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Hemi-synthesis of three new armed antibiotics analogs of Calcimycin (A23187) and determination of theirs acidity constants by potentiometric method

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Abstract

A new series of three Calcimycin derivatives **CD3**, **CD5** and **CD7** have been synthesizedby the condensation reaction of Calcimycin (A23187) with ethyl 5-chloro-5-oxopentanoate, ethyl 7-chloro-7-oxoheptanoate and 9-chloro-9-oxononanoate in an equimolar ratio. The potentiometric studies show the presence of four species following the increase in pH, LH3+, LH2, LH- and L2-. We used a preferment program of simulation Hyperquad which is very fast and gives acidity constants with a good correlation coefficient σ. Distribution curves show a good predominance of the species LH2 for all analogues at biological pH. Adjunction of aliphatic carboxylic arm at the benzoxazole nitrogen dramatically increases the acidity of the carboxylic function of 3 log units, which is enormous. This is mainly due to the attractive effect of the amide in α benzoxazole group, which relocates the link, than the aliphatic carboxylic function. Profile structure are reinforced by three binding sites, the benzoxazole group by the mean of the carboxylic function and the pyrazole nitrogen and acetopyrole group by the intermediary of the keto function. The hydrogen bond breaking-established in two sides around of the benzoxazole group by steric hindrance may also explain the increased acidity of the acid function of the benzoxazole moiety. The spectroscopic data (IR NMR) and crystallographic study confirm these findings.

Keywords: Antibiotics (A23187), hemi-synthesis, Calcimycin analogs, potentiometry, acidity constant pKa, Hyperquad.

1. Introduction

Calcimycin (A23187), an ionophore carboxylic polyether antibiotic (Fig. 1) with calcium carrier properties, has attracted considerable attention in biology since its discovery, as a tool for the study of calcium second messenger in living systems [1, 2]. Its non-fluorescent 4-bromo derivative was subsequently described and found suitable for the same application in the presence of fluorescent probes [3].

In view of the significant structural and biological applications of Calcimycin derivatives, we wish to report the synthesis of a new class of Calcimycin derivatives **CD3**, **CD5** and **CD7**. These compounds have been investigated for *in-vitro* antibacterial activity against Gram-positive bacterial strains.

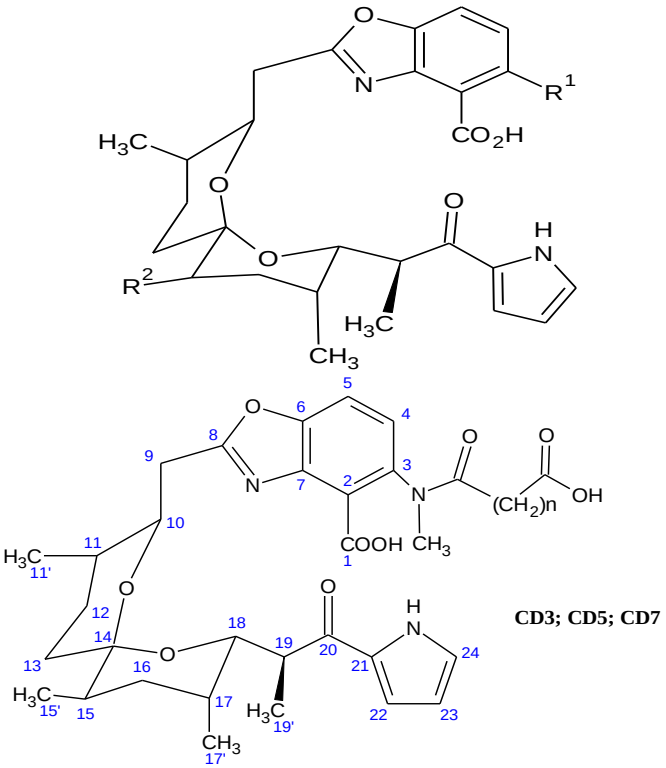
Erdahl et al. [4] recently showed that 4-bromo derivative of compound (A23187) transports Zn^{2+} and Mn^{2+} with high selectivity over Ca^{2+} and Mg^{2+} in phospholipid vesicles, and they made interesting findings concerning the stoichiometry of species involved in the transport. However, information on the impact of this natural ligand C4 substitution with lipophylic functionalised arm was lacking. Another important aspect (conformation of Calcimycin derivatives, co-ordination sites of the cation, solvation, *etc.*) should be taken in consideration and should be compared to previous study on Lasalocide [5].

The role of the benzoxazole arm was highlighted by several authors [1-3]. The coplanarity of the cycle benzoxazole with the secondary amine supports the cation mechanism of transport. It is not only maintained by hydrogen bonds of CO_2H---NHMe but also CO_2H---N (oxazolic) hydrogen bond. The physicochemical properties of the molecule changes in a drastic way by the rupture of these hydrogen bonds with methyl or *N*-acetyl substitution.the. It is in this way that we ventured to modulate the selectivity of this ionophore already

known for calcium. The addition of an arm carrying a carboxylic functionality generates an additional cavity capable of binding tosecond cation of comparable or different nature. In order to better understand the relation between the architecture of the calcimycin and its antibacterial properties, several analogues (Fig. 2) were synthesized and studied. In this article we carried out structural modifications on the level of the secondary amine of the α-benzoxazol ring by addition of a Keto-aliphatic arm with a final carboxylic function.

For all these reasons, we recently undertook the preparation of new derivatives of Calcimycin (A23187) with various supplementary lipophylic arms suitable for an increase in lipophicity. Here, we report three new calcimycin derivatives containing 5-[(-carboxy-alkanoyl-methyl-amino)] as supplementary lipophylic arms, which could reveal unusual features.

Fig. 1: Structures of some commercial antibiothics (A23187, Cezomycin and X-14885A) containing Calcimycin skeleton and new prepared Calcimycin derivatives **CD3**, **CD5** and **CD7**.



Code	**(R^1, R^2)**
A23187	(CH_3NH, CH_3)
Cezomycin	(H, CH_3)
X-14885A	(OH, H)

Code	**n**
CD3	3
CD5	5
CD7	7

The synthesis aimed once again to clarify the role of benzoxazole arm. The addition of a new aliphatic carboxylic arm will certainly increase the lipophilicity of the molecule as well as its ionophore performance. It was hypothesized that the first cavity after complexation with cations will generate another cavity capable of holding another cation. Therefore, it will be possibleto complex and to carry two cations of different valences and sizes. By doing this, it will also be possible to tune the selectivity of the new ionophores against various cations. The results of recent studies in our laboratory confirms our hypothesis.

2. Materials and Methods

2.1. Apparatus

All chemicals used were of reagents grade. All starting materials were used as received. Melting points were recorded on REICHERT melting point apparatus. Infrared spectra were recorded on PERKIN-ELMER 881spectrometer. Column chromatography was performed on Merck silica gel 0.063-0.200 nm in normal mode. Melting points (mp) were determined using a Reichert hot stage microscope. The optical rotations were measured on JASCO polarimeter model DIP-370 at 25 °C and the wavelength of the sodium D line length (δ = 589 nm). They are measured on the products in solution, and the concentration c is expressed in grams per 100 ml of solvent. The ^{1}H and ^{13}C-NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a Bruker AC 400 MHz spectrometer. CCM control has been done on silice Merck $60F_{254}$. Masse spectra were recorded on HEWLETT PACKARD 5989B Instrument (University Blaise Pascal, Clermont Ferrand, France).

