	C19—C22	1.363 (4)
C18—C25	1.378 (9)	C20—C22	1.406 (4)
C19—C23	1.504 (9)	C24—C26	1.374 (5)
C21—C27	1.347 (10)	C25—C26	1.362 (6)
C22—N26	1.117 (8)	C25—C28	1.388 (5)

TABLE 3. Selected bond angles (degree) of compounds (*R,2S*)-6b and (*R,2S*)-6c.

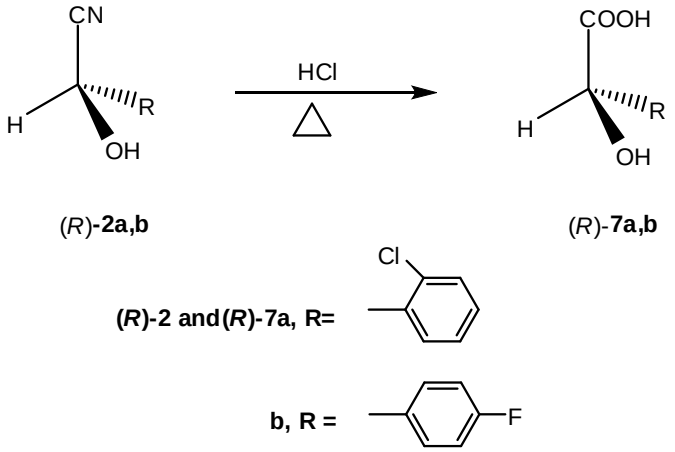
(*R,2S*)-6b		(*R,2S*)-6c	
C6—O1—C16	115.7 (5)	C6—O1—C13	115.4 (2)
C4—O3—C20	118.0 (5)	C6—C2—N15	178.3 (3)
O3—C4—C15	125.6 (6)	C12—O3—C27	117.7 (2)
C13—C5—C9	117.7 (5)	C16—C5—C28	120.6 (3)
C9—C5—C19	119.8 (5)	O1—C6—C2	104.0 (2)
O2—C6—O1	122.2 (6)	O1—C6—C5	114.8 (2)
O2—C6—C19	125.2 (6)	C2—C6—C5	110.7 (2)
O1—C6—C19	112.6 (6)	C24—O7—C29	104.9 (3)
C18—C10—C11	119.0 (6)	C4—C9—C10	119.7 (3)
C18—C10—C16	119.9 (6)	C4—C9—C23	122.7 (3)
C11—C10—C16	121.1 (6)	O3—C12—C18	125.9 (3)
O1—C16—C22	105.5 (5)	O3—C12—C21	113.8 (3)
O1—C16—C10	110.6 (5)	C18—C12—C21	120.3 (3)
C22—C16—C10	111.6 (5)	O1—C13—O11	123.4 (3)
C10—C18—C25	120.0 (7)	C26—O14—C29	105.0 (3)
C23—C19—C6	109.7 (6)	C10—C19—C22	121.2 (3)
C23—C19—C5	116.4 (5)	C4—C20—C22	118.5 (3)
C6—C19—C5	108.1 (5)	C12—C21—C23	120.7 (3)
C4—C15—C12	120.6 (6)	C19—C22—C20	121.4 (3)
C25—C21—C27	126.8 (7)	C9—C23—C21	121.3 (3)
C25—C21—F17	117.8 (9)	O7—C24—C26	110.3 (3)
C27—C21—F17	115.5 (9)	C26—C25—C28	117.0 (4)
C15—C12—C7	122.2 (6)	O14—C26—C24	109.6 (3)
N26—C22—C16	175.7 (8)	O14—C26—C25	128.6 (3)
C21—C25—C18	117.3 (7)	C24—C26—C25	121.8 (3)
C5—C13—C14	122.1 (6)	C5—C28—C25	121.5 (3)
C7—C12—C14	117.6 (5)	O7—C29—O14	108.9 (4)

TABLE 4. Selected torsion angles (degree) of compounds (*R,2S*)-6b and (*R,2S*)-6c.

(*R,2S*)-**6b**		(*R,2S*)-**6c**	
C16—O1—C6—O2	3.2(8)	C13—O1—C6—C2	149.4 (3)
C16—O1—C6—C19	-178.6(5)	C13—O1—C6—C5	-89.5 (3)
C6—O1—C16—C10	-88.7(6)	C6—O1—C13—O11	10.6 (3)
C6—O1—C16—C22	150.5(5)	N15—C2—C6—O1	157.0 (11)
C20—O3—C4—C15	3.4(9)	N15—C2—C6—C5	33.0 (11)
C20—O3—C4—C24	-177.4(6)	C27—O3—C12—C21	-175.8 (4)
O3—C4—C15—C12	-179.3(6)	C16—C5—C6—O1	130.2 (4)
O3—C4—C24—C8	179.1(6)	C16—C5—C6—C2	-112.4 (4)
C19—C5—C9—C7	-178.6(6)	C6—C5—C16—C24	175.1 (4)
C19—C5—C13—C14	178.3(5)	C28—C5—C6—O1	-53.1 (3)
C9—C5—C19—C6	70.1(7)	C28—C5—C6—C2	64.2 (3)
C9—C5—C19—C23	-165.9(6)	C6—C5—C28—C25	-175.3 (5)
C13—C5—C19—C6	-108.4(6)	C2—C6—O1—C13	149.4 (3)
C13—C5—C19—C23	15.6(8)	O3—C12—C21—C23	179.0 (4)
O1—C6—C19—C5	-104.3(5)	C26—O14—C29—O7	-10.8 (3)
O1—C6—C19—C23	127.8(5)	C29—O14—C26—C24	6.2 (3)
O2—C6—C19—C5	73.8(7)	C24—C16—C5—C6	175.1 (4)
O2—C6—C19—C23	-54.1(8)	C5—C16—C24—O7	-179.8 (5)
C16—C10—C11—C27	178.8(6)	C19—C22—C20—C4	0.4 (3)
C11—C10—C16—O1	-40.8(8)	C21—C23—C9—C4	-178.4 (5)
C11—C10—C16—C22	76.3(8)	C9—C23—C21—C12	-0.7 (3)
C18—C10—C16—O1	139.8(6)	O7—C24—C26—O14	0.7 (3)
C18—C10—C16—C22	-103.1(7)	O7—C24—C26—C25	-178.6 (5)
C16—C10—C18—C25	179.4(6)	C28—C25—C26—O14	179.2 (6)
O1—C16—C22—N26	164(11)	C24—C26—O14—C29	6.2 (3)
C10—C16—C22—N26	43(11)	O14—C26—C25—C28	179.2 (6)
F17—C21—C25—C18	179.8(7)	C5—C28—C25—C26	0.3 (3)
F17—C21—C27—C11	178.5(7)	O14—C29—O7—C24	11.2 (3)

Hydrolysis of cyanohydrins 2a-c to the corresponding α-hydroxy carboxylic acids 7-a-c.

Upon heating the optically active cyanohydrins (*R*)-2a,b and / or the racemic analogues (*R*,S)-2a-c with concentrated hydrochloric acid, they are hydrolyzed to the respective α-hydroxycarboxylic acids (*R*)-7a,b (Scheme 3) and/or (*R*,*S*)-7a-c in high percentage yields (*cf*. experimental). (*R*)-2-Chloromandelic acid (*R*)-7a is the key intermediate for the synthesis of the anti-thrompotic (anti-platelet) agent clopidogrel [33] (Fig. 3) which is sold in the form of its bisulphate salt under the original trade name Plavix®.



