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Nouvelles synthesès de dérivés hetérocycliques pour applications biologiques (Céphalosporines, Coumarines et Indoles)

New synthesis of heterocyclic derivatives for biological applications (Cephalosporins, Coumarins and Indoles)

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To my daughters, parents and wife for their love and support

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SUMMARY

Semisynthetic cephalosporins, coumarins and indoles derivatives are very valuable compounds in the biochemical branch. The firsts commonly used in the world health system against bacterial infections; the second and last ones, presents in nature and life sciences with wide spectra of biological activities and uses. Due their important applications it is necessary to count with appropiated methods of synthesis in order to obtain them. Shorter reactions time with good yields and as always as possible, in a friendly environment work up, are the main aspects to shoot down the costs of the final products.

In this work we develop some improved synthetic procedures in order to obtain some cephalosporanic antibiotics of third generation, coumarins and (1-acetyl-indol-3-yl) acetates, the most part of them reducing steps and time with a sensitive increase in yields; others, introducing some heterogeneus catalysts bringing the final products up with similar yields to those from literature with not toxic waste to treat.

RESUMEN

Los derivados de las cefalosporinas semisintéticas, cumarinas e indoles son compuestos con gran utilidad en el campo de la bioquímica. Los primeros, comunmente utilizados en el sistema de salud mundial contra infecciones bacterianas, los segundos y los últimos anteriormente mencionados, presentes en la naturaleza y en las ciencias biológicas con amplio espectro de actividades biológicas y usos. Debido a las importantes aplicaciones de estos, es necesario contar con métodos de síntesis apropiados con el objetivo de obtenerlos. La disminución del tiempo de reacción, los buenos rendimientos y siempre que sea posible, un procesamiento de la reacción amigable para el medio ambiente, son los principales aspectos a tener en cuenta para disminuir el costo de los productos finales.

En éste trabajo desarrollamos algunos procedimientos de síntesis mejorados con el objetivo de obtener algunos antibióticos cefalosporánicos de tercera generación, cumarinas y acetatos de (1-acetil-indol-3-ilo). La mayoría de los compuestos fueron sintetizados reduciendo etapas y el tiempo de reacción con un sensible aumento de los rendimientos obtenidos; otros, mediante la introducción de catalizadores heterogeneos con rendimientos similares a los reportados en la literatura pero a diferencia de ésta, sin residuales tóxicos a tratar.

ABBREVIATIONS

7-ACA	7-Amino cephalosporanic acid
Ac	Acetyl
ATMA	(Z)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetic acid
Bn	Benzyl
BSA	N,O-bis(Trimethylsilyl) acetamide
CATMA	7β-[2-(2-chloroacetylamino thiazol-4-yl)-(*Z*)-2-methoxy imino acetamido]-3-acetoxy methyl-3-cephem-4-carboxylic acid
CIGB	Centro de Ingeniería Genética y Biotecnología
DCA	Dicyclohexylamine
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DMA	N,N-Dimethylacetamide
DMF	N,N-Dimethylformamide
DMSO	Dimethylsulfoxide
Et	Ethyl
EI	Electron Ionization
FDA	Food & Drug Administration
HPLC	High Performance Liquid Chromatography
HOBT	Hydroxy benzotriazole
iPE	Isopropylether
MAEM	(*Z*) -2- [2-Aminothiazol -4- yl] -2-methoxy imino acetic acid 2-mercaptobenzo thiazolyl thioester
Me	Methyl
min	Minute
m.p:	Melting Point
MS	Mass Spectra
MSA	Methansulfonic acid
NMR	Nuclear Magnetic Resonance
Ph	Phenyl
Py	Pyridine
rep.	Reported
r.t.	Room temperature
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofurane
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
Tr	Triphenyl methyl
Ts	p-Toluenesulfonyl

CHAPTER I

SYNTHESIS OF CEPHALOSPORANIC ANTIBIOTICS OF THIRD GENERATION

1. INTRODUCTION

Since some years ago the synthesis of antibiotics is a branch of pharmaceutical chemistry to which some investments have been made in order to develop new compounds with antimicrobial activity. In 1945, Brotzu discovered the fungus *Cephalosporium acremonium* which produce a substance with antimicrobial activity denominated Cephalosporin C which is the raw material in the preparation of the 7-aminocephalosporanic acid (7-ACA), starting point in the synthesis of some cephalosporanic antibiotics[1].

The cephalosporanic antibiotics are classified in generations depending of their antimicrobial action and the moment in which they had been created. In this way, cephalosporins of first generation are mainly effective against Gram (+) germs but they are susceptible to β-lactamases and at the same time they have an inadequated capacity of penetration through the bacterian walls, important aspects which reduce their antimicrobial potency.

