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Synthesis and Biological Evaluation of Some Novel Chiral Carbamates and 4-imino-2-oxazolidinones Derived from Selected Optically Active Cyanohydrins

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ABSTRACT

The reaction of tert-butyl isocyanate (2) with the optically active cyanohydrins (R)-1a,b was accompanied with inversion of configuration giving the chiral (S)-enantiomers of the respective carbamate derivatives 3a,b. On the other hand, reactions of aryl isocyanate reagents Ar-N=C=O 5a-d with cyanohydrins (R)-1a-c gave the corresponding optically active 4-imino-2-oxazolidinone derivatives 7a-h in the form of their S-configuration. Moreover, the same reactions were also applied for the racemic cyanohydrins 1a-c and/or 1d to afford the corresponding carbamates (R,S)-3a,b, (R,S)-4 and/or 4-imino-2-oxazolidinones (R,S)-7b,d-h as racemic mixtures. Structures of the new products were elucidated with compatible micro analytical and spectroscopic (IR, ^{1}H-NMR, ^{13}C-NMR and MS) measurements. The X-ray crystallographic analysis provided an efficient tool in confirming the structure and configuration of the new chiral compounds. The antimicrobial activity of selected new derivatives against four bacterial species (Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa) and two fungi (Aspergillus flavus and Candida albicans) were evaluated. Moreover, some of the new products were screened for their in vitro antitumor activity against the human solid cancer cell lines, Human Carcinoma (HCT116), Human Hepatocellular Liver Carcinoma (HepG2) and Human Breast Adenocarcinoma (MCF-7) cell lines. Generally, most of the investigated compounds have shown moderate to high activities in comparison with the standard drugs. The structure-activity relationship (SAR) has been also discussed.

Keywords: Cyanohydrins, Chiral carbamates, Chiral 4-imino-2-oxazolidinones, Antimicrobial activity, Anticancer activity

INTRODUCTION

Carbamates are important intermediates in the synthesis of many valuable compounds in pharmaceutical, medicinal, agrochemical and polymer chemistry [1-3]. They possess many biologically potent properties such as antimicrobial [4-7], antiviral [8,9], antioxidant [10], anticonvulsant [11], antidiabetic [12], antitumor [13], anticancer [14], antihypertensive [15] and enzyme inhibitor [16] activities. Many drugs contain the carbamate moiety in their structures, such as, ritonavir (Norvir®) (Figure 1) which is an antiretroviral in combination with other medications [17] and chlorphenesin carbamate (Maolate®) (Figure 1) which is used for the treatment of pain associated with skeletal muscle trauma [18]. Moreover, the carbamate derivative of gemcitabine (Gemzar®), the anticancer drug, was found also to have a cytotoxic anti-neoplastic activity [19]. In addition, carbamate derivatives are useful scaffolds in the synthesis of many insecticides such as carbofuran (Furadan®) [20] (Figure 1), carbaryl (Sevin®) [21], fenoxycarb (Logic®) [22] and methiocarb (Mesurol®) [23]. In organic synthesis and peptide chemistry, among the various amino-protecting groups, carbamates are commonly used to enhance their chemical stability toward acids, bases and hydrogenation [24,25].

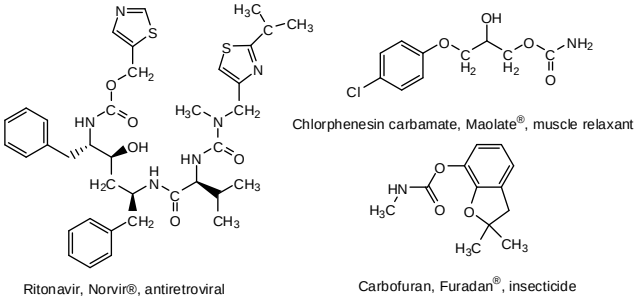


Figure 1: Some bioactive carbamate derivatives

Moreover, 2-oxazolidinones represent also an important class of compounds. They show good antibacterial properties where they act against a wide spectrum of Gram-positive and some Gram-negative pathogenic bacteria [26-30]. These compounds act by inhibiting the bacterial protein biosynthesis [31,32]. Linezolid is the first oxazolidinone derivative with antibacterial action that has been introduced into clinical use by Pharmacia Corp. (now Pfizer) and marketed under the trade name Zyvox® (Figure 2) [33-35]. Linezolid has good activity against many important multidrug resistance Gram-positive human pathogens, including Methicillin-resistance *Staphylococcus aureus* (MRSA), Vancomycin-resistance *Enterococcus faecium* (VER) and Penicillin-resistance *Streptococcus pneumoniae* [33]. In addition, some marketed 2-oxazolidinone derivatives include tedizolid (Sivextro®, antibacterial) [36], rivaroxaban (Xarelto®, Anticoagulant) (Figure 2) [37], toloxatone (Humoryl®, antidepressant) [38] (Figure 2) and zolmitriptan (Zomig®, for treatment of migraine) (Figure 2) [39].

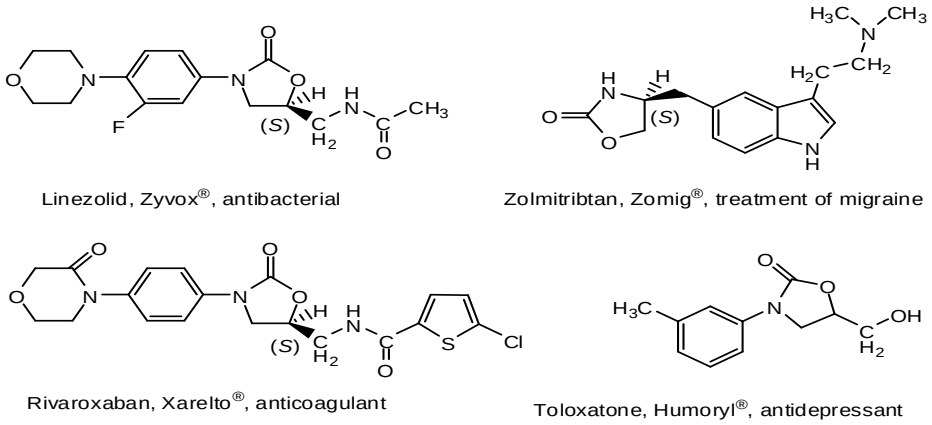


Figure 2: Selected pharmaceutical compounds containing the 2-oxazolidinone structural core

Others of this class of compounds have entered development such as ranbezolid [40], posizolid [41], radezolid [42] and eperezolid [43] (antibacterials) and befloxatone (a reversible inhibitor of monoamine oxidase A) [44]. In addition to their versatile biological activities [26-37, 39-44], the enantiomerically pure 2-oxazolidinones are also used as chiral axillaries in asymmetric synthesis of many natural products and pharmacologically active compounds [45-47]. The well-established activity-chirality relationship [48-51], the importance of enantiomeric purity of drugs [49,52], the need for new effective antimicrobial agents to overcome the problem of bacterial drug resistance [53,54] and the requirement of more efficient preparative pathways to enantiomerically pure drugs have prompted us to continue our efforts [55-57] to synthesize new chiral organic compounds with potential biological activities. Thus in this study, we have investigated the reactions of the optically active cyanohydrins (*R*)-1a-d and/or their racemic forms (Figure 3) with some selected isocyanate reagents and evaluated the antimicrobial and anticancer activities of the new carbamate and/or 4-imino-2-oxazolidinone derivatives.

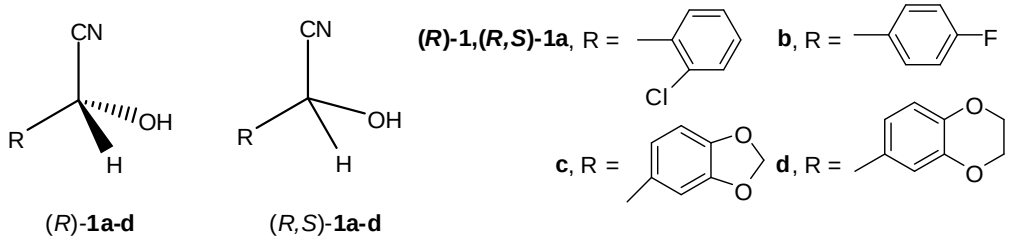


Figure 3: The chiral and/or racemic cyanohydrins 1a-d

MATERIALS AND METHODS

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 starting optically active cyanohydrins (*R*)-1a-d and/or their racemic forms were prepared as reported [57]. The isocyanate reagents 2 and 5a-d are commercially available. The new carbamates 3a,b; 4 and 4-imino-2-oxazolidinones 7a-h have been synthesized according the reported method [55,56]. 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 (TLC) on glass plates (20 × 20 cm) coated with silica gel 60 with fluorescent indicator F_{254}. Melting points were 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, Egypt. The specific rotation $[\alpha]_{D/25}=\alpha/c.\ l$ is expressed in (°.L)/(Kg.dm) where, α is the measured angular rotation, l (path length)=1 dm and c (concentration) is expressed in kg/l. Infrared spectra were performed in KBr discs using: JASCO FTIR-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 ^1H and 125 MHz for ^{13}C) (National Research Centre, Egypt) and /or Varian Mercury Vx-300 BB (running at 300 MHz for ^1H 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 Dimethyl Sulphoxide (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 follows: 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 SIR92 program [58] and refined using ma*X*us [59].

The molecular graphics were made with ORTEP [60]**.** 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 1541707, 1541708, 1541713. 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

General procedure for the synthesis of carbamates (S)-3a,b ; (R,S)-3a,b and (R,S)-4

To a stirred mixture of the appropriate optically active cyanohydrin (*R*)-1a,b or the racemic form (*R,S*)-1a, b, d (0.01 mol) and triethylamine (10 µl) in dry toluene (10 ml) was added a solution of *ter*-butyl isocyanate (2) (0.012 mol) in toluene (5 ml) at 0°C under argon atmosphere. After stirring the reaction mixture for further 48 h 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 ethanol to give the respective carbamate derivative.