*2.2. General procedure for the synthesis of compounds (**2a-2c**)*
Under inert atmosphere of Argon, to a cold (0 °C) magnetically stirred solution of acid (2.5 g, 15.6 mmol, 1 eq.) in ethanol (12.5 mL) was added, drop by drop, 0.2 mL (2 eq) of thionyl chloride $SOCl_2$. The resultant mixture was stirred for 24 hours at room temperature (20 °C). The excess of $SOCl_2$ and solvent were eliminated by evaporation at rotaevaporator. The reaction mixture was neutralized with a saturated solution of $NaHCO_3$ (10 mL), the organic phase is extracted with ethyl acetate (100 mL) then washed with saturated NaCl (10 mL) and dried over anhydrous magnesium sulfate, acetate solution, finally solution was filtered and evaporated. The purity of product **2b** was checked by TLC. The same method could be applied for the preparation of other ligand **2c**. Compound **2a** was available in laboratory.

*2.2.1. Diethyl heptanedioate: (**2b**)*
Yalow oïl. Yield: 83%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.10 (q, J = 7,0 Hz, 4H, CH_2 ester); 2.27 (t, J = 7,5 Hz, 4H, H2 + H6); 1.62 (m, 4H, H3 + H5); 1.33 (m, 2H, H4); 1.23 (t, J = 7.0 Hz, 6H, CH_3 ester).^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 173.5 (C1 + C7); 60.2 (CH_2 ester); 34.1 (C2 + C6); 28.5 (C4); 24.5 (C3 + C5); 14.2 (CH_3 ester).

*2.2.2. Diethyl nonanedioate: (**2c**)*
Yalow oïl. Yield : 97%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.08 (q, J = 7.0 Hz, 4H, CH_2 ester); 2.24 (t, J = 7.5 Hz, 4H, H2 + H8); 1.57 (m, 4H, H3 + H7); 1.28 (s, 6H, H4 + H5 + H6); 1.21 (t, J = 7,0 Hz, 6H, CH_3 ester). ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 173.7 (C1 + C9); 60.1 (CH_2, ester); 34.2 (C2 + C8); 28.8 (C3 + C7); 28.8 (C5); 24.8 (C4 + C6); 14.1 (CH_3, ester).

*2.3. General procedure for the synthesis of compounds **(3a-3c)***
At room temperature, to a magnetically stirred solution, of a diester **2a, 2b or 2c** (13 mmol) in ethanol (12 mL) was added a solution of NaOH (1 M, 13 mL). The resultant mixture was refluxed for 1 hour. The completion of reaction was monitored by TLC. The reaction mixture was cooled to room temperature. Then solvent was evaporated. To crude products, a mixture of water/ether was added. The aqueous phase was acidified by a solution of HCl (pH = 1-2), then extracted by ether. The organic phase of ether was dried on anhydrous sulfate of magnesium. After elimination of solvent at rotaevaporator, a crude product was purified by column chromatography on normal silica (eluent: ethyl acetate /cyclohexane = 50/50).

*2.3.1. 7-Ethoxy-7-oxoheptanoic acid: (**3b**)*
Transparent oil. Yield : 70%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.10 (q, J = 7.0 Hz, 2H, CH_2 ester); 2.32 (t, J = 7.5 Hz, 2H, H2); 2.27 (t, J = 7,5 Hz, 2H, H8); 1.58-1.63 (m, 4H, H3 + H7); 1.30 (se, 6H, H4 + H5 + H6); 1.23 (t, J = 7.0 Hz, 3H, CH_3 ester). ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 179.9 (C1); 173.7 (C7); 60.2 (CH_2 ester); 33.9 (C2); 33.7 (C6); 28.4 (C4); 24.6 (C3); 24.2 (C5); 14.1 (CH_3 ester).

*2.3.2. 9-Ethoxy-9-oxononanoic acid: (**3c**)*
White powder. yield : 50%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.10 (q, J = 7,0 Hz, 2H, CH_2 ester); 2.32 (t, J = 7.5 Hz, 2H, H2); 2.27 (t, J = 7.5 Hz, 2H, H8); 1.58-1.63 (m, 4H, H3 + H7); 1.30 (se, 6H, H4 + H5 + H6); 1.23 (t, J = 7.0 Hz, 3H, CH_3 ester). ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 179.9 (C1); 173.9 (C9); 60.2 (CH_2 ester); 34.3 (C2); 33.9 (C8); 28.8 (C3 + C5 + C7); 24.8 (C4); 24.6 (C6); 14.2 (CH_3 ester).

*2.4. General procedure for the synthesis of compounds (**4a-4c**)*
*2.4.1. 7-chloro-7-oxoheptanoate: (**4b**)*
A une solution de **3b** (0,3 g, 1,6 mmol, 1 eq) dans 4,2 ml de dichloroéthane sec, on ajoute goutte à goutte 1,2 équivalents de chlorure d'oxalyle $(COCl)_2$ (1,92 mmol, 165 µl) dans 2,5 ml de dichloroéthane sec. On laisse agiter à la température ambiante une nuit sous atmosphère d'argon. Après évaporation du solvant on isole 0,33 g d'une huile orange (rendement quantitatif) que l'on caractérise sous la forme de son ester méthylique par addition de quelques gouttes de MeOH sec sur une prise d'essai de l'huile orange sous atmosphère d'argon (IR CCl_4 ν_{max} : 1745cm^{-1}et1735cm^{-1}).
*2.5. General procedure for the synthesis of compounds (**5a-5c**)*
The magnetically stirred solution To a volume of (10 mL) acetique anhydride, (35 mmol) acid was added. The resultant mixture was stirred and refluxed for 6 hours.. The reaction mixture was cooled to room temperature. Then it was stirred during 48 hours. The solvent was evaporated. The target product was obtained by recrystallisation in acetonitrile.

*2.5.1. Oxocane-2,8-dione: (**5b**)*
White powder. Yield : 86%. M. p. = 55- 56° C. IR ν(cm^{-1}): 2925 (CH_2); 1814, 1750 (C=O); 1230 (C-O). NMR ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.46 (t, J = 7.0 Hz, 4H, H2 + H6); 1.66 (m, 4H, H3 + H5); 1.42 (m, 2H, H4). ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 169.2 (C1 + C7); 33.6 (C2 + C6); 28.9 (C3 + C5); 23.7 (C4).

*2.5.2. Oxecane-2,10-dione: (**5c**)*
White powder. Yield: 88%. M. p. = 58- 59°C. IR ν(cm^{-1}): 2920 (CH_2); 1810, 1740 (C=O); 1211 (C-O). ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 168.6 (C1 + C9); 33.7 (C2 + C8); 27.6 (C3 + C7); 27.3 (C4 + C6); 22.9 (C5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.16 (m, 4H, H2 + H8); 1.32 (m, 4H, H3 + H7); 1.05 (m, 6H, H4 + H5 + H6).