In order to revert these drawbacks some synthetic transformations have been done to the 7-ACA, obtaining the cephalosporins of second generation as result. These products keep their activity against Gram (+) germs and some Gram (-) germs at the same time. However, due to the increasing of infections caused by Gram (-) bacteria some efforts were made directed to find wide spectra cephalosporanic antibiotics favoring the discovery and development of a third generation of this kind of compounds, among them cefotaxime sodium salt, cefpodoxime proxetil and cefdinir.[1,2,3]

1.1 SYNTHESIS OF CEFOTAXIME SODIUM SALT

1.1.1 STATE OF THE ART

Cefotaxime sodium salt was the first cephalosporanic antibiotic of third generation developed. It has an antimicrobial potency between ten and one hundred times superior against Gram (-) bacteria and similar power against Gram (+) bacteria compared with those of second generation, a wide spectra of antibacterianne activity, high resistance to β-lactamases and low secondary effects[1,4]. This product is used by parenteral way in the treatment of infections like meningitis, septicemi, peritonitis, infections of the respiratory and genito-urinary tract, skin, bones and

articulations among others[4] and it can be useful as intermediate in the synthesis of cefpodoxime proxetil[5,6].

From a structural point of view (Fig.1), the main characteristic of cefotaxime is the presence of the radical 2-aminothiazol-4-yl linked through the (methoxyimino) acetamido group to the amino function of 7-ACA. This combination conducted, in an unexpected way, to obtain a compound with better properties to those of first and second generations, especially a good activity against Gram (-) bacteria and better stability in front of ß-lactamases generated by microorganisms.[1]

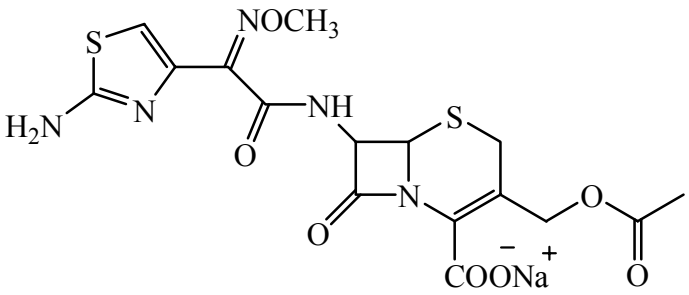
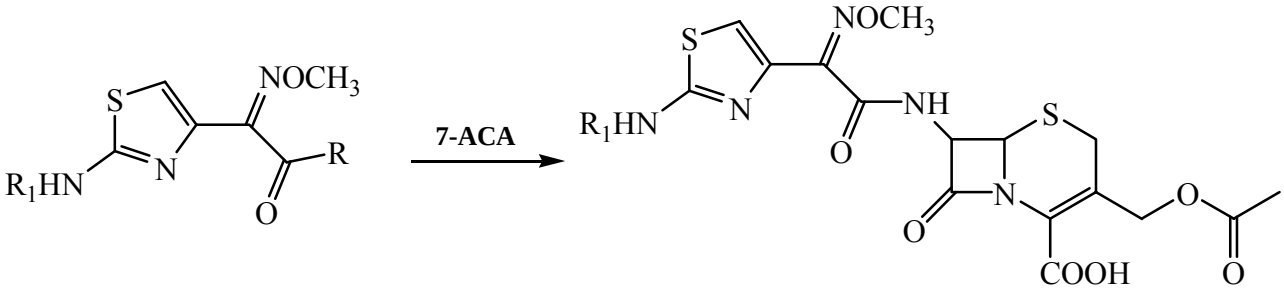


Fig.1 Cefotaxime sodium salt

1.1.1.1 *Cefotaxime acid form*

Cefotaxime could be obtained by acylation of 7-ACA with a conveniently activated derivatives of (2-aminothiazol-4-yl)-2-(methoxyimino) acetic acid (Scheme 1)[7,8,9,10,11,12]. The high reactivity of these substances makes necessary to protect the 2-aminothiazol-4-yl radical amino function in order to avoid formation of side products that could be formed due to the competition with the primary amino group of 7-ACA.[1]



R= Cl, R_1= O-Tr[7]

R= O-Isobutyl formate, R_1= Tr[8,11]

R= Cl, R_1= O-Trichloroethoxycarbonyl[9,10]

R= OH, R_1= O-Tertbutyloxycarbonyl[12]

Scheme 1

The development of new reactive derivatives of (2-aminothiazol-4-yl)-2-(methoxyimino) acetic acid, such as hydroxybenzotriazol esters (HOBT)[13],[14],[15] and thioesters[16],[17] has made possible to carry out the acylation reaction of 7-ACA in an advantageous manner without blocking the amino function of thiazolidic radical[1].