(S)-(2-Chlorophenyl)(cyano)methyl tert-butylcarbamate (S)-3a: Colourless crystals, $[\alpha]_{D/25}$=+9.0 (*c* 0.00333, acetone), m.p. 100-102°C (Ethanol), yield 95%. IR (KBr, ν_{max}, cm^{-1}): 3363 (N−H), 3028 (C−H, aromatic), 2970, 2935, 2870 (C−H, aliphatic), 2248 (C≡N), 1740 (C=O, ester), 1594, 1574 (C=C, aromatic), 1267, 1214 (C−O, ester), 1068 (HC−O, ether), 764 (C−Cl, aromatic). ^{1}H-NMR ($CDCl_3$, δ ppm): 1.34 (s, 9H, C $(CH_3)_3$), 4.88 (1H, NH, D_2O exchangeable), 6.67 (s, 1H, NC−CH), 7.36-7.45 (m, 3H, aromatics), 7.66 (d, J_{HH}=7.6 Hz, 1H, aromatic). MS (70 eV, EI) m/z (%): 266 $[M]^{\bullet+}$ (< 5%). Anal. Calcd. (%) for $C_{13}H_{15}ClN_2O_2$ (266.72): C, 58.54; H, 5.67; Cl, 13.29; N, 10.50. Found (%): C, 58.60; H, 5.64; Cl, 13.20; N, 10.45.

(R,S)-(2-Chlorophenyl)(cyano)methyl tert-butylcarbamate (R,S)-3a: Colourless crystals, m.p. 102-104°C, yield 98%. For further characterizations see (*S*)-3a.

(S)-Cyano(4-fluorophenyl)methyl tert-butylcarbamate (S)-3b: Yellow crystals, $[\alpha]_{D/25}$= +15.0 (c 0.00333, acetone), m.p. 66-68°C (Ethanol), yield 80%. IR (KBr, ν_{max}, cm^{-1}): 3343 (N−H), 3039 (C−H, aromatic), 2980 (C−H, aliphatic), 2322 (C≡N), 1713 (C=O ester), 1608 (C=C, aromatic), 1270, 1223 (CO, ester), 1165 (C−F, aromatic), 1061 (HC−O, ether). ^{1}H-NMR ($CDCl_3$, δ ppm): 1.35 (s, 9H, C−$(CH_3)_3$), 4.87 (1H, NH, D_2O exchange- able), 6.35 (s, 1H, NC−CH), 7.12 (d, J_{HH}=8.6 Hz, AA`BB` system, 2H, aromatic-H ortho to the F atom), 7.49 (d, J_{HH}=8.6 Hz, AA`BB` system, 2H, aromatic-H meta to the F atom). MS (70 eV, EI) m/z (%): 250 $[M]^{\bullet+}$ (< 5%). Anal. Calcd. (%) for $C_{13}H_{15}FN_2O_2$ (250.27): C, 62.39; H, 6.04; F, 7.59; N, 11.19. Found: C, 62.31; H, 6.06; N, 11.15.

(R,S)-Cyano(4-fluorophenyl)methyl tert-butylcarbamate (R,S)-3b: Yellow crystals, $[\alpha]_{D/25}$=0.0 (c 0.0025, acetone), m.p. 70-72°C (Ethanol), yield 85%. For further characterizations see (S)-3b.

(R,S)-Cyano(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)methyl tert-butylcarbamate (R,S)-4: Colourless crystals, $[\alpha]_{D/25}$=0.0 (*c* 0.0025, acetone), m.p. 114-116°C, yield 72%. IR (KBr, ν_{max}, cm^{-1}): 3374 (N−H), 3060 (C−H, aromatic), 2973 (C−H, aliphatic), 2361 (C≡N), 1731 (C=O, carbamate), 1591 (C=C, aromatic), 1290, 1214 (C−O, ester), 1067 (HC−O, ether). ^{1}H-NMR (DMSO-d_6, δ ppm): 1.19 (s, 9H, $C(CH_3)_3$), 4.23 (s, 4H, O−CH_2−CH_2−O), 6.35 (s, 1H, NC−CH), 6.93 (d, J_{HH}=7.6 Hz, 1H, aromatic), 6.97 (d, J_{HH}=7.6 Hz, 1H, aromatic), 7.01 (s, 1H, aromatic), 7.48 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR ($CDCl_3$, δ ppm): 28.63 ($(CH_3)_3$), 51.04 (C−$(CH_3)_3$), 62.42 (CH−O), 64.28 (CH_2−O), 116.74 (C≡N), 117.79, 120.77, 125.49, 125.83, 129.33, 143.77 (aromatic carbons), 145.01 (C=O). MS (70 eV, EI) m/z (%): 290 $[M]^{\bullet+}$ (< 5%). Anal. Calcd. (%) for $C_{15}H_{18}N_2O_4$ (290.13): C, 26.06; H, 6.25; N, 9.65. Found: C, 25.99; H, 6.27; N, 9.61.

General procedure for the synthesis of 4-iminooxazolidin-2-ones (S)-7a-h and (R,S)-7b, d, e, f, g, h

To a stirred mixture of the appropriate racemic and/or optically active cyanohydrin 2a-c (0.01 mol) and triethylamine (10 µl) in dry toluene (10 ml) was added a solution of the appropriate isocyanate reagent 5a-d (0.012 mol) in toluene (5 ml) at 0°C under argon gas atmosphere. After stirring the reaction mixture for further 48 h 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 ethanol to give the respective oxazolidinone derivatives 7a-h.

(5S)-5-(2-Chlorophenyl)-3-(4-chlorophenyl)-4-iminooxazolidin-2-one (S)-7a: Colourless crystals, $[\alpha]_{D/25}$=+165 (*c* 0.004, DMSO), m.p. 102-104°C (Ethanol), yield 77%. IR (KBr, ν_{max}, cm^{-1}): 3310 (N−H), 3092, 3067 (C−H, aromatic), 2975 (C−H, aliphatic), 1783 (C=O, exocyclic), 1687 (C=N, exocyclic), 1595 (C=C, aromatic), 760 (C−Cl, aromatic). ^{1}H-NMR (DMSO-d_6, δ ppm): 6.36 (s, 1H, HC−O), 7.45-7.68 (m, 8H, aromatics), 8.69 (1H, NH, D_2O exchangeable). MS (70 eV, EI) m/z (%): 320 $[M]^{\bullet+}$ (30%), 322 $[M^{\bullet+} + 2]$ (20), 324 $[M^{\bullet+} + 4]$ (< 3%). Anal. Calcd. for $C_{15}H_{10}Cl_2N_2O_2$ (321.16): C, 56.10; H, 3.14; Cl, 22.08; N, 8.72. Found (%): C, 56.21; H, 3.12; Cl, 21.87; N, 8.75.

(5S)-5-(2-Chlorophenyl)-3-(2,4-dichlorophenyl)-4-iminooxazolidin-2-one (S)-7b: Colourless crystals, $[\alpha]_{D/25}$ =+55.0 (*c* 0.004, acetone), m.p. 146-148°C (Ethanol), yield 68%. IR (KBr, ν_{max}, cm^{-1}): 3265 (N−H), 3089 (C−H, aromatic), 2957 (C−H, aliphatic), 1778 (C=O, exocyclic), 1682 (C=N, exocyclic), 1586 (C=C, aromatic), 1203, 1135 (C−O, ester), 1033 (HC−O, ether), 755 (C−Cl, aromatic). ^{1}H-NMR (DMSO-d_6, δ ppm): 6.54 (s, HC−O), 7.50–7.90 (m, 7H, aromatic), 8.78 (1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ ppm): 83.08 (CH−O), 132.89, 133.69, 134.68, 135.55, 135.89, 136.62, 137.03, 138.03, 138.74, 139.24, 140.20, 140.86 (aromatic carbons), 158.56, 165.09 (C=N, C=O). MS (70 eV, EI) m/z (%): 354 $[M]^{\bullet+}$ (< 2%), 356 $[M^{\bullet+}$ +2] (< 1%), 358 $[M^{\bullet+}$ +4] (< 1%). Anal. Calcd. for $C_{15}H_9Cl_3N_2O_2$ (355.60): 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.

(R,S)-5-(2-Chlorophenyl)-3-(2,4-dichlorophenyl)-4-iminooxazolidin-2-one (R,S)-7b: Colourless crystals, m.p. 149-151°C, yield 65%. For further characterization see (S)-7b.

(5S)-5-(2-Chlorophenyl)-3-(3,4-dichlorophenyl)-4-iminooxazolidin-2-one (S)-7c: Colourless crystals, $[\alpha]_{D/25}$=+180 (*c* 0.00333, DMSO), m.p. 110-112°C, yield 71%. IR (KBr, ν_{max}, cm^{-1}): 3309 (N−H), 3092 (C−H, aromatic), 2950 (C−H, aliphatic), 1807 (C=O, exocyclic), 1674 (C=N, exocyclic), 1592 (C=C, aromatic), 1194 (C−O, ester), 1036 (C−O, ether), 749 (C−Cl, aromatic). ^{1}H-NMR (DMSO-d_6, δ ppm): 6.41 (s, 1H, HC−O), 7.45-7.91 (m, 7H, aromatics), 8.83 (1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ ppm): 83.14 (HC−O), 132.38, 132.78, 133.21, 133.98, 135.20, 135.78, 135.90, 136.23, 136.86, 137.29, 137.56, 138.82 (aromatic carbons), 159.13, 165.26 (C=N, ***C***=O). MS (70 eV, EI) m/z (%): 354 $[M]^{\bullet+}$ (< 5%). Anal. Calcd. for $C_{15}H_9Cl_3N_2O_2$ (355.60): C, 50.66; H, 2.55; Cl, 29.91; N, 7.88. Found (%): C, 50.71; H, 2.51; Cl, 29.85; N, 7.80.

(5S)-5-(2-Chlorophenyl)-4-imino-3-(2-methoxyphenyl)oxazolidin-2-one (S)-7d: Colourless crystals, $[\alpha]_{D/25=}$− 4.5 (*c* 0.00666, acetone), m.p. 102°C, yield 62%. IR (KBr, ν_{max}, cm^{-1}): 3307 (N−H), 3064 (C−H, aromatic), 2994, 2940, 2843 (C−H, aliphatic), 1781 (C=O, exocyclic), 1680 (C=N, exocyclic), 1598 (C=C, aromatic), 1198, 1108 (C−O, ester), 1030 (C−O, ether), 747 (C−Cl, aromatic). ^{1}H-NMR ($CDCl_3$, δ ppm): 3.85 (s, 3H, OCH_3), 6.37 (s, 1H, HC-O), 6.50 (bs, 1H, NH, D_2O exchangeable), 7.06-7.53 (m, 8H, aromatics). ^{13}C-NMR ($CDCl_3$, δ ppm): 56.00 (OCH_3), 79.50 (CHO), 112.68, 121.45, 127.67, 129.75, 130.27, 130.78, 131.40, 131.53, 154.27, 155.36 (aromatic carbons), 159.77, 163.42 (C=N, C=O). MS (70 eV, EI) m/z (%): 317 $[M+H]^+$ (9%), 319 $[(M+H)+2]^+$ (3%). Anal. Calcd. for $C_{16}H_{13}ClN_2O_3$ (316.74): C, 60.67; H,4.14; Cl, 11.19; N, 8.84. Found (%): C, 60.75; H, 4.11; Cl, 10.98; N, 8.78.