*2.6 . Synthesis of Calcimycin A23187 and derivatives **CD-3**, **CD5** and **CD7***
2.6.1. Synthesis of Calcimycin A23187
The preparation of calcimycin was prepared in our laboratories as described previously described by our group [22].
White powder. M. p. = 184-185°C. IR ν(cm^{-1}) : 3347 (N-H); 2960-2921 (O-H, CO_2OH); 1705 (C=O, CO_2OH); 1636-1638 (C=O, α-ketopyrrole); 1600 (C=C, benzynic ring); 1565-1557-1537 (C=N); 1400-1464 (C-O); 1252-1256 (C-O, CO_2OH); 1100-1165 (C-H of benzoxazole); 1077 (aliphatic C-N); 982-996 (C-O of epoxyde). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 13.00 (s, 1H, CO_2H); 9.74 (s, 1H, NH pyrol); 8.10 (s, 1H, NH amino); 7.59 (d, J = 9.0 Hz, 1H, H5); 7.05 (m, 1H, H24); 6.92 (m, 1H, H22); 6.65 (d, J = 9.0 Hz, 1H, H4); 6.24 (m, 1H, H23); 4.27 (m, 1H, H10); 3.69 (dd, J = 10.0 Hz, J = 2.0 Hz, 1H, H18); 3.20 (m, 1H, H19); 3.07 (d, J = 7.0 Hz, 1H, H9A); 2.97 (d, J = 5,0 Hz, 3H, CH_3 amino); 2.93 (d, J = 7.0 Hz, H9B); 1.74-1.00 (M, 9H, H11 + H12 + H13 + H15 + H16 + H17); 0.97 (J = 7.0 Hz, 3H, H15'); 0.91 (d, J = 7.0 Hz, 3H, H19'); 0.87 (d, J = 6,0 Hz, 3H, H17'); 0.86 (J = 7.0 Hz, 3H, H11'). ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 193.9 (C20); 168.2 (C1); 166.1 (C8); 150.8 (C3); 141.6 (C7); 140.7 (C6); 133.1 (C21); 124.5 (C24); 116.9 (C5); 116.4 (C22); 110.1 (C23); 108.4 (C4); 98.5 (C14); 97.9 (C2); 72.7 (C18); 68.3 (C10); 42.5 (C19); 35.1 (C16); 32.4 (C9); 32.2 (C15); 30.0 (CH_3amino); 28.6 (C11); 28.3 (C17); 25.7 (C12); 25.4 (C13); 16.2 (C15'); 13.2 (C19'); 11.4 (C11'); 10.7 (C17').

*2.6.2. General procedure for the synthesis of compounds **CD3**, **CD5** and **CD7***
In a 50 ml two-necked flask fitted with a hinged cap skirt and a magnetic stirring under an argon atmosphere, away from the light, a solution is prepared from **5a**, **5b** or **5c** (1.91 mmol , 1 eq) and 10 mL of anhydrous pyridine. At 0 °C was added dropwise using a syringe to a solution of 1 g (1.91 mmol, 1 eq) calcimycin in 10 mL of anhydrous pyridine. The reaction mixture is then stirred at room temperature for two days until the disappearance of the anhydride. The pyridine was removed in a rotary evaporator, the residue was taken up in ether (30 mL) and washed with HCl (0.1 N) three times (6 mL) , the ether layer was dried over anhydrous magnesium sulfate and then filtered . After removing the solvent on a rotary evaporator, the crude product is purified by column chromatography on normal silica (eluent ethyl acetate) to give a white powder after acidification and extraction with ether.

2.6.2.1. 5-[(-carboxybutanoylmethyl-amino)-2-[3,9,11-trimethyl-8-[(1S)-1-methyl-2-oxo-2-(1H-pyrrol-2-yl)ethyl]-1,7-dioxaspiro[5.5]undec-2-ylmetyl]-4-benzoxazole-4-carboxylic acid **(CD3)**

White powder. Yield : 64%. M. p. = 120-121°C. $[\alpha]^{25}_D$ = 24.0° (C = 2.5 ; $CHCl_3$). SM (I.E.) m/z: 638 ($[M + H]^+$); 636 ($[M – H]^-$); 660 ($[M + Na]^+$). IR ν(cm^{-1}): 3500-3000 (O-H of CO_2OH)>NH (pyrolic ring); 1711 (C=O, CO_2H); 1635 (C=O, α-Ketopyrrole + -CONR); 1565 (N-H); 1408 à 1481 (C-O or O-H, CO_2H); 1238 (C-O, CO_2H); 1105 et 1176 (CH, benzoxazole); 1075 (aliphatic C-N); 987 (C-O, epoxyde). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.73 (d, J = 8,5 Hz, 1H, H4); 7.20 (d, J = 8,5 Hz, 1H, H5); 7.01 (se, 1H, H24); 6.85 (se, 1H, H22); 6.12 (se, 1H, H23); 3.94 (m, 1H, H10); 3.29 (m, 1H, H18); 3.18 (d, J = 9.0 Hz, 3H, CH_3 amide); 3.07-2.92 (M, 4H, H9 + H19); 2.23- 0.89 (M, 15H, H11 + H12 + H13 + H15 + H16 + H17 + H2' + H3' + H4'); 0.84 (d, J = 6,5 Hz, 3H, H11'); 0.78 (d, J = 5.0 Hz, 6H, H15' + H17'); 0.63 (d, J = 7.0 Hz, 3H, H19'). ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 194.9- 194.8 (C20); 177.0 (C1'); 172.9- 172.8 (C1); 169.1-169.0 (C8); 164.5-164.3 (C5'); 149.9-149.8 (C3); 141.2-141.0 (C7); 139.8-139.7 (C6); 132.7 (C21); 126.1-126.0 (C24 + C5); 120.2-119.9 (C2); 118.4-118.2 (C22); 114.6-114.4 (C4); 110.2 (C23); 98.4 (C14); 73.0-72.9 (C18); 68.7-68.6 (C10); 42.2 (C19); 37.2 (CH_3 amide); 34.9 (C16); 32.9-32.8 (C2'); 32.7 (C9); 32.5-32.4 (C4'); 32.2 (C15); 29.4-29.2 (C11); 28.2 (C17); 25.4 (C12); 25.1 (C13); 20.0 (C3'); 16.0 (C15'); 12.6 (C19'); 11.2 (C11'); 10.6 (C17').

2.6.2.2. 5-[(-carboxy-hexanoyl-methyl-amino)-2-[3,9,1-trimethyl-8-[(1S)-1-methyl-2-oxo-2-(1H-pyrrol-2-yl)ethyl]-1,7-dioxaspiro[5.5]undec-2-yl-metyl]-4-benzoxazole-4-carboxylic acid **(CD5)**
White powder. Yield: 84%. M. p. = 114- 115 °C. IR ν(cm^{-1}): 3000 -3500 (O-H, CO_2H), >NH (pyrrolic ring); 1720 (C=O, CO_2H); 1636 (C=O α-ketopyrrole of -CONMeR); 1567 (N-H); 1408-1481 (C-O or O-H, CO2H or CH_3 or CH_2 or CH_3 of CH_3-N); 1243 (C-O, CO_2H); 1076 (aliphatic C-N); 988 (C-O, epoxyde). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.74 (d, J = 9.0 Hz, 1H, H4); 7.20 (d, J = 9.0 Hz, 1H, H5); 7.02 (m, 1H, H24); 6.88 (m, 1H, H22); 6.16 (m, 1H, H23); 4.01 (m, 1H, H10); 3.30 (m, 1H, H18); 3.19 (d, J = 10,0 Hz, 3H, CH_3 amide); 3.13-2.91 (M, 4H, H9 + H19); 2.30-0.95 (M, 19H, H11 + H12 + H13 + H15 + H16 + H17 + H2' + H3' + H4' + H5' + H6'); 0.80 (d, J = 6.0 Hz, 6H, H11' + H17'); 0.73 (d, J = 7,0 Hz, 3H, H15'); 0.67 (d, J = 7,0 Hz, 3H, H19'). ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 194.8- 194.7 (C20); 177.9-177.8 (C1'); 173.3-173.2 (C1); 168.9 (C8); 163.9-163.8 (C7'); 149.8 (C3); 141.4 (C7); 140.4 (C6); 132.8 (C21); 126.2 (C24); 126.1-126.0 (C5); 119.1-119.0 (C2); 118.3-118.1 (C22); 114.8-114.7 (C4); 110.3 (C23); 98.5 (C14); 73.0 (C18); 68.6-68.5 (C10); 42.3-42.2 (C19); 37.2 (CH_3 amide); 35.0- 34.9 (C16); 33.7-33.6 (C6'); 33.3 (C2'); 32.6 (C9); 32.3 (C15); 29.3 (C11); 28.3-28.1 (C4' + C17); 25.5 (C12); 25.2 (C13); 24.5-24.3 (C5'); 24.0 (C3'); 16.0 (C15'); 12.8 (C19'); 11.2 (C11');