Scheme 3. Synthesis of the chiral α-hydroxycarboxylic acids (*R*)-7a,b.

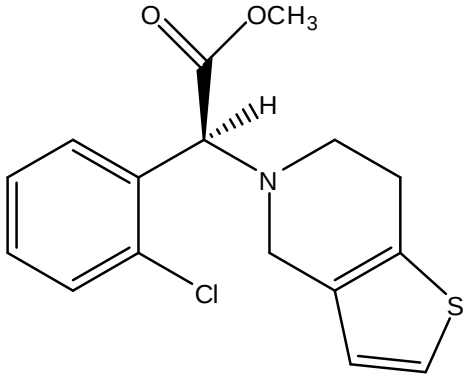


Fig. 3. The anti-platelet agent clopidogrel (Plavix®).

Elementary and spectroscopic measurements are in good support for the assigned structures. For example, structural reasonings for (*R*)-2-(4-fluorophenyl)-2-hydroxyacetic acid (*R*)-7b ($[\alpha]_{D/25}$= –189.2, *c* 0.00333, acetone) are:

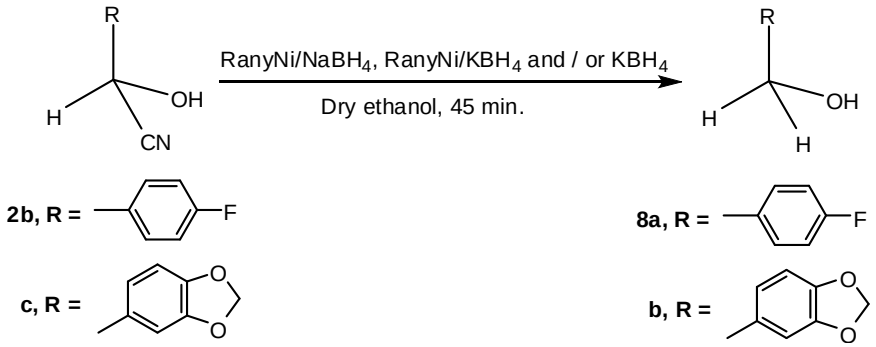
(a) Elementary analysis and molecular weight determination corresponded to $C_8H_7FO_3$, MS: m/z 170 ($M^{\bullet+}$, 0.1 %).

(b) The IR spectrum (KBr, cm^{-1}) revealed strong absorption bands at 3446 (O—H), 1722 (C=O), 1610 (C=C) and 1245, 1064 (C–O, stretching). The ^1H NMR spectrum (DMSO-d_6, δ ppm) showed two doublet signals at 7.13 and 7.42 ppm each with J_{HH} = 8.6 Hz (aromatic AA`BB` system) due to the *ortho* (2H) and *meta* (2H) protons to the fluorine atom. D_2O exchangeable singlets appeared at 12.66 (1H, COO*H*, carboxylic) and at 5.91 (1H, O*H*, alcoholic). The methine proton (O-C*H*–COOH) appeared as a sharp singlet at 5.01 ppm.

Action of selected reducing agents on cyanohydrins 2b,c

Upon treatment of cyanohydrins (*R,S*)-2b,c with Raney®-Ni / $NaBH_4$, Raney®-Ni / KBH_4 and / or KBH_4 in dry ethanol the reaction proceeded with a reductive hydrodecyanation [34,35] and total removal of the nitrile group yielding the respective primary alcohols 8a,b (Table 5). Mass spectrometry for (benzo[*d*]-[1,3]dioxol-5-ylmethanol (piperonyl alcohol) 8b corresponded to $C_8H_8O_3$ (MS: m/z 152, $M^{\bullet+}$, 100 %). Its IR spectrum (KBr, cm^{-1}) revealed absorption bands at 3325 (O—H), 3000 (C—H, aromatic), 2906 (C—H, aliphatic), 1604, 1499 (C=C, aromatic) and 1249 (C—O, stretching, alcohol). Its ^1H NMR spectrum ($CDCl_3$, δ ppm) showed signals at 1.92 (O*H*, D_2O exchangeable), 4.55 (s, 2H, CH_2–O) and at 5.95 (s, 2H, O–CH_2–O).

TABLE 5. Hydrodecyanation of cyanohydrins (*R*,S)-2b,c to the respective primary alcohols 8a,b.



Entry	Substrate (mmole)	Reducing Agent (mmole)	Temp.	Time (min.)	Product	Yield (%)
1	(*R,S*)-**2b** (10)	Raney Ni / KBH_4 (40 / 10)	r. t.	45	**8a**	60
2	(*R,S*)-**2b** (10)	KBH_4 40	r. t.	45	**8a**	54
3	(*R,S*)-**2c** (5)	Raney Ni / $NaBH_4$ (20 / 5)	r. t.	45	**8b**	65

Biological evaluation

The antimicrobial evaluation of selected naproxen derivatives 6

The newly synthesized naproxen derivatives (*R,2S*)-6a-c and (*R,2S*)(*S,2S*)-6a,b,d were screened *in vitro* against two Gram +ve bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram–ve bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two fungal species (*Aspergillus flavus* and *Candida albicans*) by using the modified Kirby–Bauer disc diffusion method [36]. Ampicillin and Amphotericin B were taken as reference drugs for the antibacterial and antifungal screenings, respectively. The results expressed as the

inhibition zone diameter (mm / mg sample) are compiled in Table 6 and represented in Fig. 4. In general, the tested compounds showed slight activity (9 mm/mg sample) and / or were found to be inactive against the four bacterial species. For example, only the diastereomeric mixtures (*R,2S*)(*S,2S*)-6a,b,d have shown activity against *Bacillus subtilis*. The diastereomeric pure derivatives (*R,2S*)-6a-c were found to be inactive against the four bacterial species except for compound (*R,2S*)-6b which showed a slight activity (9 mm/mg sample) against *Escherichia coli* only. Moreover, *Pseudomonas aeruginosa* was found to be sensitive to (*R,2S*) (*S,2S*)-6b,d, while *Staphylococcus aureus* is slightly sensitive to (*R,2S*)(*S,2S*)- 6d. Compound (*R,2S*)-6a is inactive against the four bacterial species, meanwhile its diastereomeric mixture analog (*R,2S*)(*S,2S*)- 6a showed slight activity against *Bacillus subtilis* (Gram +ve) and *Escherichia coli* (Gram –ve) bacterial species. This might reflect the well established relation between the stereochemistry of a given compound and its biological activity [24-27]. On the other hand, all the investigated compounds did not exert any activity against the two fungal species *Aspergillus flavus* and *Candida albicans*.

TABLE 6. The antimicrobial activity of compounds 6a–d expressed in inhibition zone diameter (mm / mg Sample).