(R,S)-5-(2-Chlorophenyl)-4-imino-3-(2-methoxyphenyl)oxazolidin-2-one (R,S)-7d: Colourless crystals, m.p. 106-108°C, yield 55%. For further characterizations see (S)-7d.

(5S)-3-(3,4-Dichlorophenyl)-5-(4-fluorophenyl)-4-iminooxazolidin-2-one (S)-7e: Pale yellow crystals, $[\alpha]_{D/25}$=+4 (c 0.0025, acetone), m.p. 130°C, yield 90%. IR (KBr, ν_{max}, cm^{-1}): 3306 (N−H), 3090, 3047 (C−H, aromatic), 2927 (C−H, aliphatic), 1776 (C=O, exocyclic), 1677 (C=N, exocyclic), 1600 (C=C, aromatic), 1226 (C−O, ester), 1127 (C−F), 1043 (HC−O, ether), 747 (C−Cl, aromatic). ^{1}H-NMR (DMSO-d_6, δ ppm): 6.15 (s, 1H, CH−O), 7.28 (d, J_{HH}=8.6 Hz, AA`BB` system, 2H, aromatic-H ortho to the F atom), 7.57 (d, J_{HH}=8.6 Hz, 1H, aromatic), 7.64 (d, J_{HH}=8.6 Hz, AA`BB` system, 2H, aromatic-H meta to the F atom), 7.78 (d, J_{HH}=8.6 Hz, 1H, aromatic), 7.90 (s, 1H, aromatic), 8.69 (1H, NH, D_2O exchangeable). MS (70 eV, EI) m/z (%): 338 $[M]^{\bullet+}$ (25%). Anal. Calcd. for $C_{15}H_9Cl_2FN_2O_2$ (339.15): C, 53.12; H, 2.67; Cl, 20.91; N, 8.26. Found (%): C, 53.07; H, 2.69; Cl, 20.85; N, 8.18.

(R,S)-3-(3,4-Dichlorophenyl)-5-(4-fluorophenyl)-4-iminooxazolidin-2-one (R,S)-7e: Pale yellow crystals, m.p. 127-128°C, yield 77%. For further characterizations see (*S*)-7e.

(5S)-5-(Benzo[d][1,3]dioxol-5-yl)-3-(4-chlorophenyl)-4-iminooxazolidin-2-one (S)-7f: Colourless crystals, $[\alpha]_{D/25}$=+115 (*c* 0.004, acetone), m.p. 142-143°C, yield 80%. IR (KBr, ν_{max}, cm^{-1}): 3305 (N−H), 3065 (C−H, aromatic), 2995, 2912 (C−H, aliphatic), 1784 (C=O, exocyclic), 1679 (C=N, exocyclic), 1246, 1201 (C−O, ester), 1030 (HC−O, ether), 781 (C−Cl, aromatic). ^{1}H-NMR (DMSO-d_6, δ ppm): 6.03, 6.04 (2s, 3H, CH_2−O and CH−O), 6.98 (d, J_{HH}=8.6 Hz, 1H, aromatic), 7.01 (d, J_{HH}=8.6 Hz, 1H, aromatic), 7.13 (s, 1H, aromatic), 7.47-7.55 (m, 4H, aromatics), 8.45 (s, 1H, NH, D_2O exchangeable). MS (70 eV, EI) m/z (%): 330 $[M]^{\bullet+}$ (50%) and 332 $[M^{\bullet+}+2]$ (20%). Anal. Calcd. for $C_{16}H_{11}ClN_2O_4$ (330.72): C, 58.11; H, 3.35; Cl, 10.72; N, 8.47. Found (%): C, 58.20; H, 3.34; Cl, 10.60; N, 8.43.

(R,S)-5-(Benzo[d][1,3]dioxol-5-yl)-3-(4-chlorophenyl)-4-iminooxazolidin-2-one (R,S)-7f: Colourless crystals, m.p. 140-142°C, yield 85%. For further characterizations see (S)-7f.

(5S)-5-(Benzo[d][1,3]dioxol-5-yl)-3-(2,4-dichlorophenyl)-4-iminooxazolidin-2-one (S)-7g:"Colourless crystals, $[\alpha]_{D/25}$=+154 (*c* 0.005, acetone), m.p. 128°C (Ethanol), yield 78%. IR (KBr, ν_{max}, cm^{-1}): 3303 (N−H), 3076 (C−H, aromatic), 2993, 2914 (C−H, aliphatic), 1764 (C=O, exocyclic), 1680 (C=N, exocyclic), 1496 (C=C, aromatic), 1206, 1124 (C−O, ester), 1035 (HC−O, ether), 756 (C−Cl, aromatic). ^{1}H-NMR (DMSO-d_6, δ ppm): 6.05, 6.12 (2s, 3H, CH_2−O and CH−O), 6.97-7.01 (m, 3H, aromatics), 7.20 (s, 1H, aromatic), 7.63 (d, J_{HH}=8.6 Hz, 1H, aromatic), 7.87 (s, 1H, aromatic), 8.57 (s, 1H, NH, D_2O exchangeable). MS (70 eV, EI) m/z (%): 364 $[M]^{\bullet+}$ (1%), 366 $[M^{\bullet+}+2]$ (<1%), 268 $[M^{\bullet+}+4]$ (< 1%). Anal. Calcd. for $C_{16}H_{10}Cl_2N_2O_4$ (365.17): C, 52.63; H, 2.76; Cl, 19.42; N, 7.67. Found (%): C, 52.57; H, 2.77; Cl, 19.37; N, 7.64.

(R,S)-5-(Benzo[d][1,3]dioxol-5-yl)-3-(2,4-dichlorophenyl)-4-iminooxazolidin-2-one (R,S)-7g: Colourless crystals, m.p. 125-126°C, yield 67%. For further characterizations see (S)-7g.

(5S)-5-(Benzo[d][1,3]dioxol-5-yl)-3-(3,4-dichlorophenyl)-4-iminooxazolidin-2-one (S)-7h: Colourless crystals, $[\alpha]_{D/25=}$+137.5 (*c* 0.005, acetone), m.p. 156-158°C, yield 90%. IR (KBr, ν_{max}, cm^{-1}): 3294 (N−H), 3088, 3070 (C−H, aromatic) 2966, 2902 (C−H, aliphatic), 1778 (C=O, exocyclic), 1681 (C=N, exocyclic), 1614, 1591 (C=C, aromatic), 1227, 1184 (C−O, ester), 1032 (HC−O, ether), 759 (C−Cl, aromatic). ^{1}H-NMR (DMSO-d_6, δ ppm): 6.043, 6.049 (2 s, 3H, CH_2−O and CH−O), 6.90 (d, J_{HH}=7.6 Hz, 1H, aromatic), 7.05 (d, J_{HH}=7.6 Hz, 1H, aromatic), 7.17 (s, 1H, aromatic), 7.57 (d, $J_{HH=}$8.6 Hz, 1H, aromatic), 7.76 (d, $J_{HH=}$8.6 Hz, 1H, aromatic), 7.91 (s, 1H, aromatic), 8.50 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ ppm): 84.75 (CH−O), 106.64 (O-CH_2-O), 113.23, 113.73, 127.42, 127.88, 132.66, 133.70, 136.09, 137.9, 152.93, 153.32, 153.64, 156.39 (aromatic carbons), 159.16 (C=N), 166.77 (C=O). MS (70 eV, EI) m/z (%): 364 $[M]^{\bullet+}$ (55%), 368 $[M^{\bullet+}+4]$ (8%). Anal. Calcd. for $C_{16}H_{10}Cl_2N_2O_4$ (365.17): C, 52.63; H, 2.76; Cl, 19.42; N, 7.67. Found (%): C, 52.60; H, 2.74; Cl, 19.20; N, 7.69.

(R,S)-5-(Benzo[d][1,3]dioxol-5-yl)-3-(3,4-dichlorophenyl)-4-iminooxazolidin-2-one (R,S)-7h: Colourless crystals, m.p. 152-154°C, yield 95%. For further characterizations see (S)-7h.

THE BIOLOGICAL EVALUATION

The antimicrobial sensitivity test

Antimicrobial activity of the tested samples was determined using a modified Kirby−Bauer disc diffusion method [61]. Briefly, 100 µl of the test bacteria or 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 [62]. 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 were inoculated with filamentous fungi *as Aspergillus flavus* at 25°C for 48 h; Gram-positive bacteria as *Staphylococcus aureus, Bacillus subtilis*; Gram-negative bacteria as *Escherichia coli, Pseudomonas aeruginosa* were incubated at 35-37°C for 24-48 h and yeast as *Candida albicans* were incubated at 30°C for 24-48 h. Standard discs of ampicillin (Antibacterial agent), amphotericin B (Antifungal agent) served as positive controls for antimicrobial activity but filter discs impregnated with 10 µlof solvent (DMSO) were used as a negative control. Blank paper discs with a diameter of 8.0 mm were impregnated with 10 µl 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 is measured in millimeters with a sterilized slipping caliper.

Determination of Minimum Inhibitory Concentration (MIC_{90}) for compound (S)-3a against *S. aureus*

The minimum concentration of a compound which inhibits 90% of the tested microorganism growth when compared to control (no treatment) is known as MIC_{90}. It is determined by using agar dilution method [63].

Briefly, stationary-phase cultures of bacteria were prepared at 37°C and they were used to inoculate a fresh 5.0 ml culture to an OD_{600} value (Optical density of the sample measured at wave length of 600 nm) of 0.05. The 5.0 ml cultures were then incubated at 37°C until an OD_{600} of 0.10 was achieved from which standardized bacterial suspensions were prepared to a final cell density of 6×10^5 CFU/ml. Serial dilutions from the treatments (0-320 mg/ml) were prepared and mixed with 5.0 ml of the standardized bacteria suspension then added to the plates and incubated for 24 h at 37°C. The CFU were, counted for each dilution.