10.6 (C17'). $[\alpha]^{25}_{D}$ = 16.0° (C = 2.5; $CHCl_3$). SM (I.E.) m/z: 666 ($[M + H]^+$); 664 ($[M – H]^-$); 688 ($[M + Na]^+$); 704 ($[M + K]^+$).

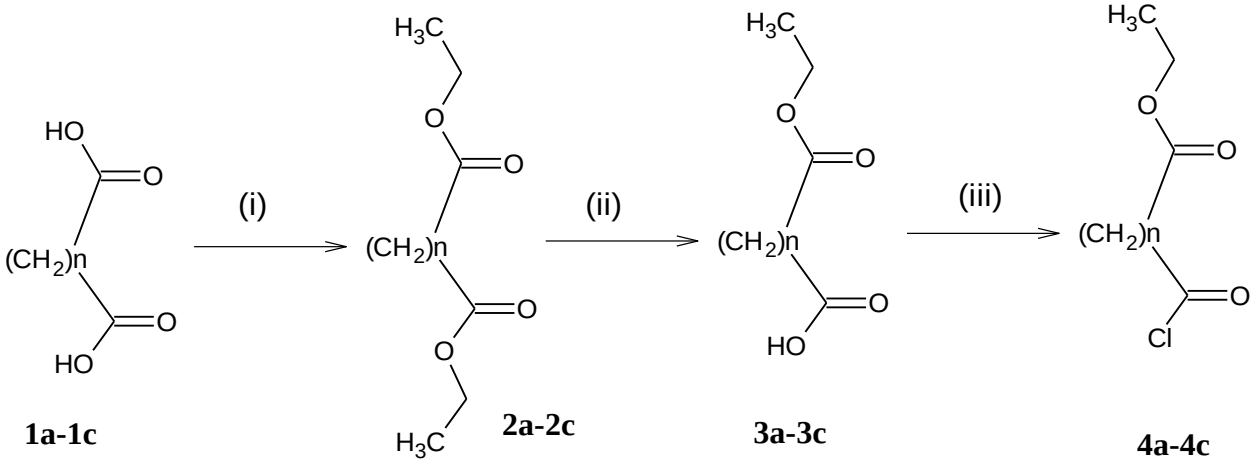
2.6.2.3. 5-[(-carboxyoctanoylmethyl-amino)-2-[3,9,11-trimethyl-8-[(1S)-1-méthyl-2-oxo-2-(1H pyrrol-2-yl)ethyl]-1,7-dioxaspiro[5.5]undec-2-ylmetyl]-4-benzoxazole-4-carboxylic acid **(CD7)**
White powder. Yield: 68%. M.p. = 116-117 °C. IR ν(cm^{-1}) : 3000, 3500 (O-H, carboxylic), >NH (pyrrolic ring); 1707 (C=O, carboxylic); 1621 (C=O, α-ketopyrrole of amide); 1408-1481 (C-O or O-H of CO_2H or CH_3, CH_2 or CH_3 of CH_3-N); 1243 (C-O, carboxylic acid); 1076 (C-N aliphatic); 988 (C-O, epoxyde); ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 194.8 (C20); 178.8 (C1'); 173.6-173,5 (C1); 169.0 (C8); 164.4-164.3 (C9'); 149.8 (C3); 141.3-141.2 (C7); 140.2 (C6); 132.7 (C21); 126.2 (C24); 126.1-125.8 (C5); 119.6 (C2); 118.4 (C22); 114.7-114.6 (C4); 110.3 (C23); 98.4 (C14); 72.9 (C18); 68.6-68.5 (C10); 42.2-42.1 (C19); 37.3 (CH_3 amide); 34.9- 34.8 (C16); 33.9 (C2' + C8'); 32.6 (C9); 32.2 (C15); 29.4 (C11); 28.7 (C4' + C5' + C6'); 28.2 (C17); 25.5 (C12); 25.1 (C13); 25.0- 24,5 (C3' + C7'); 16.0 (C15'); 12.7 (C19'); 11.2 (C11'); 10.6 (C17'). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.81 (d, J = 9.0 Hz, 1H, H4); 7.25 (d, J = 9.0 Hz, 1H, H5); 7.09 (se, 1H, H24); 6.94 (se, 1H, H22); 6.21 (se, 1H, H23); 4.04 (m, 1H, H10); 3.36 (m, 1H, H18); 3.26 (se, 3H, CH_3 amide); 3.20-2.95 (M, 4H, H9 + H19); 2.34-1.10 (M, 23H, H11 + H12 + H13 + H15 + H16 + H17 + H2' + H3' + H4' + H5' + H6' + H7' + H8'); 0.86 (d, J = 7.0 Hz, 6H, H15' + H19'); 0.73 (d, J = 6.0 Hz, 3H, H11'); 0.68 (d, J = 6,0 Hz, 3H, H17'). $[\alpha]^{25}_{D}$ = 21.0° (C = 5.0 ; $CHCl_3$). SM (I.E.) m/z: 694 ($[M + H]^+$); 716 ($[M + Na]^+$).

3. Results and discussion

3.1. Synthesis of the desired Calcimucin derivatives

The Calcimycin acid (A23187) derivatives were prepared in a four-step reaction. Calcimycin derivatives **CD3**, **CD5** and **CD7** were first prepared on a limited preparative scale in 64, 84 and 68% yields, respectively, by a condensation of Calcimycin precursor (A23187) with compounds **4a-4c**. The compounds **4a-4c** were in turn obtained in quantitative yields as yellow oils, from compounds **3a-3c**, as shown in Scheme 1.

Scheme 1: Synthesis of starting chemicals **2-4**. (i) $SOCl_2$/ EtOH (0 °C). (ii) NaOH/Δ. (iii) $(COCl)_2/C_2H_5Cl_2$ (20-25 °C).