Standards and Tested Compounds		Inhibition Zone Diameter (mm / mg sample)					
		Bacteria				Fungi	
		Gram–positive		*Gram–negative*			
		B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***A. flavus***	***C. albicans***
Standards	**Ampicillin** Antibacterial agent	20	18	19	20	---	---
	Amphotericin B Antifungal agent	---	---	---	–––	16	19
The Tested Compounds	(*R,2S*)-6a	0	0	0	0	0	0
	(*R,2S*)(*S,2S*)-6a	9	0	9	0	0	0
	(*R,2S*)-6b	0	0	9	0	0	0
	(*R,2S*)(*S,2S*)-6b	9	0	9	9	0	0
	(*R,2S*)-6c	0	0	0	0	0	0
	(*R,2S*)(*S,2S*)-6d	9	9	0	9	0	0

< 7 mm(non active), 7-9 mm (slightly active), 10-12 mm(moderately active), ≥ 13 mm (highly active).

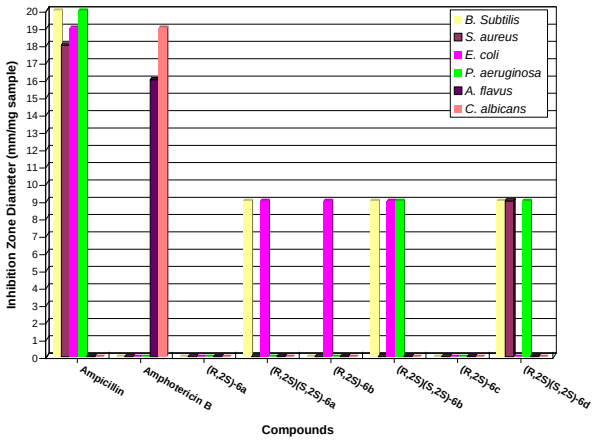


Fig. 4. The antimicrobial evaluation for derivatives 6a-d.

The antitumor screening for compounds (R,2S)-6b,c and(R,2S)(S,2S)-6b

Three of the newly synthesized compounds, *(R,2S)*-6b,c and *(R,2S)(S,2S)*-6b, were screened for their *in vitro* cytotoxic and growth inhibitory activities against human colon (HCT 116), liver (HepG2) and breast (MCF-7) cancer cell lines using the sulforhodamine B (SRB) method [37] in comparison with doxorubicin (DOX) as a reference drug [38]. The cytotoxic activities are expressed as IC_{50} values µg / mL (the concentration required for 50 % inhibition of cell viability) and are represented in Fig. 5. The relation between the surviving fraction and the concentrations of the investigated compounds is graphically plotted in Fig. 6-8 to get the survival curves for the tumor cell lines HCT 116, HepG2 and MCF-7, respectively. A gradual decrease in viability of cancer cells was observed with increasing concentration of the tested compounds.

TABLE 7. The cytotoxic activities expressed as IC_{50} values (µg / ml) of the tested compounds against HCT116, HepG2 and MCF−7 tumor cell lines.

Compound	IC_{50} values (µg / ml)		
	HCT116	HepG2	MCF−7
(*R,2S*)-6b	22.20	18.70	15.00
(*R,2S*)(*S,2S*)-6b	15.20	12.10	15.80
(*R,2S*)−6c	20.30	19.60	12.00
DOX	4.19	5.87	4.13

The investigated compounds were found to be active against the three cell lines. However, their cytotoxic activities are less than that of doxorubicin where they recorded higher IC_{50} values. Among the synthesized tested compounds, (*R,2S*)(*S,2S*)-6b was found to be the most potent against HCT 116 and HepG2 where it recorded IC_{50} values of 15.2, 12.1 (Table 7, Fig. 5), respectively.

Moreover, the diastereomeric mixture (*R,2S*)(*S,2S*)-6b is more potent than its diastereomeric pure form (*R,2S*)-6b (IC_{50} : 22.2 and 18.7) against HCT 116 and HepG2 cell lines, respectively. Again, this may also strengthen the correlation between activity and stereochemistry. For MCF-7, compound (*R,2S*)-6c was found to be the most potent among the tested synthesized compounds where it recorded IC_{50} value of 12.00. In general, the descending order of cytotoxic activity against HCT116 is DOX > (*R,2S*)(*S,2S*)-6b > (*R,2S*)-6c > (*R,2S*)-6b, against HepG2 is DOX >(*R,2S*)(*S,2S*)-6b >(*R,2S*)-6b > (*R,2S*)-6c and against MCF−7 is DOX > (*R,2S*)-6c >(*R,2S*)-6b >(*R,2S*)(*S,2S*)-6b.

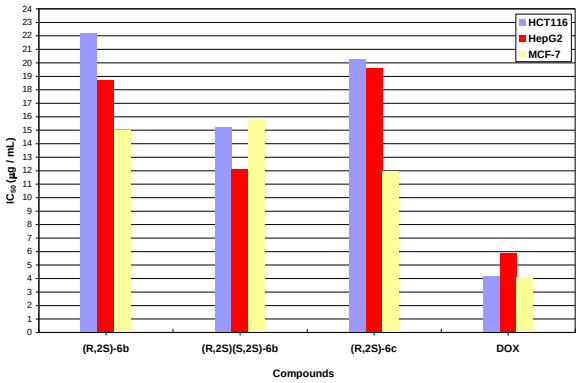


Fig. 5. The *in vitro* cytotoxicity of the tested compounds expressed as IC_{50} values against HCT 116, HepG2 and MCF-7 human cancer cell lines.

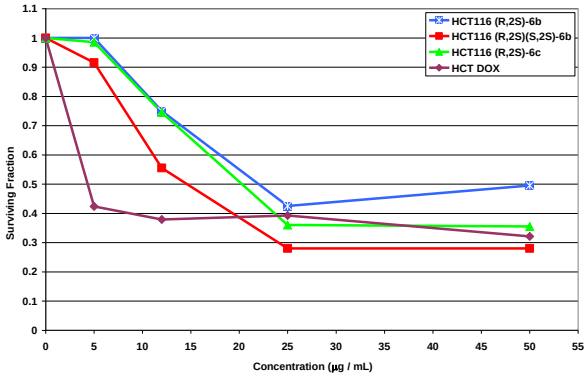


Fig. 6. The surviving fraction as a function of concentrations of the investigated compounds compared with doxorubicin (reference drug) against HCT116 tumor cell lines.

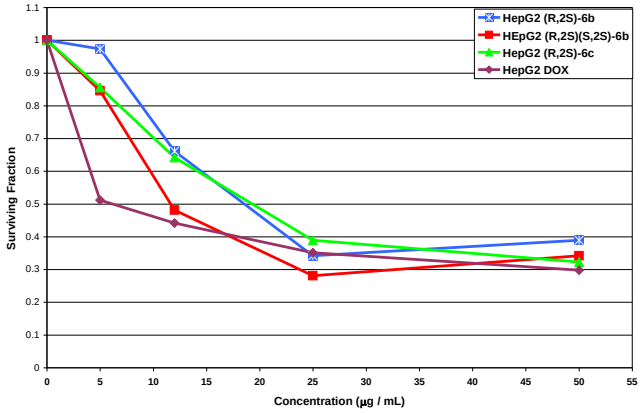


Fig. 7. The surviving fraction as a function of concentrations of the investigated compounds compared with doxorubicin (reference drug) against HepG2 tumor cell lines.