***In vitro* cytotoxicity assay**

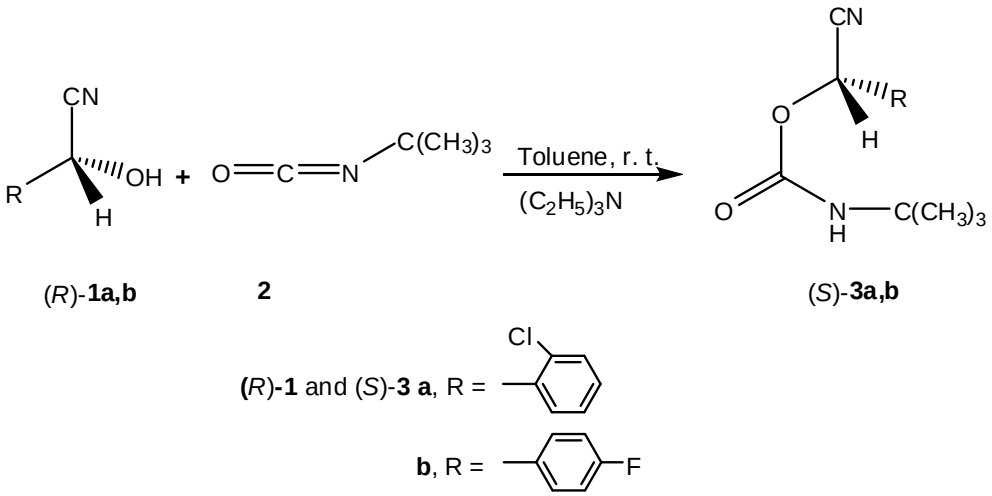
Materials: Sulfo-Rhodamine-B stain (SRB) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), fetal bovine serum (FBS), doxorubicin (the reference drug, DOX) [64], RPMI-1640 medium, trypsin, penicillin, DMSO, trypan blue stain, streptomycin, sodium bicarbonate, acetic acid, Trichloroacetic 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°C) 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 SRB stain assay according to the reported standard procedure [65]. Cells were seeded in 96-multiwell microtiter plates at a concentration of 5×10^4-10^5 cell/well in a fresh medium for 24 h 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 h. Control cells were treated with vehicle alone. Four wells were used for each individual drug concentration. After 24, 48 and 72 h treatment, the cells were fixed with 50 µl of cold 50% trichloroacetic acid for 1 h at 4°C. 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 Enzyme-linked Immunosorbent Assay (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 were 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.

RESULTS AND DISCUSSION

Chemistry

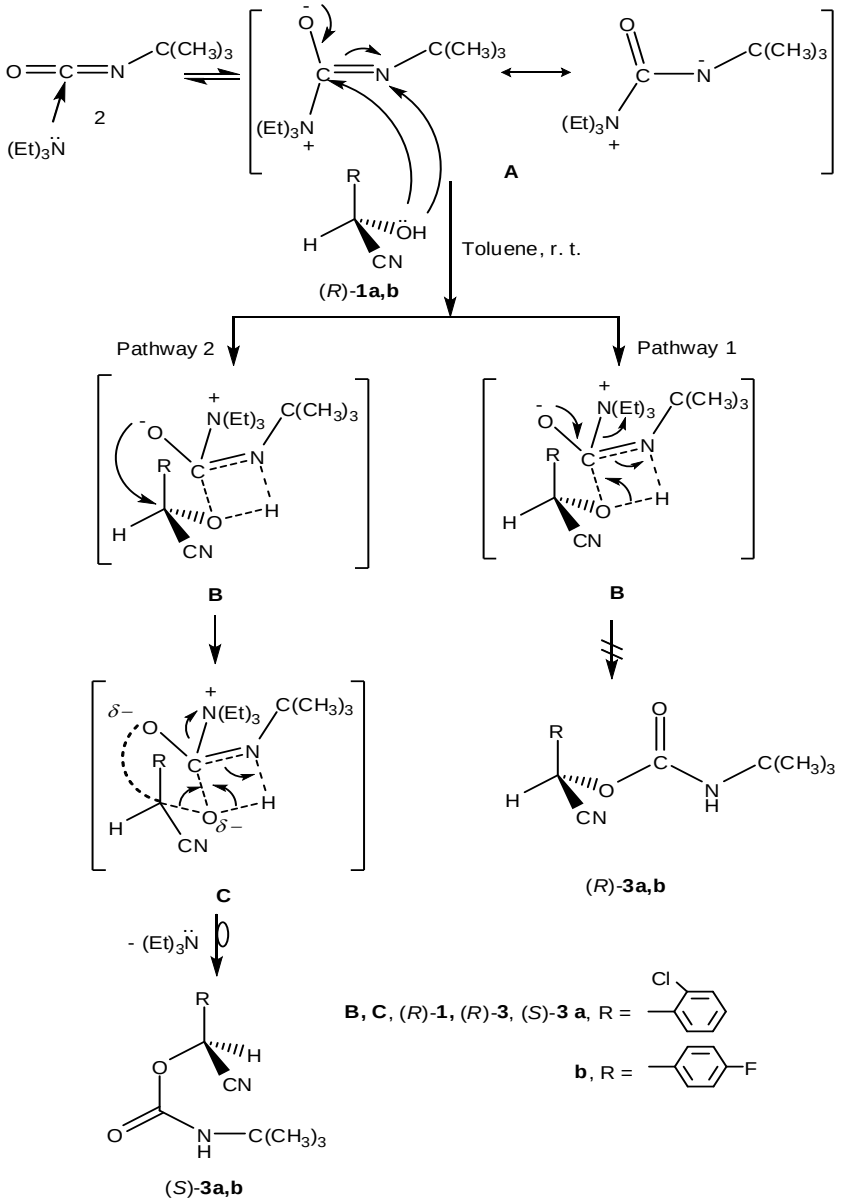
Now we have reacted the optically active cyanohydrins (*R*)-1a,b [57] with *tert*-butyl isocyanate (2) in toluene at r. t. in the presence of a few drops of triethylamine according to a given procedure (c.f. Experimental) [55,56]. It was found that the reaction has proceeded with inversion of configuration to give the *S*-enantiomers of the corresponding carbamate derivatives 3a,b (Schemes 1,2). The mechanism depicted in Scheme 2 can be applied to explain the stereo chemical outcome of the reaction.



Scheme 1: The stereo chemical behavior of the optically active cyanohydrins (*R*)-1a,b

Towards tert-butyl isocyanate (2)

In presence of triethylamine, the isocyanate reagent 2 form a salt like A [66] which is attacked by the appropriate cyanohydrin (*R*)-1a,b in a concerted fashion to give intermediate B [67,68]. Apparently, such cyclic intermediate B enables the negatively charged oxygen atom to undergo a back side intramolecular nucleophilic attack (internal S_N2 reaction) [69] on the chiral carbon atom (Scheme 2, Pathway 2) to give another cyclic intermediate like C instead of spontaneous rearrangement to the corresponding carbamate (*R*)-3a,b (Scheme 2, Pathway 1). Completion of carbonyl oxygen–cyanohydrin carbon bond formation and cyanohydrin carbon-cyanohydrin oxygen bond breaking gives carbamates 3a,b where the entering group is the same as the leaving group (Pathway 2, Scheme 2). However, compounds 3a,b were obtained in the (*S*)-configuration instead of (*R*)-configuration due to the nucleophilic back side substitution.



Scheme 2: A suggested mechanism for the formation of carbamates (*S*)-3a,b

Compound (*S*)-3a, namely (*S*)-(2-chlorophenyl)(cyano)methyl *tert*-butylcarbamate as an example, was separated as colorless crystals and recorded $[\alpha]_{D/25}$ value of +9.0 (*c* 0.00333, acetone). It was given the assigned stereo chemical structure due to the following reasons: (a) Compatible microanalyses and molecular weight determination (MS) corresponded to a molecular formula of $C_{13}H_{15}ClN_2O_2$ (266.72). (b) The IR spectrum (KBr, ν_{max}, cm^{-1}) of (*S*)-3a lacks the absorption due to O−H group which appeared in compound (*R*)-1a at 3409 cm^{-1} [57]. However, the spectrum showed absorption bands at 3363 (N−H), 2970, 2935, 2870 (C–H, aliphatic), 2248 (C≡N), 1740 (C=O, ester), 1267, 1214 (C−O, ester) and 1068 (HC−O, ether). (c) The ^{1}H-NMR spectrum ($CDCl_3$, δ ppm) showed two singlet signals at 1.34 and 6.67 ppm which could be attributed to the tert-butyl -$C(CH_3)_3$ (9H) and methine protons NC–CH (1H), respectively [70]. The proton on nitrogen NH appeared as a D_2O exchangeable singlet at 4.88 ppm. The aromatic protons (4H) recorded a multiplet in the region 7.36-7.45 ppm (3H) and a doublet (J_{HH}=7.6 Hz) at 7.66 ppm (1H). Similarly, compound (S)-3b recorded the molecular peak, in its mass spectrum, at m/z 250 (< 5%) which corresponds to $C_{13}H_{15}FN_2O_2$. Its IR spectrum showed bands at 3343 (N−H), 2980 (C−H, aliphatic), 2322 (C≡N) and 1713 (C=O, ester). The $C(CH_3)_3$ (9H) and NC−CH (1H) protons appeared, in the ^{1}H NMR spectrum of (S)-3b, as two singlets at 1.35 and 6.35 ppm, respectively. The aromatic AA`BB` system [70] appeared as two doublets (J_{HH}=8.6 Hz) at 7.12 and 7.49 ppm. The NH proton showed a D_2O exchangeable singlet at 4.87 ppm. The X-ray crystallographic analysis confirmed the formation of carbamates 3a,b in the (S)- configuration as estimated from their ORTEP overviews in Figure 4. The crystal structure and data refinement, selected bond lengths, bond angles and torsion angles of carbamates (S)-3a,b are represented in Tables 1-4 respectively. In compound (S)-3a, the torsion angles (º) C6−O3−C4−O5, C8−N2−C4−O5, C6−O3−C4−N2 and C8−N2−C4−O3 have values of 2.8(2), –2.5(3), –178.1 and 178.4(1), respectively, which are very close to 0° and or 180°. Such planarity may enhance the electron delocalization involving the O3-C4(O5)-N2 portion of the molecule rendering the N2−C4 and O3−C4 bonds to gain some double bond character. Thus, the N2−C4 bond was found to have a distance value of 1.332 (18) Å which is in between 1.47 Å for C−N single bond and 1.28 Å for C=N double bond [71]. Moreover, O3−C4 bond have a distance of 1.386 (17) Å which is in between 1.43 Å and 1.18 Å for C−O and C=O bonds, respectively. However, the C4−O5 bond has gained very slight single bond character [71] where it recoded a bond distance value of 1.20 Å.