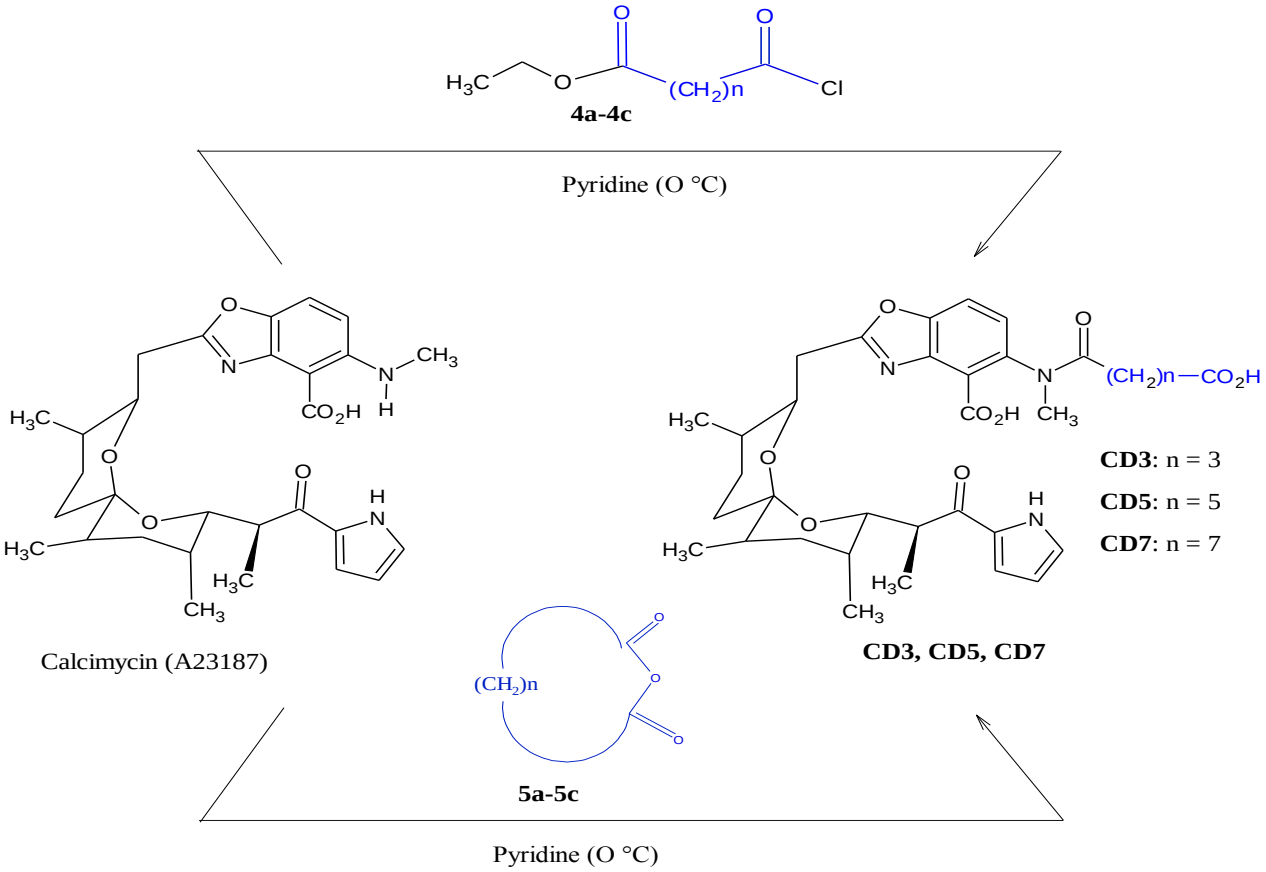
1a-1c **2a-2c** **3a-3c** **4a-4c**

All derivatives were soluble in ethanol, DMF, and DMSO at room temperature and in methanol on heating only. These three new calcimycin derivatives were characterized by means of ^{1}H, ^{13}C NMR studies, optical measurements ($[\alpha]^{25}_{D}$) and FT-IR analysis.

3.2. Spectroscopic analyses

3.2.1. IR Spectra

The characteristic bands of IR spectra of antibiotics **CD3**, **CD5**, **CD7** and their parent molecule A23187 are reported in experimental section. The IR spectra of all the new compounds exhibited the bands at 3000-3500, 1700-1720, 1565-1537, 1400-1480 and 1070-1080 cm^{-1} respectively due to (N-H/OH) and (C=O/C=N) vibrations of pyrol/benzoxazole/carboxylate amoieties (Table 1).



Scheme 2. Synthesis of Calcimycin derivatives **CD3**, **CD5** and **CD7**.

Table 1: Selected IR data of **CD3**, **CD5** and **CD7**.

Compd.	n	Physical properties			IR υ (cm^{-1})				
		MW (g/M)	Tf (°C)	$[\alpha]^{25}$ (°)	O-H/ N-H	(C=O) CO_2H	(C=O) Acy-Pyz/ N-CO	C-O CO_2H	C-N Pyz/ Oxazol
CD3	3	637	120	24.0	3000 3500	1711	1635	1408 1481	1075
CD5	5	665	114	16.0	3000 3500	1720	1636	1408 1481	1076
CD7	7	693	116	21.0	3000 3500	1707	1621	1408 1481	1076
A23187	---	523	184	---	3347	1705	1637	1408 1481	1077

Compared to the calcimycin, IR Spectra clearly show a non-negligible variation of the resonance frequency only for groups (C = O) of the acidic function CO_2H and the Pyz Acyl-(N-CO) group (Table 1). This can be attributed to the significant structural changes only on those sites.

3.2.2. ^1H NMR spectra

The ^1H NMR spectral data of the ligands **CD3**, **CD5** and **CD7** and their parent molecule A23187 are recorded in the experimental part. The exhibited signals of all the protons due to heteroaromatic/aromatic groups were found, as to be in their expected region. The spectra of all Calcimycin derivatives **CD3**, **CD5** and **CD7** displayed protons H4, H5 due to C_4–H and C_5–H group of 1,3-benzoxazole moiety at 7.73–7.81 and at 7.20–7.25 ppm respectively, as doublets ($J_{H4\text{-}H5}$ = 8.51-9.01 Hz). The strong down field shift of H4, H5 protons indicated electro-attractor effect of the new amido/carboxylic acid arm or their intramolecular C=O/H4 bonding. The spectrum of A23187 exhibited the 1,3benzoxazole C_4–H and C_5–H protons as two doublet at 7.59 and 6.65 ppm respectively (Table 2).

Table 2: Selected ^{1}H NMR data (400 MHz, $CDCl_3$) of **CD3**, **CD5** and **CD7**.

Compd.	**n**	**δ ppm (J in Hz)**		
		NH Amino	H4 δ (J_{H-H})	H5 δ (J_{H-H})
CD3	3	---	7.73 (d, 8.5 Hz)	7.20 (d, 8.5 Hz)
CD5	5	---	7.74 (d, 9.0 Hz)	7.20 (d, 9.0 Hz)
CD7	7	---	7.81 (d, 9.0 Hz)	7.25 (d, 9.0 Hz)
A23187	---	8.10	7.59 (d, 9.0 Hz)	6.65 (d, 9.0 Hz)

3.2.3. ^{13}C NMR spectra

The ^{13}C NMR spectral data (Table 3) are reported along with their possible assignments in the experimental section and all the carbons were found in the expected regions. The conclusion obtained from these studies provides further support to the mode of inductive effect of arm on 1,3-benzoxazole moiety explained in their IR and ^{1}H NMR spectral data. In order to get more information about the general effect of new arm on species repartition in solution with various pH, pKa are determined by potentiometry and regrouped for comparison (Table 4).

Table 3: Selected ^{13}C NMR data ($CDCl_3$, 400 MHz) of calcimycin derivatives **CD3**, **CD5** and **CD7**.