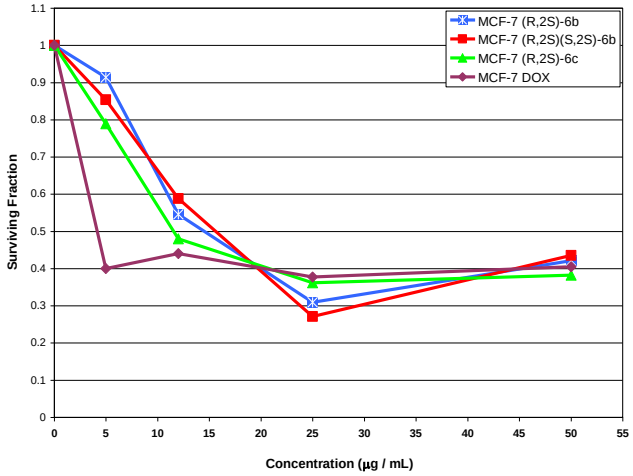


Fig. 8. The surviving fraction as a function of concentrations of the investigated compounds compared with doxorubicin (reference drug) against MCF−7 tumor cell lines.

Experimental

General

Reactions with air-sensitive reagents were carried out in flame-dried glassware under dry argon atmosphere. Solvents were dried and purified according to the usual procedures. The reacting aldehydes are commercially available and were purified directly before use. The almond meal and the isolated cyanohydrins were stored at -15 °C. (*R*)-Oxynitrilase enzyme (PaHNL)

[EC4.1.2.10] was extracted [39], assayed and its activity was measured [40] according to the given procedures. *p*H-Values were determined by Precisa Digital pH-Meter pH 900 with Ag/AgCl electrode. The activity of the enzyme was measured by Schimadzu UV-2401 PC UB-VIS Recording Spectrophotometer. The reactions were followed and the purity of the isolated products were controlled by TLC using silica gel with fluorescent indicator F_{254} coated on aluminum sheets of layer thickness 0.2 mm [Merck]. 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 F_{254}. Melting points are measured on Stuart SMP1 apparatus and are uncorrected. The angular rotations were measured on AA-65 Series Automatic Polarimeter, Optical Activity Ltd (England), National Research Centre. The specific rotation $[\alpha]_{D/25} = \alpha / c \,.\, l$ expressed in (°.L) / (Kg.dm) where α is the measured angular rotation, l (path length) = 1 dm and c (concentration) expressed in Kg / L. Infrared Spectra were performed either neat or in KBr discs using: JASCO FT/IR-300E Fourier Transform Infrared Spectrophotometer (National Research Centre, Egypt). The spectra were reported in cm^{-1}. The NMR spectra were recorded on: JEOL ECA-500 (running at 500 MHz for ^{1}H and 125 MHz for ^{13}C) (National Research Centre, Egypt) and / or Varian Mercury Vx-300 BB (running at 300 MHz for ^{1}H and 75 MHz for ^{13}C) (Micro Analytical Unit, Cairo University, Egypt) equipments. The spectra were obtained from deuterated chloroform ($CDCl_3$) and / or deuterated dimethylsulphoxide (DMSO-d_6) and the chemical shifts were reported in δ ppm units downfield from tetramethylsilane (TMS) as an internal standard. Splitting patterns were designated as follow: s = singlet; bs = broad singlet, d = doublet; m = multipult; q = quartet; t = triplet. The Mass Spectra were recorded on Shimadzu Qp-2010 Plus Spectrometer at 70 eV (Electron Impact). The elemental microanalyses were carried out at the Micro Analytical Centre, Cairo University, Egypt. X-Ray diffraction: the intensity data were performed with a Kappa-CCD Enraf Nonius FR 590 Single Crystal Diffractometer. The structures were solved by direct methods using the *SIR*92 program [41] and refined using ma*X*us [42]. The molecular graphics were made with ORTEP [43]. Crystallographic data (CIF) for the structures reported in this article have been deposited in the Cambridge Crystallographic Data Centre (CCDC) as supplementary publication No. CCDC 1523515 and 1523609. 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). The antimicrobial evaluation was carried out at the Micro Analytical Centre, Cairo University, Cairo, Egypt. The antitumor activity was carried out at the Cancer Biology Department, National Cancer Institute, Cairo University, Egypt.

Chemistry

Synthesis of the racemic cyanohydrins

General procedure [44]

In a three necked flask equipped with a mechanical stirrer and a dropping funnel, a saturated solution of sodium metabisulphite (150g in 200 ml of distilled

water) was added drop wise to a mixture of the appropriate aldehyde 1a-d (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 minutes), the reaction mixture was stirred for further 12 hours 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 material, the ether filtrate was evaporated well *in vacuo* to leave the corresponding racemic cyanohydrins (*R,S*)-2a-d.

(R,S)-2-(2-Chlorophenyl)-2-hydroxyacetonitrile (R,S)-2a
Yellow oil, yield 85 %. For further characterizations see (*R*)-2a.
(R,S)- 2-(4-Fluorophenyl)-2-hydroxyacetonitrile (R,S)-2b
Yellow oil, yield 85 %. For further characterizations see (*R*)-2b.
(R,S)-2-(benzo[d][1,3]dioxol-5-yl)-2-hydroxyacetonitrile (R,S)-2c
Yellow oil, yield 80 %. For further characterizations see (*R*)-2c.

(R,S)-2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-hydroxyacetonitrile (R,S)-2d
Colourless oil, yield 70 %. IR (neat, ν_{max}, cm^{-1}): 3435 (O—H), 3032 (C—H, aromatic), 2914 (C—H, aliphatic), 2240 (C≡N), 1612 (C=C, aromatic), 1030 (C—O, alcohol). ^{1}H NMR ($CDCl_3$, δ ppm): 4.07 (s, 1H, O*H*, D_2O exchangeable), 4.12 – 4.23 (m, 4H, O−CH_2−CH_2−O), 5.34 (s, 1H, NC−C*H*), 6.83 - 7.90 (m, 3H, aromatics). Anal. Calcd. (%) for $C_{10}H_9NO_3$ (191.18): C, 62.82; H, 4.74; N, 7.33. Found (%): C, 62.74; H, 4.77; N, 7.28.

Synthesis of the Racemic Cyanohydrins (R,S)-3a-c using Acetone Cyanohydrin as a Transcyanating Agent [29]

To a stirred solution of the appropriate aldehyde (0.1 mole) in diisopropyl ether (20 ml), acetone cyanohydrin (3) was added dropwise followed by sodium hydroxide (15 ml of 1M solution) at room temperature. The reaction mixture was stirred for 6 hours then followed-up as described in the previous general procedure to give the racemic cyanohydrins (*R,S*)-2a-c in yields of 81, 78 and 73 %, respectively.

Synthesis of the Optically Active Cyanohydrins

Step 1: Preparation of Hydrogen Cyanide Solution [45]

Orthophosphoric acid (0.2 mole, 13.7 ml) was added drop wise 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 minutes at 0°C. After stirring the reaction mixture for further 15 minutes, the cooling bath was removed and the ethereal layer was separated.

Step 2: Addition of Hydrogen Cyanide Solution to the aldehydes 1a-c: General Procedure [46]

The ethereal solution of hydrogen cyanide was added dropwise at 0 °C under stirring by a mechanical stirrer to a mixture of the crude enzyme extract [39,40] and the appropriate aldehyde 1a-c in diisopropyl ether (30 ml). After completion of the addition (30 min.), the reaction mixture was stirred for further 24 hours. 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 minutes. The ethereal layer was separated, washed with distilled water (2 × 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 cyanohydrins (*R*)-2a-c.