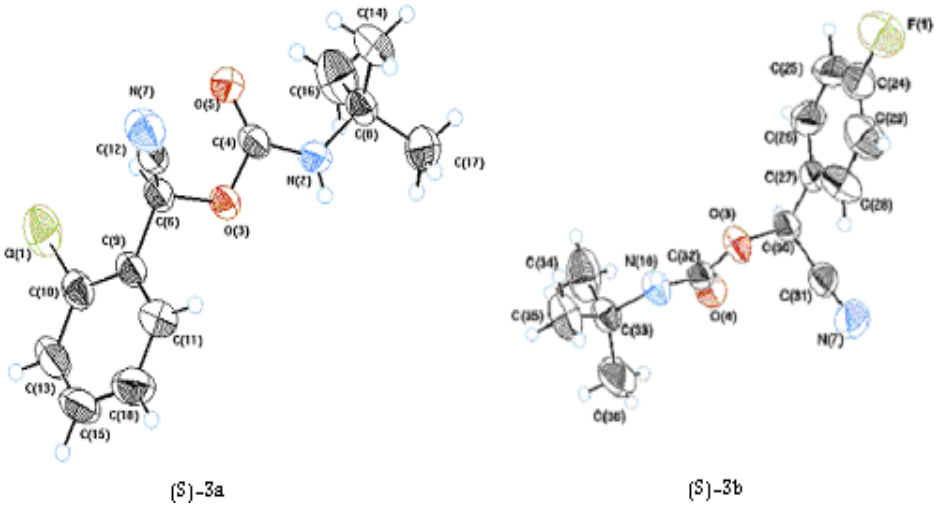


Figure 4: ORTEP overviews of carbamates (*S*)-3a and (S)-3b

Table 1: Crystal structure and data refinement of carbamates (*S*)-3a and (*S*)-3b

	Compounds	
	(*S*)-3a	**(*S*)-3b**
Empirical formula	$C_{13}H_{15}ClN_2O_2$	$C_{13}H_{15}FN_2O_2$
Formula weight	266.728	250.273
Crystal system/Space group	Monoclinic/$P2_1/a$	Triclinic/$P\bar{1}$
a/Å	11.4281 (4)	9.8849 (6)
b/Å	9.5449 (3)	11.4937 (6)
c/Å	12.8653 (5)	13.4419 (10)
α/º	90.00	64.689 (2)
β/º	100.5601 (14)	89.136 (2)
γ/º	90.00	90.003 (2)
V/Å^3	1379.58 (8)Å^3	1380.4 (2)
Z	4	2
D_{calc} (g/cm^3)	1.284 Mg m^{-3}	0.602
μ (mm^{-1})	0.273	0.046
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	0 – 35.15	0/34.92
Reflections collected	6262	8724
Independent reflections	5969	8525
Data/restraints/parameters	5969/0/163	8525/0/325
Goodness of fit on F^2	0.655	0.753
Final R indices [I > 2σ (I)]	0.0487	0.0692
R indices (all data)	0.1864	0.3036
Largest difference peak/hole	0.185/– 0.235	0.185/ –0.158

Table 2: Selected bond lengths (Å) of carbamates (*S*)-3a and (*S*)-3b

(*S*)-3a		**(*S*)-3b**	
Cl1—C10	1.729 (18)	O3—C32	1.380 (4)
N2—C4	1.332 (18)	O3—C30	1.438 (4)
N2—C8	1.473 (18)	O4—C32	1.208 (4)
O3—C4	1.386 (17)	C32—N10	1.332 (4)
O3—C6	1.420 (17)	N10—C33	1.482 (4)
C4—O5	1.200 (17)	C30−C31	1.483 (5)
C6—C12	1.482 (2)	C30—C27	1.501 (4)
C6—C9	1.519 (19)	C33—C36	1.497 (5)
N7—C12	1.136 (2)	C27—C26	1.366 (5)
C8—C17	1.507 (2)	F1—C24	1.371 (5)
C9—C11	1.383 (2)	C31—N7	1.135 (4)

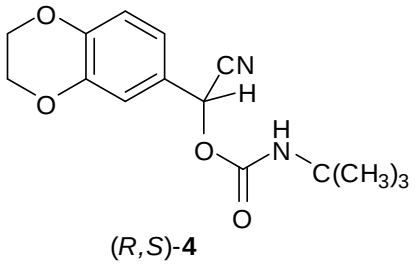
Table 3: Selected bond angles (°) of compounds (*S*)-3a and (*S*)-3b

(*S*)-3a		(*S*)-3b	
C4—N2—C8	125.50 (12)	C32—O3—C30	114.60 (2)
C4—O3—C6	115.13 (11)	O4—C32—N10	127.70 (3)
O5—C4—N2	129.14 (14)	O4—C32—O3	122.60 (3)
O5—C4—O3	122.23 (13)	N10—C32—O3	109.7 (3)
N2—C4—O3	108.62 (12)	C32—N10—C33	125.4 (3)
O3—C6—C12	110.40 (12)	O3—C30—C31	108.20 (3)
O3—C6—C9	106.89 (11)	O3—C30—C27	108.60 (3)
C12—C6—C9	109.78 (12)	C31—C30—C27	111.40 (3)
N2—C8—C17	106.42 (13)	N10—C33—C36	110.30 (3)
N2—C8—C14	109.83 (13)	N10—C33—C34	108.80 (3)
C17—C8—C14	109.76 (16)	C36—C33—C35	110.70 (3)
N2—C8—C16	110.17 (14)	C26—C27—C28	119.00 (4)
C11—C9—C10	118.59 (14)	C26—C27—C30	119.00 (3)
C11—C9—C6	119.93 (13)	C28—C27—C30	122.00 (3)
C9—C10—Cl1	120.60 (12)	N7—C31—C30	178.30 (4)
N7—C12—C6	176.97 (16)	C25—C24—F1	118.50 (5)

Table 4: Selected torsion angles (°) of compounds (*S*)-3a and (*S*)-3b

(*S*)-3a		(*S*)-3b	
C8−N2−C4−O3	178.4 (1)	C30−O3−C32−O4	3.1 (5)
C8−N2−C4−O5	–2.5 (3)	C30−O3−C32−N10	–178.0 (3)
C4−N2−C8−C14	62.1 (2)	C32−O3−C30−C27	160.2 (3)
C4−N2−C8−C17	–179.2 (2)	C32−O3−C30−C31	–78.6 (4)
C6−O3−C4−N2	–178.1 (1)	O3−C32−N10−C33	172.1 (3)
C6−O3−C4−O5	2.8 (2)	O4−C32−N10−C33	–9.0 (6)
C4−O3−C6−C9	163.8 (1)	C32−N10−C33−C35	–176.6 (3)
C4−O3−C6−C12	–76.8 (2)	C32−N10−C33−C36	62.9 (5)
O3−C6−C9−C11	56.3 (2)	C32−N10−C33−C34	–59.2 (4)
C12−C6−C9−C10	115.8 (2)	O3−C30−C27−C28	77.4 (4)
C12−C6−C9−C11	–63.5 (2)	C31−C30−C27−C28	–41.8 (5)
O3−C6−C12−N7	–149.0 (3)	C31−C30−C27−C26	136.8 (4)
C9−C6−C12−N7	–32.0 (3)	O3−C30−C31−N7	–78 (16)
C6−C9−C10−Cl1	1.3 (2)	C27−C30−C31−N7	41.0 (16)
C11−C9−C10−Cl1	–179.4 (1)	C30−C27−C28−C29	179.9 (4)
C6−C9−C11−C18	179.2 (2)	F1−C24−C29−C28	179.6 (5)

Similarly, the racemic cyanohydrins (R,S)-1a,b,d [57] were reacted with isocyanate 2 to give the respective racemic carbamate derivatives (R,S)-3a,b and 4 (c.f. Experimental). The racemic carbamate 4 (Figure 5) was separated as colourless crystals in a 72% yield. It showed no optical activity where it recorded a specific rotation $[\alpha]_{D/25}$ value of 0.0 (c 0.0025, acetone). Compatible microanalyses and molecular weight determination (MS) of (R,S)-4 corresponded to a molecular formula of $C_{15}H_{18}N_2O_4$ (290.13). Its IR spectrum showed strong bands at 3374 (N−H), 2361 (C≡N) and 1731 (C=O, carbamate) cm^{-1}. The ^{1}H-NMR spectrum (DMSO-d_6, δ ppm) of (R,S)-4 showed four singlet signals at 1.19 (9H, $C(CH_3)_3$), 4.23 (4H, $-CH_2-CH_2-$), 6.35 (1H, NC−CH) and 7.48 (s, 1H, NH, D_2O exchangeable). The three aromatic protons appeared at δ values 6.93 (d, J_{HH}=7.6 Hz), 6.97 (d, J_{HH}=7.6 Hz) and 7.01 (s) ppm. The ^{13}C-NMR ($CDCl_3$) of (R,S)-4 has recorded signals at δ values of 28.63 ($(CH_3)_3$), 51.04 ($C-(CH_3)_3$), 62.42 (CH−O), 64.28 (CH_2−O), 116.74 (C≡N), 117.79, 120.77, 125.49, 125.83, 129.33, 143.77 (aromatic carbons) and 145.01 (C=O) ppm.



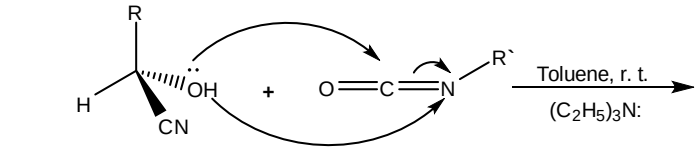
(*R*,*S*)-**4**

Figure 5: The racemic carbamate derivative (*R*,*S*)-4

On the other hand, when the optically active cyanohydrins (*R*)-1a-c were reacted with the isocyanate reagents R-N=C=O 5a-d (where R=aryl) in toluene at r. t. in the presence of a few drops of triethylamine, the respective 4-imino-2-oxazolidinone derivatives (*S*)-7a-h were formed (Scheme 3). The assigned stereochemistry and oxazolidinone structure were given to compounds (*S*)-7a-h due to the reasons outlined below. Apparently, the initially formed (*S*)-enantiomers of carbamates 6a-h, through intermediates like A and B, undergo an intramolecular heterocyclization to give the corresponding 4-imino-2-oxazolidinones (*S*)-7a-h (Scheme 3).