Carbon	**A23187**	**CD3**	**CD5**	**CD7**
C_1	168.2	177.0	177.9	178.8
C_2	97.9	120.1	119.1	119.6
C_3	150.8	149.9	149.8	149.8
C_4	108.4	114.5	114.8	114.7
C_5	116.4	---	126.1	126.0
C_6	140.7	139.8	140.4	140.2
C_7	141.6	141.1	141.4	141.3
C_8	166.1	169.1	168.9	169.0
C_9	32.4	32.7	32.6	32.6
C_{10}	68.3	68.7	68.6	68.6
C_{11}	28.6	29.3	29.3	29.4
C_{12}	25.7	25.4	25.5	25.5
C_{13}	25.4	25.1	25.2	25.1
C_{14}	98.5	98.4	98.5	98.4
C_{15}	32.2	32.2	32.3	32.2
C_{16}	35.1	34.9	35.0	34.9
C_{17}	28.3	28.2	28.3	28.2
C_{18}	72.7	73.0	73.0	72.9
C_{19}	42.5	42.2	42.3	42.2
C_{20}	193.9	194.9	194.8	194.8
C_{21}	133.1	132.7	132.8	132.7
C_{22}	116.4	118.4	118.3	118.4
C_{23}	110.1	110.2	110.3	110.3
C_{24}	124.5	126.1	126.2	126.2
$C_{1'}$	---	172.9	173.3	173.6
$C_{2'}$	---	32.9	33.3	33.9
$C_{3'}$	---	20.0	24.0	25.0
$C_{4'}$	---	32.5	28.3	28.7
$C_{5'}$	---	164.5	24.5	28.7
$C_{6'}$	---	---	33.7	28.7
$C_{7'}$	---	---	163.9	25.0
$C_{8'}$	---	---	---	33.9
$C_{9'}$	---	---	---	164.4
$C_{11'}$	11.4	11.2	11.2	11.2
$C_{15'}$	16.2	16.0	16.0	16.0
$C_{17'}$	10.7	10.6	10.6	10.6
$C_{19'}$	13.2	12.6	12.8	12.7
$NHCH_3$	30.0	---	---	---
CH_3 amide	---	37.2	37.2	37.3

Replacing the external 5-methyl-amino group by 5-[(carboxy-alkanoyl-methyl-amino)] as supplementary lipophylic arms, as in compound **CD3**, does not significantly affect the structure of the molecule. ^{1}H and ^{13}C NMR data (Tables 2, 3) are similar to those of parent molecule (A23187) and are in agreement with the armed structures

CD3, CD5 and **CD7.**

In order to get more insight into the structure/ properties relationship, we envisaged varying the terminal arm at C-3 position of the benzoxazole group by changing the number of methylene bridges (n). It is worth noting that the three new compounds are not more active than parent molecule A23187. Are they relatively less toxic? To reply to this crucial query, all new compounds should be tested in some laboratory without paying fee of tests. Unfortunately we have limited financial support at Morocco, particularly at Oujda. *In vitro* Brine Shrimp bioassay should be carried out to study the cytotoxic properties of these new antibiotics.

In Table 3; we remark a high variations of ^{13}C chemical shifts of the carbons C1 (10 ppm), C2 (22 ppm) and C4 (4 ppm) compared to calcimycin, which may be to assigned to drastic a structural changes especially on the carboxylic function benzoxazole moiety. It's also observed for chemical shift of H4 and H5. In addition, positive variations of carbons C8 (3ppm) of the one hand and C22 (2 ppm) and C24 (2 ppm) on the other can be related to the efficient involvement of nitrogen and the pyrazole moiety in cetopyrole intramolecular interactions process. In spite of this modification of the molecular structure, the diketo acid form of antiviral/ antifungal type is now coexistent with antibacterial (NH---O) pharmacophore site which is always predominant, This will be confirmed elsewhere by the Petra/ Osiris/ Molinspiration (POM) analyses (Fig. 2).

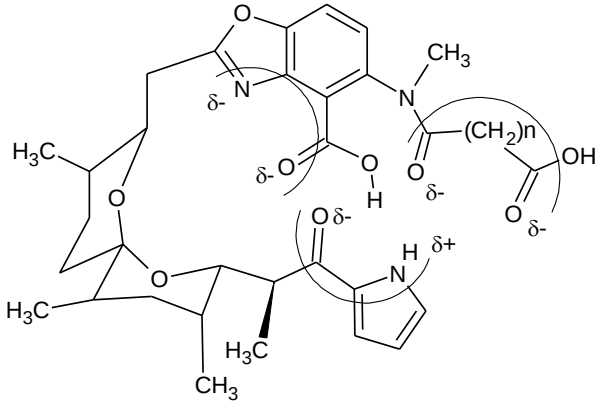


Fig. 2: Potential pharmacophore sites of new antibiotics **CD3**, **CD5** and **CD7**.

3.3. Crystalline structure

The X-ray structure of [Ni(calcimycin)$_2$] complex shows two essentially identical conformations of the two A23187 compounds in the dimeric association, in this ionophore family. In the complex, the nickel (II) cation has a distorted O_4N_2 octahedral coordination environment (Fig. 3).

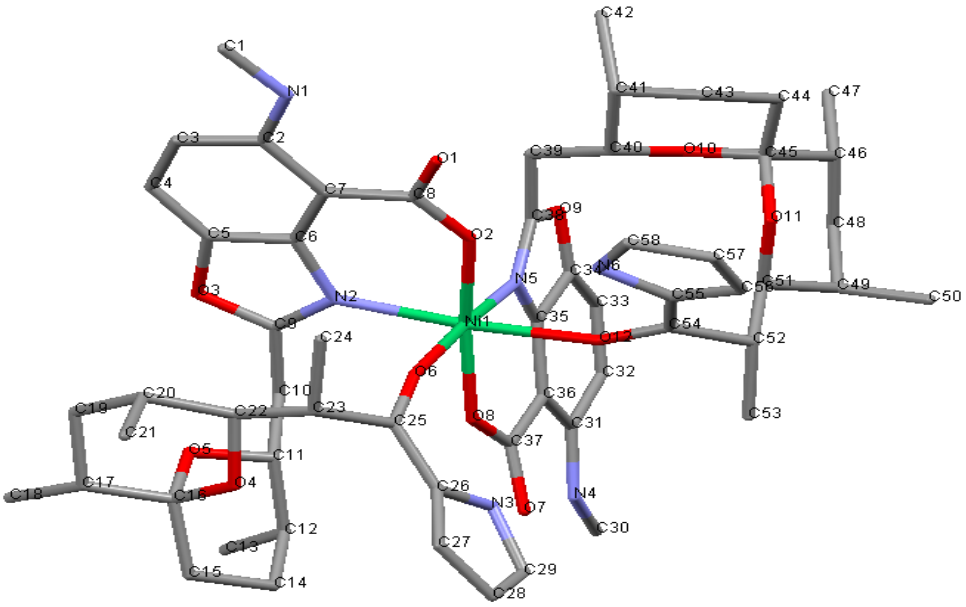


Fig. 3: ORTEP drawing of [Ni(Calcimycin)$_2$] crystal structure showing the very similar conformations in the ligands and intramolecular N—H…O bonds. H atoms have been omitted for clarity. Displacement ellipsoids are drawn at the 10% probability level [6].

This study clearly demonstrates the involvement of the carboxylate function of the benzoxazole group, the nitrogen of the pyrazole and the ketone function in complexation. For each ligand, these three sites are inevitable to have a good complexation with Nickel which has a relatively high constant for formation found in an anterior study in our laboratory [23]. Also it must be interlined that the benzoxazole ring, the carboxylic function and azole N-methyl group are in perfect planarity which positively contribution to the formation of hydrogen bonds on both sides in fact to fortify the structure.

3.4. Potentiometry

We used the zero current potentiometry for the determination of the acidity constants of caclimycine and its derivatives. The data processing is carried out by the simulation program Hyperquad which allows quick and efficient processing of suggested models.