(R)-2-(2-Chlorophenyl)-2-hydroxyethanenitrile (R)-2a

Yellow oil, enantiomeric excess (ee) 93 %, $[\alpha]_{D/25}$ = – 79.0 (*c* 0.01538, acetone) (ref. [33]: (ee) 91 %), yield 93 %. IR (neat, ν_{max}, cm^{-1}): 3409 (O—H), 3072 (C—H, aromatic), 2922 (C—H, aliphatic), 2250 (C≡N), 1624, 1595 (C=C, aromatic), 1037 (C—O, alcohol), 757 (C—Cl, aromatic). ^{1}H NMR ($CDCl_3$, δ ppm): 4.21 (s, 1H, O*H*, D_2O exchangeable), 5.81 (s, 1H, NC–C*H*), 7.32 - 7.45 (m, 3H, aromatic), 7.70 (d, J_{HH}= 6.8 Hz, 1H, aromatic).

(R)- 2-(4-Fluorophenyl)-2-hydroxyacetonitrile (R)-2b

Yellow oil, enantiomeric excess (ee) 91 %, $[\alpha]_{D/25}$= +38.4 (*c* 0. 01666, acetone) (ref. [47]: (ee) 77 %), $[\alpha]_{D/25}$ = +23.5 (*c* 0.260, $CHCl_3$)), yield 88 %. IR (neat, ν_{max}, cm^{-1}): 3417 (O—H), 3090 (C—H, aromatic), 2920 (C—H, aliphatic), 2253 (C≡N), 1606 (C=C, aromatic), 1160 (C—F, aromatic), 1039 (C—O, alcohol). ^{1}H NMR ($CDCl_3$, δ ppm): 4.09 (s, 1H, O*H*, D_2O exchangeable), 5.47 (s, 1H, NC–C*H*), 7.06 (d, J_{HH} = 7.6 Hz, AA`BB` system, 2H, aromatic-*H ortho* to the F atom), 7.42 (d, J_{HH} = 7.6 Hz, AA`BB` system, 2H, aromatic-*H meta* to the F atom).

(R)-2-(benzo[d][1,3]dioxol-5-yl)-2-hydroxyacetonitrile (R)-2c

Yellow oil, enantiomeric excess (ee) 93 %, $[\alpha]_{D/25}$= +26.4 (*c* 0.01666, acetone), yield 80%. IR (neat, ν_{max}, cm^{-1}): 3415 (O—H), 3003 (C—H, aromatic), 2904 (C—H, aliphatic), 2247 (C≡N), 1617 (C=C, aromatic), 1036 (C—O, alcohol). ^{1}H NMR ($CDCl_3$, δ ppm): 4.02 (s, 1H, O*H*, D_2O exchangeable), 5.35 (s, 1H, NC–C*H*), 5.94 (s, 2H, O–CH_2–O), 6.76 (d, J_{HH}= 8.6 Hz, 1H, aromatic), 6.89 – 6.91 (m, 2H, aromatic). Anal. Calcd. (%) for $C_9H_7NO_3$ (177.16): C, 61.02; H, 3.98; N, 7.91. Found (%): C, 61.14; H, 3.95; N, 7.87.

Preparation of Optically Active Cyanohydrins using Acetone Cyanohydrin as a Transcyanating Agent [29]

Acetone cyanohydrin (3) (0.015 mole, 1.2 ml) was added to a mixture of the appropriate aldehyde 1a-c 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 cyanohydrins (*R*)-2a-c in yields of 78 %, 73 % and 69 %, respectively.

Determination of the enantiomeric excess of cyanohydrines (R)-2a-c through derivatization with naproxen chloride [31]

Step 1: Preparation of naproxen chloride (S)-5

Naproxen® (*S*)-4 (obtained from commercially available tablets after extraction with chloroform) (0.03 mole, 7g) was refluxed with freshly distilled oxalyl chloride (0.03 mole, 3.8 g , 2.6 ml) in dry hexane for 2 hours under dry argon atmosphere. The volatile materials were removed *in vacuo* to leave naproxen chloride (*S*)-5 as a pale yellow residue.

Step 2: Addition of naproxen chloride

A solution of naproxen chloride (*S*)-5 (from step 1), in 10 ml of dry methylene chloride, was added dropwise to a mixture of the appropriate cyanohydrin (*R*)-2a-c and / or (*R,S*)-2a,b,d (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 hours 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 plates by eluting with petroleum ether 60-80 °C/ acetone (85: 15).

(2S)-((R)-(2-chlorophenyl)(cyano)methyl)-2-(6-methoxynaphthalen-2-yl) propanoate (R,2S)-6a

Diastereomeric excess (de) 92 %, $[\alpha]_{D/25}$ = + 42.0 (*c* 0.00166, acetone). Colourless crystals, m.p. 90 - 91 °C, yield 60 %. IR (KBr, ν_{max}, cm^{-1}): 3059 (C—H, aromatic), 2939 (C—H, aliphatic), 1741 (C=O, ester), 1602 (C=C, aromatic), 1213 (C—O, ester), 1025 (C—O, ether), 767 (C—Cl, aromatic). ^{1}H NMR ($CDCl_3$, δ ppm): 1.64 (d, J_{HH} = 6.9 Hz, 3H, HC-C*H*$_3$), 3.91 (s, 3H, O-C*H*$_3$), 3.94 (q, J_{HH} = 6.9 Hz, 1H, C*H*-CH$_3$), 6.71 (s, 1H, O-C*H*-CN), 7.12 – 7.69 (m, 10H, aromatics). ^{13}C NMR ($CDCl_3$, δ ppm): 18.5 (*C*H$_3$), 45.1 (*C*H-C=O), 55.42 (O-*C*H-CN), 60.6 (O*C*H$_3$), 119 (*C*≡N), 105.6, 115.4, 126.1, 126.3, 127.5, 128.9, 129.4, 130.1 (aromatic *carbons*), 157.9 (=*C*-OCH$_3$), 172.4 (*C*=O). MS (70 eV, EI) m/z (%): 379 $[M]^{\bullet+}$ (14 %). Anal. Calcd. (%) 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, 9.25; N, 3.63.

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

Colourless crystals, m.p. 88-90 °C, $[\alpha]_{D/25}$ = + 16.0 (*c* 0.0025, acetone), yield 70 % . IR (KBr, ν_{max}, cm^{-1}): 3050 (C—H, aromatic), 2938 (C—H, aliphatic), 1743

(C=O, ester), 1604 (C=C, aromatic), 1216 (C—O, ester), 1027 (C—O, ether), 771 (C—Cl, aromatic). ^{1}H-NMR ($CDCl_3$, δ ppm): 1.63 (d, J_{HH}= 7.6 Hz, 3H, C-C*H*$_3$), 3.92 (s, 3H, O-C*H*$_3$), 3.97 (q, J_{HH} = 7.6 Hz, 1H, C*H*-CH$_3$), 6.69 (s, 1H, O-C*H*-CN, (*S,2S*)-diastereomer) [6.71 (s, 1H, O-C*H*-CN, (*R,2S*)-diastereomer)], 7.11–7.59 (m, 10H, aromatics). MS (70 eV, EI) m/z (%): 379 [M]$^{\bullet+}$ (48 %) and 381 [M$^{\bullet+}$+2] (12 %).