1.1.1.2 *Cefotaxime sodium salt*

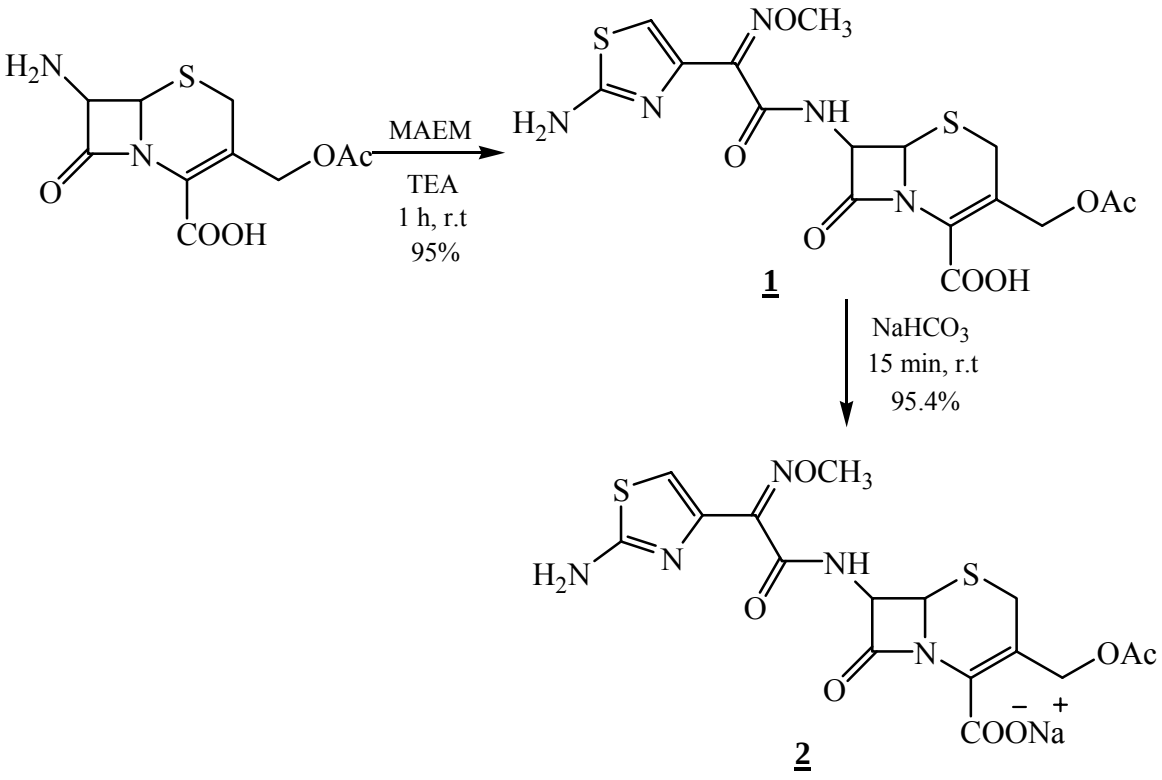
The Cefotaxime obtained by synthetic procedures is not soluble in water and should be transformed in an alcaline salt in order to be used for clinical purposes. This process is performed by neutralization with either sodium hydrogenocarbonate[18],[19] or salts of carboxylic acids of higher pKa (sodium acetate or sodium 2-ethylhexanoate) and further isolation and purification from alcoholic solutions[9].

The purpose of this part of the work is to report a new procedure for the synthesis of cefotaxime, starting from the 7-ACA and a thioester of ATMA (MAEM), a commercially available reagent that does not need preliminary modifications to react.

1.1.2 RESULTS AND DISCUSSION

The developed procedure for cefotaxime (Scheme 2) consists in the reaction between 7-ACA and MAEM in DCM as solvent and using TEA both to dissolve 7-ACA and catalyze the reaction. The process was carried out at r.t. Monitoring by TLC the advancement of the reaction, showed it was found complete within 1 h. Cefotaxime was obtained in the form of the corresponding triethylammonium salt, separated from the reaction mixture by means of simple extraction with water, while 2-mercaptobenzothiazole, obtained as by-product, remained in the organic phase. The aqueous extracts were acidified to obtain the acid form and washed with water, ethanol and diethyl ether.

In the next step, the addition of sodium hydrogen carbonate in aqueous ethanol gave the sodium salt of cefotaxime [8,20,21].



Scheme 2

A brief summary of the different methods reported for the synthesis of cefotaxime is shown in Table 1 in which the reaction conditions, the used reagents and obtained yields in each process are taken into account for comparison.

Methods C and D use acylation of 7-ACA with the acid chloride of ATMA or ATMA anhydride (methods A and B) but E, where the formyl derivative, give better yields than those mentioned before.