Potentiometric assays are performed in a thermostated cell at 25 ± 0.1 °C (Haake D1) placed on a magnetic stirrer Metrohm E 649 category. Degassing is carried out systematically on research solutions by a stream of nitrogen to avoid possible oxidation. This nitrogen stream is purified by passage through several traps. In a first step, the nitrogen is passed through a container of Pyrogallol ($C_6H_{11}O_3$) 1M to scavenge oxygen. Then, bubbled in NaOH solution in methanol (1 M) to trap carbon dioxide. Finally, and before reaching the measuring cell, it passes into a vessel containing the study solvent (pure methanol). A base tetraethylammonium (Et_4NMeO) at 10^{-2} M, it is metered by perchloric acid $HClO_4$ solution in methanol [7] was used.

The potential difference read is measured via two electrodes: a glass electrode and the other saturated calomel reference. Many studies have shown that the glass electrode retains its reversibility with respect to the proton in methanol, even in a basic medium [8]. In fact, exchange between hydronium ions belonging to the glass membrane and those present in solution is not poisoned by the presence of ammonium cation ($C_4H_9N^+$) large. The two electrodes are always kept in methanol Reading potential is realized by means of a pH meter Metrohm 605 digital display and with precision of ± 0.1 mV kind. The additions of the base tetraethylammonium were performed using an automatic burette type Metrohm 605 Dosimat Multi - cylinder with a length of 20 cm^3. The galvanic cell is represented by the following electrochemical chain Hg | Hg_2Cl_2 | KClsaturé in (MeOH) | | methanolic solution | | glass electrode. Calibration of the glass electrode is systematically performed to verify the relationship of the Nernst potential E which is written:

It is said that if the electrode Nernst curve E = f (log $\mathbf{aH^+}$) gives a straight line with a RT/nF slope close to 59.16 mV. The value of E_0 the originally ordered depends on the diffusion potential and "asymmetric" potential that is unique to each electrode and can vary significantly from one experiment to another. By calculation, the conversion potential differences E pH is achieved, then we trace E = f (pH) which allows the determination of the characteristics of the electrode (slope and ordered the original E_0). An electrode is considered in good condition when it can scan an area of potential 400 mV on the one hand and it must follow the Nernst equation. An electrode is always usable so long as it does not exceed 20% of the value of the slope at 25 °C (59.16 mV). In relation Nernst activity $\mathbf{aH^+}$ can be confused with the concentration of very dilute medium. However, the activity coefficients are calculated at each point according to the Debye- Huckel theory of electrolytes applied to the 1: 1 and 2: 1 [9, 10].

$$\log\gamma_{\pm} = -\left(A\,|z_+||z_-|\,\sqrt{I}\right) / \left(1+|z_+||z_-|\,Bq\,\sqrt{I}\right)$$

z^+, z^- : charge of species; I: ionic force average≈ 2,5 $\sum_{i=0}^{i=n} Ci$ for divalent cations; A= 1,895 and Bq= 4,362 (in MeOH).

3.4.1. Choice of study solvent

The ligands investigated insolubility in water, associated with the need of a solvent which can be defined a pH scale led to choose the study of methanol as solvent. In the literature, most studies have been conducted in methanol considered the nearest to the water solvent. It is therefore quite important bibliographic data. This is a polar protic solvent in which electrolytes are separated.

3.4.2. Preparation of the base: methoxide tetraethylammonium (Et4NMeO)

Preparing the base methanolates tetraethylammonium has been cited in several books and publications we limit to name a few [9, 11].

The preparation procedure is as follows:

$$(Et)_4N^+I^- + \frac{1}{2}\,Ag_2O \xrightarrow{\mathbf{MeOH}} (Et)_4N^+MeO^- + AgI\downarrow + \frac{1}{2}\,H_2O$$

Tetraethylammonium methoxide (quaternary ammonium base) is prepared by the action of silver iodide on tetraethylammonium oxide dissolved in methanol. The reaction is carried out at 0 °C under nitrogen atmosphere for

3h. After removal of the silver iodide by filtration on sintered glass. The colorless solution obtained is assayed potentiometrically with a perchloric acid solution in methanol of known concentration perfectly.

For determining thermodynamic acidity constants, we choose a powerful program of simulation Hyperquad which is an easy program to handle. This program was developed by a group of Italian researcher from several earlier versions [12-15]. The formation constant is estimated by using the least squares method. They found a wide application in the chemical and biochemical field. For this program the systematic errors should be minimized when handling experimentally caution. These errors can arise from the calibration electrodes, the dilution phenomenon, weighing the samples, the variation of temperature in the measurement cell or the quality of methanol used.

The principle of this program is to find a model composed of a set of species that coincide on the one hand , the experimental curves and those simulated on the other hand metering convergence of the sum of squared residuals σ to 1 [15]. The refinement of formation constants is determined by a number of iterations, any time a model is acceptable when σ less than 3 is very satisfactory [14]. This is due to differences in pH between the calculated and experimental points and multiple uncertainties experience.

2.4.3. Acidity Constants

Calcimycin has two acid functions, they shall be appointed by LH_2, its derivatives have a more concerning the additive carboxylic function. The determination of acidity constants of different ligands **CDn** (n = 3, 5 and 7) synthesized is an essential study to understand the behavior of ligands in solution. This is a preliminary study of later studies on complexation equilibrium we are considering in the near futur. Equilibrium EA, EB are connected to the dissociation of the two acid functions and aliphatic benzoxazolic respectively, while the balance EC is bonded to the protonation of the nitrogen oxazole . Acidification of the solution containing the ligand $\mathbf{CD_n}$ by a strong acid $HClO_4$ in methanol allows highlighting this balance and thus brings up LH^{3+} species in the calculations. For this, several types (a, b and c) of experiments were performed, the direct assay with the base, the back titration with perchloric acid, and acidification of the ligands and the assay basis. The results show the prominent presence of 4 species LH_3^+, LH_2 , LH^- and L^{2-} following balances below. In addition, we can say that the parent compound and derivatives did not undergo any degradation from strongly acid and strongly basic vis- versa medium. Constants were measured for the following three equilibriums:

$$LH_2 \rightleftharpoons LH^- + H^+ \qquad K_{a_1}^0 = \frac{(LH^-)\,(H^+)}{(LH_2)} \qquad \text{EA}$$

$$LH^- \rightleftharpoons L^{2-} + H^+ \qquad K_{a_2}^0 = \frac{(L^{2-})\,(H^+)}{(LH^-)} \qquad \text{EB}$$

$$LH_3^+ \rightleftharpoons LH_2 + H^+ \qquad K_{a_3}^0 = \frac{(LH_2)\,(H^+)}{(LH_3^+)} \qquad \text{EC}$$

K_{ai}^0 = thermodynamic acidity constant, **(X)**: Activity of species in solution.