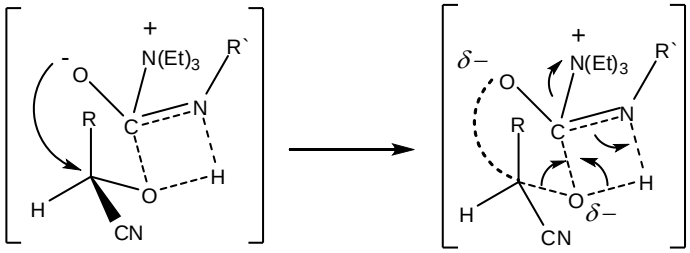
Compound (*S*)-7a, namely (*5S*)-5-(2-chlorophenyl)-3-(4-chlorophenyl)-4-iminooxazolidin-2-one, taken as an example, was obtained as colourless crystals in a 77% yield and has recorded a specific rotation value $[\alpha]_{D/25}$ of +165 (*c* 0.004, DMSO). Compatible microanalyses of (*S*)-7a corresponded to a molecular formula of $C_{15}H_{10}Cl_2N_2O_2$ (321.16).

Its IR spectrum (KBr, ν_{max}) showed strong absorption bands at 1783 and 1687 cm^{-1} that could be attributed to exocyclic C=O and C=N bonds, respectively [70]. The spectrum showed also bands at 3310 (N−H), 3092, 3067 (C−H, aromatic), 2975 (C−H, aliphatic), 1595 (C=C, aromatic) and 760 (C−Cl, aromatic). The ^{1}H-NMR spectrum of (*S*)-7a showed two singlets at 6.36 and 8.69 ppm attributed to the methine N=C-CH−O proton and the D_2O exchangeable proton on nitrogen, respectively. The aromatic protons (8H) appeared as a multiplet in the δ region 7.45-7.68 ppm.

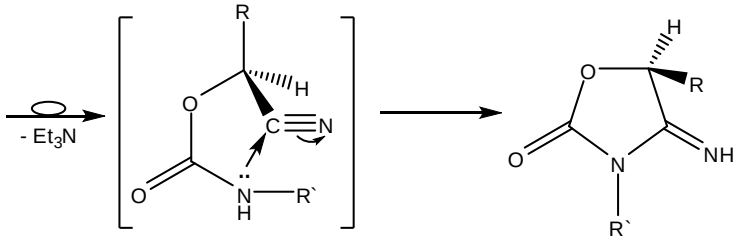


(*R*)-**1a**, R = 2-chlorophenyl
b, R = 4-fluorophenyl
c, R = benzo[*d*][1,3]dioxol-5-yl

5a, R` = 4-Cl-C_6H_4
b, R` = 2,4-$(Cl)_2$-C_6H_3
c, R` = 3,4-$(Cl)_2$-C_6H_3
d, R` = 2-MeO-C_6H_4



A **B**



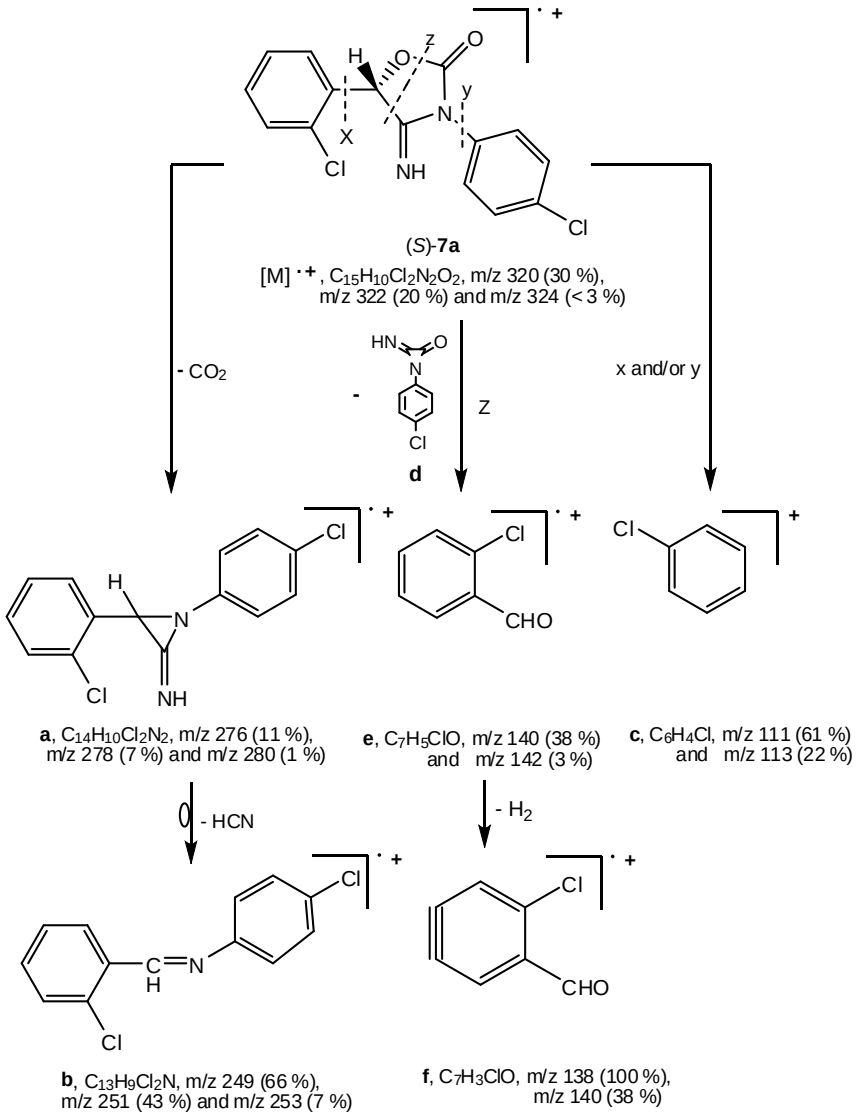
(*S*)-**6a-h** (*S*)-**7a-h**

A, **B**, (*S*)-**6** and (S)-**7 a,** R = 2-chlorophenyl, R` = 4-Cl-C_6H_4
b, R = 2-chlorophenyl, R` = 2,4-$(Cl)_2$-C_6H_3
c, R = 2-chlorophenyl, R` = 3,4-$(Cl)_2$-C_6H_3
d, R = 2-chlorophenyl, R` = 2-MeO-C_6H_4
e, R = 4-fluorophenyl, R` = 3,4-$(Cl)_2$-C_6H_3
f R = benzo[*d*][1,3]dioxol-5-yl, R` = 4-Cl-C_6H_4
g R = benzo[*d*][1,3]dioxol-5-yl, R` = 2,4-$(Cl)_2$-C_6H_3
h, R = benzo[*d*][1,3]dioxol-5-yl, R` = 3,4-$(Cl)_2$-C_6H_3

Scheme 3: Synthesis of the chiral 4-imino-2-oxazolidinones (*S*)-7a-h

The mass spectrum of (*S*)-7a (Scheme 4) revealed the molecular ion peak at m/z 320 (30%, based on $2Cl^{35}$), 322 (20%, based on Cl^{35}+Cl^{37}) and 324 (< 3%, based on $2Cl^{37}$). Expulsion of CO_2 molecule from $[M]^{+\bullet}$ can afford the radical cation a at m/z 276 (11% based on $2Cl^{35}$), 278 (7% based on Cl^{35}+Cl^{37}) and 280 (1%, based on $2Cl^{37}$). Such mass spectrometry fragmentation pattern is characteristic for several lactones [72,73]. Rearrangement of a followed by elimination of a neutral HCN molecule can yield the radical cation b at m/z 249 (66% based on $2Cl^{35}$), 251 (43% based on Cl^{35}+Cl^{37}) and 253 (7%, based on $2Cl^{37}$). The fragmentation of $[M]^{+\bullet}$ at axes x and/or y can give cation c, at m/z 111 (61% based on Cl^{35}) and 113 (22% based on Cl^{37}). Fragmentation at axis z with loss of a molecule like, d can give the radical cation e, at m/z 140 (38% based on Cl^{35}) and 142 (3%, based on Cl^{37}). Elimination of H_2 molecule from e can afford a radical cation like f at m/z 138 (100% based on Cl^{35}) and 140 (38% based on Cl^{37}).

Moreover, the oxazolidinone ring formation as well as the (*S*)-configuration of the chiral carbon-5 atom of (*S*)-7a were confirmed by X-ray crystallographic analysis. An ORTEP overview of (*S*)-7a is outlined in Figure 6. The crystal structure and data refinement, selected bond lengths, bond angles and torsion angles of (*S*)-7a are represented in Tables 5-8, respectively. The oxazolidinone ring in compound (*S*)-7a was found to be very nearly planar as it is evident from the values of the five torsion angles C17–N6–C14–O15, C16–O15–C14–N6, C14–O15–C16–C17, O15–C16–C17–N6 and C14–N6–C17–C16 [1° (1), 3° (1), -5° (1), 5° (1) and -4° (1), respectively] which are very close to 0°. Moreover, the torsion angles C16–O15–C14–O5 (179° (1)) and C14–N6–C17–N7 (176° (1)) with values almost equal to 180° are suggesting the presence of the C=O and C=N bonds, respectively, in the same plane of the ring. Apparently, such nearly coplanar structure of the 4-imino-2-oxazolidinone ring in 7a is due to the flattening effect exerted by the two sp^2 carbon atoms of the C=O and/or C=N bonds [74-76]. Moreover, the O15−C14, N6−C14 and N6−C17 bond distances of 1.338 (11), 1.397 (11) and 1.367 (11) Å, respectively, are indicative of some double bond character [71], suggesting an electron delocalization through the ring.



Scheme 4: The mass fragmentation pattern of compound (*S*)-7a

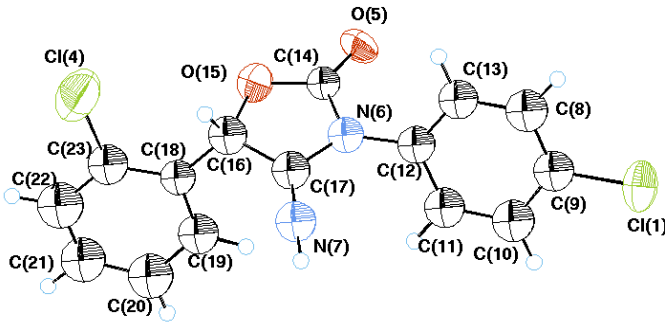


Figure 6: ORTEP overview of oxazolidinone (*S*)-7a

Biological evaluation

Antimicrobial activity

The carbamate derivatives (*S*)-3a,b and their racemic forms (*R*,*S*)-3a,b as well as the racemic carbamate (*R*,*S*)-4 were screened for their antibacterial activity against two Gram-positive bacterial species namely, *B. subtilis*, *S. aureus* and two Gram-negative bacterial species namely, *E. coli* and *P. aeruginosa* (Table 9 and Figure 7) by using a modified Kirby−Bauer disc diffusion method [61].