(2S)-((R)-cyano(4-fluorophenyl)methyl)-2-(6-methoxynaphthalen-2-yl) propanoate (R,2S)-6b

Colourless crystals, m.p.105-107 °C, diastereomeric excess (de) 91 %, $[\alpha]_{D/25}$ = + 48.0 (*c* 0.0025, acetone), yield 85 %. IR (KBr, ν_{max}, cm^{-1}): 3056 (C—H, aromatic), 2977, 2940 (C—H, aliphatic), 2260 (C≡N), 1744 (C=O, ester), 1603 (C=C, aromatic), 1224 (C—O, ester), 1137 (C—F), aromatic 1019 (C—O, ether). ^{1}H NMR ($CDCl_3$, δ ppm): 1.61 (d, J_{HH} = 6.7 Hz, 3H, CH−C*H*$_3$), 3.88 - 3.90 (m, 4H, C*H*−CH$_3$ and O-C*H*$_3$), 6.40 (s, 1H, O-C*H*-CN), 6.94 – 7.62 (m, 10H, aromatics). MS (70 eV, EI) m/z (%): 363 [M]$^{\bullet+}$ (5 %). Anal. Calcd. (%) for $C_{22}H_{18}FNO_3$ (363.38): C, 72.72; H, 4.99; F, 5.23; N, 3.85. Found (%): C, 72.66; H, 5.01; N, 3.80.

(2S)-((R,S)-cyano(4-fluorophenyl)methyl)-2-(6-methoxynaphthalen-2-yl) propanoate (R,2S),(S,2S)-6b

Colourless crystals, m.p.100-102 °C, $[\alpha]_{D/25}$ = + 56.0 (*c* 0.0025, acetone), yield 80 %. IR (KBr, ν_{max}, cm^{-1}): 3077 (C—H, aromatic), 2986 (C—H, aliphatic), 2267 (C≡N), 1746 (C=O, ester), 1605 (C=C, aromatic), 1226 (C—O, ester), 1138 (C—F, aromatic), 1026 (C—O, ether). ^{1}H NMR (DMSO-d$_6$, δ ppm): 1.47 (d, J_{HH} = 7.6 Hz, 3H, CH−C*H*$_3$), 3.82 (s, 3H, O−C*H*$_3$), 4.07 (q, J_{HH} = 7.6 Hz, 1H, C*H*−CH$_3$), 6.71 (s, 1H, O-C*H*-CN), 7.19 –7.72 (m, 10H, aromatics).

(2S)-((R)-benzo[d][1,3]dioxol-5-yl(cyano)methyl)-2-(6-methoxynaphthalen-2-yl) propanoate (R,2S)-6c

Diastereomeric excess (de) 93 %, $[\alpha]_{D/25}$ = +18.8 (*c* 0.00266, acetone). Colourless crystals, yield 75%, m.p. 96 – 98 °C. IR (KBr, ν_{max}, cm^{-1}): 3072 (C—H, aromatic), 2997, 2965 (C—H, aliphatic), 2254 (C≡N), 1747 (C=O), 1605 (C=C, aromatic), 1238 (C—O, ester), 1027 (C—O, ether). ^{1}H NMR ($CDCl_3$, δ ppm): 1.62 (d, J_{HH} = 6.9 Hz, 3H, CH−C*H*$_3$), 3.92 - 3.95 (m, 4H, C*H*−CH$_3$ and O–C*H*$_3$), 5.94 (s, 2H, O−C*H*$_2$–O), 6.31 (s, 1H, O−C*H*–CN), 6.69 (d, J_{HH} = 7.8 Hz, 1H, aromatic), 6.76 – 6.81 (m, 2H, aromatic), 7.11 – 7.16 (m, 2H, aromatic), 7.33 (d, J_{HH}=7.6 Hz, 1H, aromatic), 7.55 (s, 1H, aromatic), 7.63 – 7.69 (m, 2H, aromatic). MS (70 eV, EI) m/z (%): 389 [M]$^{\bullet+}$ (100 %, Base peak). Anal. Calcd. (%) for $C_{23}H_{19}NO_5$ (389.40): C, 70.94; H, 4.92; N, 3.60. Found (%): C, 70.86; H, 4.95; N, 3.54.

(2S)-((R,S)-cyano(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)methyl)-2-(6-methoxynaphthal-en-2-yl) propanoate (R,2S)(S,2S)-6d

Colourless crystals, m.p. 98-100 °C, $[\alpha]_{D/25}$ = + 38.6 (*c* 0.00155, acetone), yield 98 %. IR (KBr, ν_{max}, cm^{-1}): 3060 (C—H, aromatic), 2935 (C—H, aliphatic), 2240 (C≡N); 1744 (C=O), 1600 (C=C), 1289 (C—O, ester), 1065 (C—O, ether). ^{1}H NMR (DMSO-d_6, δ ppm): 1.45 (d, J_{HH} = 6.7 Hz, 3H, CH−C*H*$_3$), 3.83 (s 3H, O−C*H*$_3$), 4.06 (q, J_{HH}= 6.7 Hz, 1H, C*H*−CH$_3$), 4.17 – 4.22 (m, 4H, O−C*H*$_2$−C*H*$_2$−O), 6.55 (s, 1H, O−C*H*−CN), 6.82 −6.95 (m, 3H, aromatics), 7.13 (t, J_{HH} = 6.7 Hz, 1H, aromatic), 7.26 – 7.33 (m, 2H, aromatics), 7.64 – 7.76 (m, 3H, aromatics). ^{13}C NMR (DMSO-d_6, δ ppm): 23.3 (*C*H$_3$−CH), 49.1 (*C*H−C=O), 60.3 (O−*C*H3), 67.8 (N≡C-*C*H-O), 69.1, 69.2 (O−*C*H$_2$–*C*H$_2$–O), 110.9, 121.5, 121.8, 122.1, 122.6, 123.9, 125.8, 129.7, 130.8, 131.2, 132.2, 133.4, 134.2, 138.5, 139.7, 148.6, 149.9 (*C*≡N and aromatic *carbons*), 162.4 (=*C*−OCH$_3$), 177.5 (*C*=O). MS (70 eV, EI) m/z (%): 403 $[M]^{\bullet+}$ (6%). Anal. Calcd. (%) for $C_{24}H_{21}NO_5$ (403.43): C, 71.45; H, 5.25; N, 3.47. Found (%): C, 71.37; H, 5.27; N, 3.43.

Hydrolysis of cyanohydrins (R)-2a,b and (R,S)-2a-c to the respective α-hydroxy-carboxylic acids (R)-7a,b and (R,S)-7a-c

General procedure

A solution of the appropriate optically active and / or racemic cyanohydrin (0.03 mole) in concentrated hydrochloric acid (50 ml) was stirred for 16 hours at room temperature, then refluxed for 5 hours. 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. After removing the solid material, the filtrate was evaporated *in vacuo* and the residual substance was collected and recrystallized from the appropriate solvent to give the respective α-hydroxycarboxylic acid.