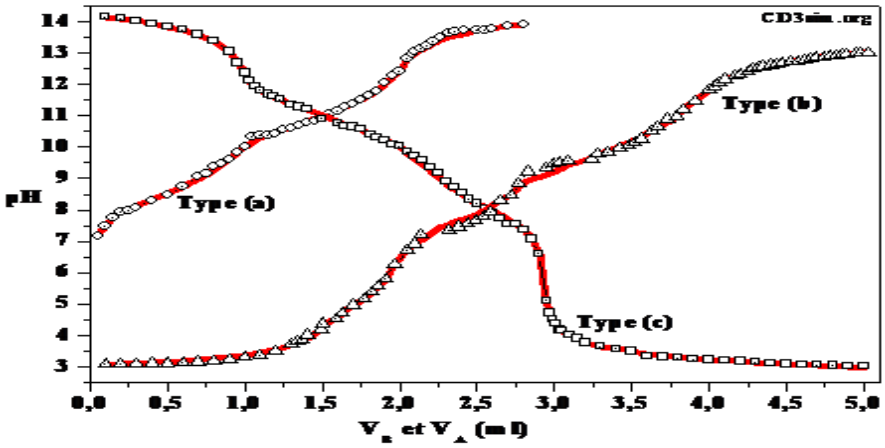


Fig. 4: potentiometric titration curves of experimental and simulated pH versus volume of base or acid ligand $\mathbf{CD_3}$.○ Dosage direct du ligand par la base: type (a); Δ Acidification of ligand and dosage by the base: type (b); □ Dosage in return by $HClO_4$ acid : type (c); (—) : Simulation.

The addition of a second aliphatic carboxylic function has a third regenerated acidity constant. The three synthesized analogs (**CD3**, **CD5** and **CD7**) behave similarly acid medium until the basic medium. In direct assays and

by the base for all the ligands **CDn** pH titration curve = f (VB) shows the presence of two jumps of pH related to the deprotonation of the two aliphatic acids and benzoxazolique functions . The monitoring of the assay by the addition of a strong acid $HClO_4$ allows protonation of the nitrogen oxazole , which explains the presence of a third pH jump on the titration curve . The embodiment of a back-titration confirms the results obtained. The acidity of the ligand sequence varies along **CD7** > **CD5** ≥ **CD3**, it is thus proportional to the elongation of the chain carboxylic acid. This can be confirmed experimentally by potentiometry VB = 0 where the initial pH varies in the same direction [pHd (**CD7**) < pHd (**CD5**) ≤ pHd (**CD3**)].

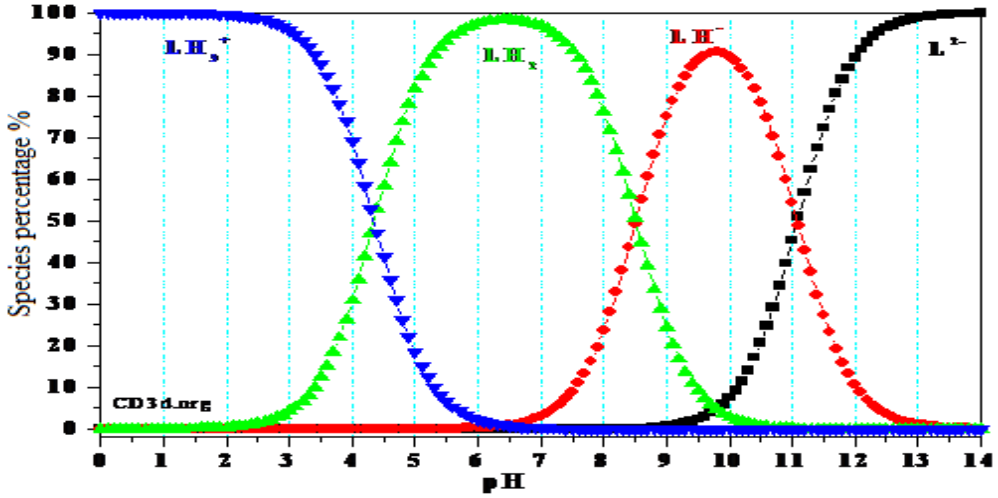


Fig. 5: Case of the **CD3** ligand**:** Distribution curve of species versus pH.

Distribution curves of the three analogues have the same look but with a slight variation in prevalence areas of each species. One can note the strong presence of the species LH_2 in the field of biological pH. Compared to new analogs, the acidity constants of calcimycin are between **CD5** and **CD7**. Indeed the oxazole nitrogen is more acid in the case of the **CD7** and less acid in the case of **CD5** and **CD3**. The A23187 benzoxazolique carboxylic function is much closer to that of **CD7** other.

Table 3. pKa values of ligands **CD3**, **CD5**, **CD7** and A 23187 determined at 25 °C and at zero ionic strength.

Compound	**pKa_3**	**pKa_2**	**pKa_1**	**σ**
A 23187	3.12 [16]	----	10.94 [16]	---
CD_3	2.78 ± 0.1	10.67 ± 0.03	8.29 ± 0.03	4.6
CD_5	3.05 ± 0.10	11.04 ± 0.05	8.41 ± 0.06	5.8
CD_7	3.87 ± 0.02	10.91 ± 0.02	8.52 ± 0.03	5.3
N-acetyl Calcimycin	---	---	8.43 [17, 18]	---
X14885A	2.34 [16]	---	8.42 [16]	---
4-bromo-Calcimycin	---	---	9.04 [19]	---
N-methyl Calcimycin	3.3 [20]	---	10.74 [20]	---
Cezomycin	< 1.5 [21]	---	8.3 [21]	---

Generally, the acidity constants of the three ligands are increasing with increasing n. If we compare the acidity constant of the pKa1 benzoxazole function we notice a drastic drop about 2.5 log units. The lengthening of the chain makes, so the carboxylic acid benzoic more this is mainly due to the amide group close to the benzoxazole fragment than the aliphatic carboxylic function quite far from inductive and mesomeric effects. This can be confirmed by the values found for the pKa of the N-acetyl calcimycin derivatives, X14885A and 4-bromo-calcimycin. These groups have a strong attracting effect which increases the acidity of the carboxylic group but with a group donor such as a methyl, the acidity decreases; it's the case of N-methyl-calcimycin. We can consider another significant effect; it is the establishment and breaking of hydrogen bond which be achieved by interaction at the two sides of benzoxazole fragment as shown in the diagram below. This is certainly the steric bulk of methyl N-acetyl groups and methyl groups responsible of the breaking hydrogen bonds.

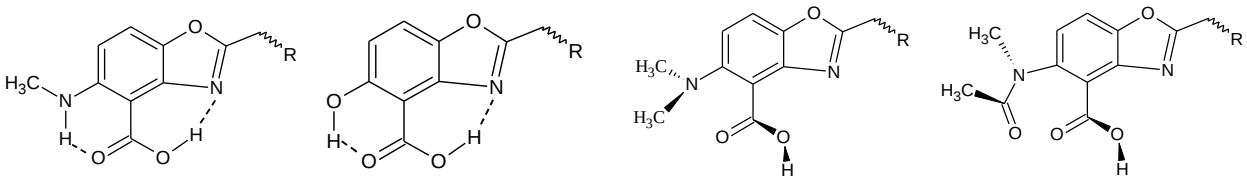


Fig. 6: Intramolecular hydrogen interraction proton with oxazolyl group.

Conclusion

The rising prevalence of multidrug-resistant microbial infections in the past few decades has become a serious health care problem. In particular, the emergence of multidrug-resistant strains of Gram-positive bacteria pathogens such as methicillin resistant Staphylococcus aureus is a problem of ever-increasing significance. Currently, we use some antibiotics discovered more than 30 years before and surprising anticancer drugs used in the 60s,in order to prevent this serious medical problem, the elaboration of the new types of the known drugs is a very critical task. The benzoxazoles constitute an important class of heterocyclic compounds exhibiting chemotherapeutic activities such as antimicrobial, antiviral, anticancer activities. In this project, we aimed to investigate synthesis, structure elucidation and antimicrobial activity of some new benzoxazoles and their analogs.

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