Table 5: Crystal structure and data refinement of compound (*S*)-7a

Empirical formula	$C_{15}H_{10}Cl_2N_2O_2$
Formula weight	321.163
Crystal system/Space group	Monoclinic/$P2_1/c$
a/Å	9.0666 (4)
b/Å	11.6928 (4)
c/Å	27.5951 (12)
α/°	90.00°
β/°	95.6323 (13)
γ/°	90.00
V/Å^3	2911.3 (2)
Z	4
D_{calc} (g/cm^3)	1.465
μ (mm^{-1})	0.225
Colour/Shape	Colourless/prismatic
Wavelength	Mo *Ka* (0.71073 Å)
Temperature	298 K
Theta range for collection/°	0–25.12
Reflections collected	5234
Independent reflections	4969
Data/restraints/parameters	4969/0/214
Goodness of fit on F^2	0.648
Final R indices [I > 2σ (I)]	0.0815
R indices (all data)	0.4186
Largest difference peak/hole	0.313/–0.280

Table 6: Selected bond lengths (Å) of compound (*S*)-7a

Cl1—C9	1.733 (11)	N6—C14	1.397 (11)
Cl4—C23	1.710 (11)	N6—C12	1.409 (11)
O15—C14	1.338 (11)	C12—C13	1.350 (12)
O15—C16	1.473 (11)	C12—C11	1.398 (13)
O5—C14	1.203 (10)	C16—C18	1.457 (12)
N7—C17	1.246 (11)	C16—C17	1.498 (13)
N6—C17	1.367 (11)	C18—C19	1.385 (12)

Table 7: Selected bond angles (º) of compound (*S*)-7a

C14—O15—C16	109.1 (8)	O15—C16—C17	103.2 (9)
C17—N6—C14	110.1 (9)	N7—C17—N6	124.1 (11)
C17—N6—C12	129.3 (10)	N7—C17—C16	128.9 (11)
C14—N6—C12	120.5 (9)	N6—C17—C16	107.0 (10)
C13—C12—N6	125.1 (11)	O5—C14—O15	123.4 (11)
C11—C12—N6	117.7 (10)	O5—C14—N6	126.2 (11)
C18—C16—O15	109.8 (8)	O15—C14—N6	110.2 (10)
C18—C16—C17	116.9 (10)	C23−C18−C16	122.8 (10)

Table 8: Selected torsion angles (º) of compound (*S*)-7a

C14−O15−C16−C18	– 130.4 (9)	C17−N6−C14−O5	– 175 (1)
C14−O15−C16−C17	– 5.0 (1)	N6−C12−C11−C10	178 (1)
C16−O15−C14−O5	179.0 (1)	N6−C12−C13−C8	179 (1)
C16−O15−C14−N6	3.0 (1)	O15−C16−C18−C19	87 (1)
C17−N6−C12−C11	60.0 (1)	O15−C16−C18−C23	– 94 (1)
C17−N6−C12−C13	– 117.0 (1)	C17−C16−C18−C19	– 30 (1)
C14−N6−C12−C11	– 120.0 (1)	C17−C16−C18−C23	149.0 (1)
C14−N6−C12−C13	64.0 (1)	O15−C16−C17−N7	– 174.0 (1)
C12−N6−C17−N7	– 4.0 (2)	O15−C16−C17−N6	5.0 (1)
C12−N6−C17−C16	176.5 (9)	C18−C16−C17−N7	– 54.0 (2)
C14−N6−C17−N7	176.0 (1)	C18−C16−C17−N6	126.0 (1)
C14−N6−C17−C16	– 4.0 (1)	C16−C18−C19−C20	180.0 (1)
C12−N6−C14−O15	– 179.6 (8)	C23−C18−C19−C20	1.0 (2)
C12−N6−C14−O5	5.0 (2)	C16−C18−C23−Cl4	2.0 (1)
C17−N6−C14−O15	1.0 (1)	C16−C18−C23−C22	– 178.0 (1)

The five tested carbamate derivatives showed high to moderate activities against the four screened bacterial species. In general, their activities against the Gram-positive and Gram-negative species are comparable. Among the five tested carbamates, compound 3a was found to be the most potent where its optically active form (*S*)-3a has recorded an inhibition zone diameter value of 14 (mm/mg) against *S. aureus* and *P. aeruginosa* while the racemic form (*R,S*)-3a has recorded the same value against *B. subtilis.* The MIC_{90} value for compound (*S*)-3a was determined using agar dilution method [63] where, it recorded a value of 59 mg/ml against *S. aureus*.

Apparently, type of the chiral carbon substituent may have some effect on the activity. For example, compound (*S*)-3a (R=2-Cl-C_6H_4) is more potent than (*S*)-3b (R=4-F-C_6H_4) against *P. aeruginosa* where they recorded inhibition zone diameter values of 14 and 10, respectively. Similarly, the racemic carbamate (*R,S*)-3a (R=2-Cl-C_6H_4) is more potent than carbamates (*R,S*)-3b (R=4F-C_6H_4) and (*R,S*)-4 (R=2,3-dihydrobenzo[*b*][1,4] dioxin-6-yl) against *B. subtilis* (Table 9 and Figure 7). However, the configuration was found to have almost no effect on the activity of the tested carbamates where no marked differences in activities were observed between the optically active forms and their respective racemic analogues against a given bacterial species. For example, the optically active carbamate (*S*)-3b is slightly more potent than its racemic analogue (*R,S*)-3b against *B. subtilis* where they recorded inhibition zone diameter values of 12 and 10 (mm/mg), respectively. However, the racemic (*R,S*)-3b is slightly more potent than (*S*)-3b against *S. aureus* (Table 9 and Figure 7). Moreover, the racemate (*R,S*)-3a is slightly more potent than its optically active form (*S*)-3a against *B. subtilis* where they recorded inhibition zone diameter values of 14 and 12 (mm/mg), respectively. Compound (*S*)-3a and its racemic analogue (*R,S*)-3a showed the same activity against *E. coli* where they both recorded an inhibition zone diameter value of 13 (mm/mg). Similarly, (*S*)-3b and (*R,S*)-3b recorded a value of 11 (mm/mg) against *E. coli*. Carbamates (*S*)-3a,b, (*R,S*)-3a,b and (*R,S*)-4 have been screened also against two fungal species namely *A. flavus* and *C. albicans*. Only the optically active carbamate (*S*)-3a and its racemic analogue (*R,S*)-3a that showed activity against *C. albicans* where they both recorded an inhibition zone diameter value of 10 (mm/mg). *A. flavus* fungs was found to be totally insensitive to the tested carbamates. The optically active 4-imino-2-oxazolidinone derivatives (*S*)-7a-h as well as the racemic forms (*R,S*)-7b, d, e, f, g, h have been also screened for their antibacterial activities. The tested oxazolidinones showed slight to high activities against the four screened bacterial species, except for compounds (*S*)-7g, h against *B. subtilis* and compounds (S)-7b, d against *S. aureus* where they were found to be inactive. The behavior of some of the tested 2-oxazolidinones against the screened bacteria may reflect the stereochemistry-activity correlation. For example, compounds (*S*)-7g and (*S*)-7h are inactive against *B. subtilis*, while their racemic analogues (*R,S*)-7g and (*R,S*)-7h showed moderate activities by recording inhibition zone diameter values of 10 and 12 (mm/mg), respectively. Similarly, compounds (*S*)-7b and (*S*)-7d are inactive against *S. aureus*, while their racemic forms (*R,S*)-7b,d are active against the same bacterial species. Apparently, the (*R*)-enantiomer of 7-b,d and 7-g,h are rather more active than their (*S*)-analogues against *S. aureus* and *B subtilis*, respectively. Moreover, (*R,S*)-7d was found to be more potent than its optically active form (*S*)-7d against *B. subtilis, E. coli* and *P. aeruginosa*. Otherwise, the configuration, nature of the substituent on the chiral carbon (R) and/or on nitrogen (R`) almost have no effect on the activities of the tested 2-oxazolidinones where they recorded comparable values. Moreover, the evaluated oxazolidinones were screened also against *A. flavus* and *C. albicans* fungi. The first was found to be insensitive to all of the investigated oxazolidinones, while the second was found to be sensitive to compounds (*S*)-7a, (*S*)-7c, (*S*)-7e, (*R,S*)-7e, (*S*)-7f, (*R,S*)-7f, (*S*)-7h and (*R,S*)-7h where they showed comparable activities (Table 9 and Figure 7).

Table 9: The antimicrobial activity of the investigated carbamate and 2-oxazolidinone derivatives expressed in inhibition zone diameter (mm/mg sample)

Standards and tested compounds		Inhibition zone diameter (mm/mg sample)					
		Bacteria				Fungi	
		Gram−positive		Gram−negative			
		Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Aspergillus flavus***	***Candida albicans***
Standards	Antibacterial agent (**Ampicillin**)	20	18	19	20	-	-
	Antifungal agent **(Amphotericin B)**	-	-	-	-	16	19
The tested compounds	(*S*)-3a	12	14	13	14	0	0
	(*R,S*)-3a	14	13	13	13	0	0
	(*S*)-3b	12	11	11	10	0	10
	(*R,S*)-3b	10	13	11	9	0	10
	(*R,S*)-4	10	12	12	12	0	0
	(*S*)-7a	12	13	11	12	0	11
	(*S*)-7b	9	0	11	11	0	0
	(*R,S*)-7b	10	10	10	11	0	0
	(*S*)-7c	11	10	11	12	0	10
	(*S*)-7d	9	0	9	10	0	0
	(*R,S*)-7d	12	12	12	12	0	0
	(*S*)-7e	13	13	12	12	0	10
	(*R,S*)-7e	13	12	13	13	0	11
	(*S*)-7f	11	11	13	12	0	12
	(*R,S*)-7f	12	11	12	12	0	12
	(*S*)-7g	0	10	10	12	0	0
	(*R,S*)-7g	10	12	12	13	0	0
	(*S*)-7h	0	13	12	12	0	13
	(*R,S*)-7h	12	12	13	13	0	13

< 7 mm (No active); 7-9 mm (Slightly active); 10-12 mm (Moderately active); ≥ 13 mm (Highly active)

Antitumor activity

The newly synthesized carbamates (*S*)-3b, (*R,S*)-3b, (*R,S*)-4, the optically active oxazolidinones (*S*)-7a,b,c,e,f,g,h as well as the racemic oxazolidinones (*R,S*)-7b,e,f,g,h were screened for their *in vitro* cytotoxic activities against the human colon (HCT116), liver (HepG2) and breast (MCF-7) cancer solid cell lines in comparison with the activity of the known anticancer drug doxorubicin (DOX) [64]. The SRB method [65] was used to assay the cytotoxicities which are expressed as IC_{50} values (μg/ml). The screening results are compiled in Table 10 and Figure 8. Moreover, the arrangement of the cytotoxic activities according to their descending orders is represented in Table 11. All of the investigated compounds were found active against the three solid cell lines where some of them showed comparable activities with the standard drug (DOX) or even more potent that it. Among the carbamate derivatives, only (*R,S*)-3b showed a comparable activity (IC_{50}=5.0 μg/ml) to DOX (IC_{50}=4.19 μg/ml) against HCT116.