(R)-2-(2-Chlorophenyl)-2-hydroxyacetic acid (R)-7a

Pale yellow crystals, m. p. 116 – 118 ºC ($CHCl_3$), $[\alpha]_{D/25}$ = −138.1 (*c* 0.00166, acetone) (Ref. [33] : m. p. 119 – 121, $[\alpha]_{D/23}$= -126 (*c* 3 %, H_2O)), yield 80 %. IR (neat, ν_{max}, cm^{-1}): 3450 (O−H), 3070 (C−H, aromatic), 2986 (C−H, aliphatic), 1732 (C=O), 1584 (C=C, aromatic), 1225 (C−O, acid), 1082 (C−O, alcohol), 756 (C−Cl, aromatic). ^{1}H NMR ($CDCl_3$, δ ppm): 5.22 (bs, 1H, O*H*, alcohol, D_2O exchangeable), 5.64 (s, 1H, O-C*H*-), 7.25 – 7.48 (m, 5H, aromatics and COO*H*). MS (70 eV, EI) m/z (%): 186 $[M^{\bullet+}]$ (2 %), 188 $[M^{\bullet+}+2]$ (0.8 %).

(R)-2-(4-Fluorophenyl)-2-hydroxyacetic acid (R,)-7b

Colourless crystals, m.p. 130-132 °C ($CHCl_3$), $[\alpha]_{D/25}$= −189.2 (*c* 0.00333, acetone), yield 95%. IR (KBr, ν_{max}, cm^{-1}): 3446 (O−H), 1722 (C=O), 1610 (C=C, aromatic), 1245 (C−O, acid), 1200 (C—F, aromatic), 1064 (C−O, alcohol). ^{1}H NMR (DMSO-d_6, δ ppm): 5.01 (s, 1H, C*H*−O), 5.91 (bs, 1H, O*H*, D_2O exchangeable), 7.13 (d, J_{HH} = 8.6 Hz, AA`BB` system, 2H, aromatic *protons ortho* to the F atom), 7.42 (d, J_{HH} = 8.6 Hz, AA`BB` system, 2H,

aromatic-*H meta* to the F atom), 12.66 (bs, 1H, COO*H*, D_2O exchangeable). MS (70 eV, EI) m/z (%): 170 $[M]^{\bullet+}$ (0.1 %). Anal. Calcd. (%) for $C_8H_7FO_3$ (170.14): C, 56.48; H, 4.15.; F, 11.17. Found (%): C, 56.40; H, 4.18.

The racemic (*R,S*)-7a,b acids have been similarly prepared in yield values of 75 and 90 % with m. p. 116 ºC and 128-130 ºC, respectively.

(R,S)-2-(Benzo[d][1,3]dioxol-5-yl)-2-hydroxyacetic acid (R,S)-7c

Brown crystals, m. p. 162-164 °C (acetone), yield 60%. IR (KBr, ν_{max}, cm^{-1}): 3448 (O—H), 2935 (C—H, aliphatic), 1720 (C=O), 1607 (C=C, aromatic), 1251 (C—O, acid), 1076 (C—O, alcohol). ^1H NMR ($CDCl_3$, δ ppm): 2.91 (bs, 1H, O*H*, D_2O exchangeable); 5.16 (s, 1H, O-C*H*-), 5.98 (s, 2H, O–CH_2–O), 6.81 (d, J_{HH} = 8.4 Hz, 1H, aromatic), 6.88 – 6.95 (m, 2H, aromatic and COO*H*), 7.27 (s, 1H, aromatic). MS (70 eV, EI) m/z (%): 197 $[M^{\bullet+}+1]$ (0.2 %). Anal. Calcd. (%) for $C_9H_8O_5$ (196.16): C, 55.11; H, 4.11. Found (%): C, 54.80; H, 4.16.

The reduction of Cyanohydrins (R,S)-2b,c

General procedures [48]

1) Raney®-Ni (moist weight 0.64 g, approximately 10 mmol), KBH_4 (2.16 g, 40 mmol) and 25 ml of absolute ethanol were placed in a 50 ml flask, then (*R,S*)-2b (1.5 g, 10 mmol), was added while stirring. After vigorous stirring at room temperature for 45 min., the reaction mixture was filtered. The organic layer was evaporated and the residue was dissolved in ethyl acetate and then washed with water. The organic layer was dried, evaporated and the product was purified by chromatography on silica gel plates to give the (*R,S*)-(4-fluorophenyl)methanol (4-fluorobenzyl alcohol) 8a.

2) Alcohol 8a was also obtained when the same procedure was conducted by using KBH_4 as a reducing agent without Raney®-Ni.

(4-Fluorophenyl)methanol 8a [49]

Pale Yellow oil, yield 60 %. IR (neat, ν_{max}, cm^{-1}): 3355 (O–H), 3040 (C–H, aromatic); 2932, 2880 (C–H, aliphatic), 1604 (C=C, aromatic), 1157 (C—F, aromatic), 1012 (C–O, alcohol). ^1H NMR ($CDCl_3$, δ ppm): 2.30 (bs, 1H, O*H*, D_2O exchangeable), 4.63 (s, 2H, O–CH_2), 7.01 (d, J_{HH} = 8.6 Hz, 2H, AA`BB` system, aromatic-*H ortho* to the F atom), 7.31 (d, J_{HH} = 8.6 Hz, 2H, aromatic-*H meta* to the F atom, AA`BB` system). MS (70 eV, EI) m/z (%): 126 $[M]^{\bullet+}$ (7 %).

3) Raney®-Ni (moist weight 0.32 g, approximately 5 mmol), $NaBH_4$ (0.8 g, 20 mmol), and 25 ml dry ethanol were placed in a 50 ml flask, then cyanohydrin (*R,S*)-2c (0.88 g, 5 mmol), was added while stirring. After vigorous stirring at room temperature for 45 min, the reaction mixture was filtered. The organic layer was evaporated and the residue dissolved in ethyl acetate, then washed with water. The organic layer was dried, evaporated and the product was obtained after chromatography on silica gel plates to give alcohol 8b.

Benzo[d][1,3]dioxol-5-ylmethanol (Piperonyl alcohol) 8b

Brown crystals, m.p. 48-50 °C (Ref. [50]: m. p. 51 °C), yield 65%. IR (KBr, ν_{max}, cm^{-1}): 3325 (O—H), 3000 (C—H, aromatic), 2909 (C—H, aliphatic), 1604, 1499 (C=C, aromatic), 1249 (C—O, alcohol). ^{1}H NMR ($CDCl_3$, δ ppm): 1.92 (bs, 1H, O*H*, D_2O exchangeable), 4.55 (s, 2H, CH_2−O), 5.95 (s, 2H, O−CH_2−O), 6.77 – 6.85 (m, 3H, aromatics). MS (70 eV, EI) m/z (%): 152 (100 %) $[M]^{\bullet+}$.

The biological evaluation

The antimicrobial sensitivity test

Antimicrobial activity of the tested samples was determined using a modified Kirby−Bauer disc diffusion method [36]. Briefly, 100 µL of the tested bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 108 cells/ml for bacteria or 105 cells/mL for fungi [51]. A 100 µL of microbial suspension was spread onto agar (Müller-Hinton agar) plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility. Plates inoculated with filamentous fungi *as Aspergillus flavus* at 25 ºC for 48 hours; Gram (+) bacteria as *Staphylococcus aureus, Bacillus subtilis*; Gram (-) bacteria as *Escherichia coli, Pseudomonas aeruginosa* they were incubated at 35-37 °C for 24-48 hours and yeast as *Candida albicans* incubated at 30 °C for 24-48 hours. Standard discs of Ampicillin (Antibacterial agent), Amphotericin B (Antifungal agent) served as positive controls for antimicrobial activity but filter discs impregnated with 10 µL of solvent (DMSO) were used as a negative control. Blank paper disks with a diameter of 8.0 mm were impregnated with 10µL of the tested chemical and placed on agar where the chemical diffuses from the disc into the agar. When an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the tested chemical. The area of no growth around the disc is known as the "Zone of inhibition" whose diameter was measured in millimeters with a sterilized slipping calipers.

In Vitro cytotoxicity assay

Materials

Sulforhodamine-B stain (SRB) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), fetal bovine serum (FBS), doxorubicin, RPMI-1640 medium, trypsin, penicillin, dimethylsulphoxide (DMSO), trypan blue stain, streptomycin, sodium bicarbonate, acetic acid, trichloroacetic acid (TCA) were obtained from Sigma Chemical Company (St. Louis, Mo, U.S.A). The selected new compounds were screened against three human tumor cell lines, namely, colon (HCT 116), liver (HepG2) and breast (MCF-7) cell lines, obtained frozen in liquefied nitrogen (-180 ºC) from the American Type Culture Collection (Rockville, MD, USA). The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing.

Method

The antiproliferative activity was measured *in vitro* using the sulforhodamine-B stain (SRB) assay according to the reported standard procedure [37]. Cells were seeded in 96-multiwell microtiter plates at a concentration of $5x10^4$-10^5 cell / well in a fresh medium for 24 hr to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO at 1 mg / mL immediately before use and diluted to the appropriate volume just before addition to the cell culture. Cells were incubated with the appropriate concentration ranges of the tested compounds and doxorubicin, completed to total of 200 µL volume / well using fresh medium for 24, 48 and 72 hr. Control cells were treated with vehicle alone. Four wells were used for each individual drug concentration. After 24, 48 and 72 hr. treatment, the cells were fixed with 50 µL of cold 50 % trichloroacetic acid for 1 hr at 4 ºC. Wells were washed 5 times with distilled water and stained for 30 min at room temperature with 50 µL of 0.4 % SRB dissolved in 1 % acetic acid. Unbounded dye was removed by four washes with 1 % acetic acid. Then, the plates were air-dried and the dye was recovered with 100 µL / well of 10 mM tris-base (pH 10.5) for 5 min on a shaker (Orbital shaker OS 20, Boeco, Germany) at 1600 rpm. The optical density (O.D.) of each well was measured spectrophotometrically at 564 nm with an ELISA microplate reader (Meter tech. Σ 960, U.S.A.). The mean background absorbance was automatically subtracted and the mean values of each drug concentration was calculated. The percentage of cell survival was calculated as follows: Survival fraction = O.D. (treated cells) / O.D. (control cells). The experiment was repeated 3 times for each cell line.

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تحضير و تفاعلات بعض السيانو هيدرينات المُخَلَطَه و النشطه ضوئيا المشتقه من ٢-كلوروبنزألدهيد و ٤-فلوروبنزألدهيد و بنزو[*d*][٣,١] داى أوكزول-٥-كاربألدهيد و ٢،٣-داى هيدروبنزو[*b*][٤,١] داىأوكزين-٦-كاربألدهيد. تقييم النشاط المضاد للميكروبات و المضاد للأورام للنواتج

هشام عبد الله عبد المنعم يوسف[١] و أحمد مؤمن المصرى[٢] و نبيله مصطفى إبراهيم[١] و إيمان حامد إمام إسماعيل[١] و محمد رفعت حسين مهران[١]

[١] قسم الكيمياء العضويه الفلزيه والعضويه شبه الفلزيه – المركز القومى للبحوث – شارع البحوث - الرقم البريدى ١٢٦٢٢ - الدقى – الجيزه و [٢] قسم الكيمياء – كليه العلوم – جامعه الزقازيق – الزقازيق – مصر.

عند تفاعل الألدهيدات ٢- كلوروبنزألدهيد (**1a**) و ٤- فلوروبنزألدهيد (**1b**) و بنزو[*d*][٣,١] داى أوكزول -٥- كاربألدهيد (**1c**) و / أو ٢،٣- داى هيدروبنزو[*b*][٤,١] داى أوكزين - ٦- كاربألدهيد (**1d**) مع مركب سيانيد الهيدروجين فى وجود إنزيم (*R*) - أوكسى نيتريلاز (*R*)-PaHNL [EC4.1.2.10] المستخلص من ثمار نبات اللوز فإنه يتكون السيانوهيدرينات النشطه ضوئياً المقابله (*R*)-**2a-c**. كما يمكن تحضير السيانو هيدرينات (*R*)-**2a-c** بتفاعل الألدهيدات **1a-c** مع مركب الأسيتون سيانوهيدرين **(3)** فى وجود الإنزيم. كما أمكن تحضيرالسيانوهيدرينات المُخَلَطَه (الراسيميه) (*R,S*)-**2a-d** بمعالجه المركبات **1a-d** بمحلول مائى من مركب سيانيد البوتاسيوم فى وجود محلول مشبع من مركب ميتابايكبريتيت الصوديوم ($Na_2S_2O_5$). و قد تم تعيين درجه النقاء الضوئى للسيانوهيدرينات النشطه ضوئياً (*R*)-**2a-c** بتفاعلها مع مركب (*S*)-كلوريد النابروكسين **5**-(*S*) لتعطى الدياستيريوميرات المقابله (*R,2S*)-**6a-c** حيث تم قياس نسب زياده هذه الدياستيريوميرات و ذلك بدراسه نتائج التحليل الطيفى لنواه ذره الهيدروجين، لكل مركب على حده، حيث وصلت النسبه لبعض منها إلى ٩٣ % . عند تسخين السيانو هيدرينات النشطه ضوئياً (*R*)-**2a,b** و النظائر المخلطه (*R,S*)-**2a-c** مع حمض الهيدروكلوريك المركز فإنه تتكون أحماض *ألفا*-هيدروكسى كاربوكسيلك المقابله **7**. علاوه على ذلك ، فإن إختزال السيانوهيدرينات (*R,S*)-**2b,c** تحت ظروف تفاعل مختلفه قد أدى إلى نزع مجموعه السيانيد و إحلالها بذره هيدروجين لتتكون الكحولات الأوليه المقابله **8a,b** . و قد تأيدت التركيبات البنائيه و الفراغيه للمركبات الجديده بواسطه التحاليل الدقيقه للعناصر و كذلك التحاليل الطيفيه مثل طيف الأشعه تحت الحمراء و طيف الرنين النووى المغناطيسى لنواه ذره الهيدروجين ونواه ذره الكربون و طيف الكتله و كذلك طيف حيود الآشعه السينيه وحيده البللوره. و قد تم دراسه نشاط المركبات **6a-d** ضد أربعه سلالات من البكتريا و سلالتين من الفطريات . علاوه على ذلك ، فقد تم عمل مسح للمركبات (*R,S*)-**6b** , (*R,2S*)(*S,2S*)-**6b** , (*R,2S*)-**6c** لدراسه نشاطها كمضادات للأورام ضد خطوط خلايا سرطانيه بشريه لسرطان القولون و الكبد و الثدى.