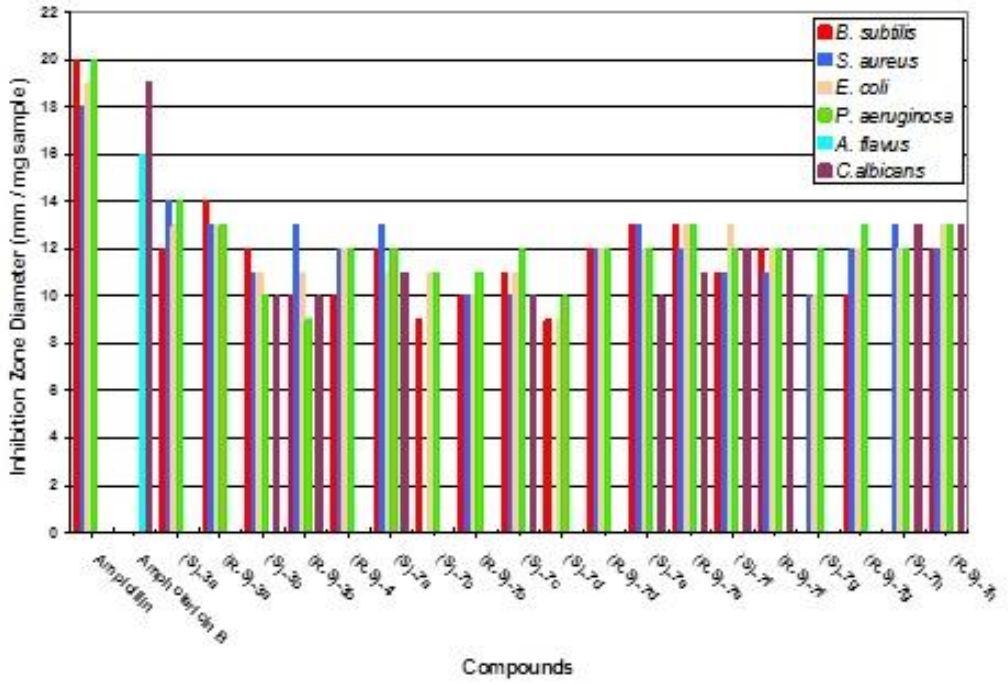


Figure 7: The antimicrobial evaluation of the investigated compounds

Among the tested oxazolidinones, the optically active derivatives (*S*)-7b, c, e, as well as the racemic forms (*R,S*)-7b,e,f,g.h were found more potent (IC_{50}=4.04, 3.89, 3.58, 5.11, 3.43, 4.50, 4.50 and 4.50 µg/ml, respectively) than DOX (IC_{50}=5.87 µg/ml) against HepG2 cell lines. Moreover, against MCF-7, compounds (*S*)-7b,e, f and (*R,S*)-7b,e exerted higher activities than the standard drug (DOX), while only (*S*)-7b and (*R,S*)-7e were more active than DOX against HCT116 cell lines (Tables 10 and 11; Figure 8). The investigation accentuates the well correlated structure-activity relationship (SAR). For example, against HCT116, the racemic carbamate (*R,S*)-3b (IC_{50}=5.00 µg/ml) is more potent than its optically active form (*S*)-3b (IC_{50}=9.53 µg/ml). Among the tested 2-oxazolidinones, the racemic derivatives (*R,S*)-7e, g, h were found more active than their respective optically active forms (*S*)- 7e, g, h against HCT116 and HepG2 carcinoma. However, compound (*S*)-7b is more active than its racemic analogue (*R,S*)-7b against the three investigated cell lines. In addition to the configuration, the antitumor activity may correlate also with the nature of the substituent on the chiral carbon atom (R) and/or nitrogen atom (R`). Thus for example, against HCT116 cell lines, oxazolidinones with different R and identical R` groups (Scheme 2) like (*S*)-7c, e, h showed different potencies which decreased in the order (*S*)-7e (4.53) > (*S*)-7h (6.93) > (*S*)-7c (7.70) (Tables 10 and 11, Figure 8). Similarly, compounds (*S*)-7a-c, as examples, with different N-substituents (Scheme 2) showed different activities against HCT116 where their potencies decreased in the order (*S*)-7b > (*S*)-7c > (*S*)-7a (Tables 10 and 11; Figure 8).

Table 10: The *in vitro* cytotoxic activities expressed as IC_{50} values (µg/ml) of the tested compounds against HCT116, HepG2 and MCF-7 human cancer solid cell lines

Compound	IC_{50} values (µg/ml)		
	HCT116	**HepG2**	**MCF−7**
DOX	4.19	5.87	4.13
(*S*)-3b	9.53	10.30	8.93
(*R,S*)-3b	5.00	12.10	7.39
(*R,S*)-4	10.30	11.50	8.31
(*S*)-7a	9.22	9.53	6.93
(*S*)-7b	3.28	4.04	3.73
(*R,S*)-7b	6.93	5.11	3.89
(*S*)-7c	7.70	3.89	5.72
(*S*)-7e	4.53	3.58	3.58
(*R,S*)-7e	3.43	3.43	3.28
(*S*)-7f	8.15	5.87	3.90
(*R,S*)-7f	8.46	4.5	8.76
(*S*)-7g	10.30	9.10	5.00
(*R,S*)-7g	5.00	4.50	4.80
(*S*)-7h	6.93	8.31	8.46
(*R,S*)-**7h**	4.80	4.50	4.65

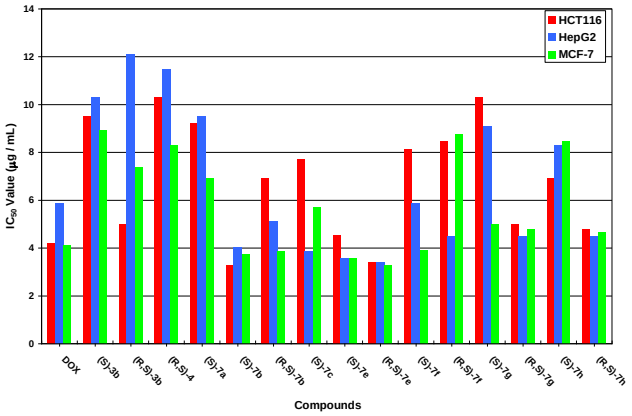


Figure 8: The *in vitro* cytotoxicity of the tested compounds expressed as IC_{50} values against HCT 116, HepG2 and MCF-7 human cancer cell lines

Table 11: The cytotoxic activities of the investigated compounds against HCT116, HepG2 and MCF-7 tumor cell lines according to their descending orders

HCT116		HepG2		MCF-7	
Compound	**IC_{50} µg/ml**	**Compound**	**IC_{50} µg/ml**	**Compound**	**IC_{50} µg/ml**
(*S*)-7b	3.28	(*R*,*S*)-7e	3.43	(*R*,*S*)-7e	3.28
(*R*,*S*)-7e	3.43	(*S*)-7e	3.58	(*S*)-7e	3.58
DOX	4.19	(*S*)-7c	3.89	(*S*)-7b	3.73
(*S*)-7e	4.53	(*S*)-7b	4.04	(*R*,*S*)-7b	3.89
(*R*,*S*)-7h	4.80	(*R*,*S*)-7f	4.50	(*S*)-7f	3.90
(*R*,*S*)-3b	5.00	(*R*,*S*)-7g	4.50	DOX	4.13
(*R*,*S*)-7g	5.00	(*R*,*S*)-7h	4.50	(*R*,*S*)-7h	4.65
(*R*,*S*)-7b	6.93	(*R*,*S*)-7b	5.11	(*R*,*S*)-7g	4.80
(*S*)-7h	6.93	(*S*)-7f	5.87	(*S*)-7g	5.00
(*S*)-7c	7.70	DOX	5.87	(*S*)-7c	5.72
(*S*)-7f	8.15	(*S*)-7h	8.31	(*S*)-7a	6.93
(*R*,*S*)-7f	8.46	(*S*)-7g	9.10	(*R*,*S*)-3b	7.39
(*S*)-7a	9.22	(*S*)-7a	9.53	(*R*,*S*)-4	8.31
(*S*)-3b	9.53	(*S*)-3b	10.30	(*S*)-7h	8.46
(*R*,*S*)-4	10.30	(*R*,*S*)-4	11.50	(*R*,*S*)-7f	8.76
(*S*)-7g	10.30	(*R*,*S*)-3b	12.10	(*S*)-3b	8.93

CONCLUSION

It was found that, the nature of substituent on the nitrogen atom of some selected isocyanate reagent controls the structure of the products that result from their reactions with cyanohydrins 1a-d. The *tert*-butyl isocyanate (2) gave the respective carbamates 3a, b and 4 whereas the aryl isocyanates 5a-d gave the corresponding 2-oxazolidinone derivatives 7a-h. Moreover, the reactions of isocyanates 2 and 5a-d with the chiral cyanohydrins (*R*)-1a-c were found to be accompanied with inversion of configuration where the (*S*)-enantiomers of the respective carbamates and/or 4-imino-2-oxazolidin- ones have been produced. The X-ray crystallographic analysis has provided an effective tool in confirming the structure and configuration of the new products. Most of the new compounds showed marked antibacterial activities which are rather close to the reference drug (Ampicillin). Moreover, some of the new products revealed pronounced *in vitro* anticancer activities when screened against the HCT116, HepG2 and MCF-7 cancer solid cell lines. The most promising results were recorded by compounds (*S*)-7b, c, e, f and (*R*,*S*)-7b, e, f, g, h where they showed IC_{50} values less than those recorded by the reference drug DOX.

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