Because of the high reactivity of these derivatives it was necessary, in all cases, to block the ATMA amino function to avoid the formation of side reactions. Although the Scheme 1 acylation reaction was fast (1-2 h), the final deprotection required much time, and decreased the yield below 65%. In the last four procedures F-I, ATMA esters and/or active amides were used as reagents. The lower reactivity of these derivatives made possible to carry out the process without blocking the ATMA amino group and undesirable side reactions were less probable.

As a consequence, the final yield is higher even more than 90% in case of methods G-I; the time required is shorter than that necessary for other methods, despite a slower reaction rate.

Table 1. Comparative results between the literature methods (A-I) and the one developed in this work (J).

Method [Ref]	Reactants	t (h)	T (°C)	Yield (%)
A[9,13]	*t*-Butyl ester of 7-ACA, acyl chloride of CATMA, DCM, pyridine, thiourea, anisole, TFA.	16	25	31
B[8]	7-ACA, tritylated derivative of ATMA, DCC, TEA, dichlorometane, formic acid.	3	20	65
C[10]	7-ACA, acid chloride CATMA, DCM, TEA, thiourea.	8	20	54
D[14]	7-ACA, acid chloride of CATMA, TEA, thiourea tetrahydrofuran.	16	25	24
E[21]	7-ACA, formyl derivative of ATMA, diphenyl phosphite, pyridine, dioxane.	2	25	72
F[15]	7-ACA, active ester of ATMA, DCM, tetrahydrofuran, TEA.	16	25	69
G[16]	7-ACA, active ester of ATMA, acetonitrile, sodium hydrogen carbonate.	6	25	93
H[19]	7-ACA, active amide of ATMA, acetonitrile, sodium hydrogen carbonate.	4	25	95
I[19]	7-ACA, MAEM, BSA.	15	25	92
J[22]	7-ACA, MAEM, DCM, TEA.	1	25	95

Two main advantages of the proposed method (J) are, that acylation takes place in 1 h and protection of the ATMA amino function is no more necessary and as result, a higher yield is obtained with a shorter reaction time (1 h). Moreover, an additional advantage of method J is the use of MAEM, a commercial reagent, as opposed to the hydroxybenzotriazole esters and/or amides of ATMA. In the procedure I, where MAEM was also used, a catalyst (BSA) was necessary and, despite this, the reaction time was a longer than that necessary for the method J. Finally the proposed method allowed the recovery of 2-mercaptobenzothiazole (**3**), which is widely used in the chemical industry[23], with a high degree of purity and high yield, constituting an additional advantage of this method.

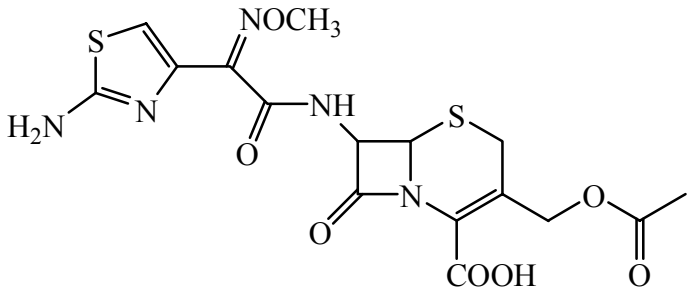
1.1.3 EXPERIMENTAL PART

General methods

TLC analyses were performed on precoated silica gel Merck GF-254 plates. The spots were visualized in a UV CAMAG lamp at λ=254 nm. ^{1}H NMR spectra were recorded at 250 MHz in a Bruker BHZ AC 250F using DMSO-d_6 as solvent and TMS as internal standard. Chemical shifts are expressed in ppm. MS were recorded in a quadrupolar mass spectrometer TRIO 1000 (Fisons Instrument) based on the electronic impact technique with EI=70 eV and DMK 400 V. pH measurements were carried out in aqueous solution on 10% w/v at 25°C in a Crison micropH 2001. Melting points were determined in a Gallenkamp apparatus and are uncorrected. The HPLC techniques were carried out in a Merck Hitachi, LaChrom model, performed by a pump L7100, a UV detector model L-7400 and an injector with a loop of 20 mL, using the Biochrom software (CIGB, Cuba). The stationary phase was a LiChrosorb RP-18 (5 mm; Merck) column of 250 x 4 mm coupled with a pre-column RP-18 (Merck). As mobile phase was used a mixture of methanol-water-acetic acid (30:70:0.1, v:v) adjusted to pH=3.40 with glacial acetic acid. The work-flow was 0.75 mL min^{-1}. Detection was made at λ=254 nm. Prepared cefotaxime sodium salt was compared with an authentic sample.

1.1.3.1 *Preparation of cefotaxime acid form*

***3-Acetoxymethyl-7-[2-(2-amino-thiazol-4-yl)-2-methoxyimino-acetylamino]-8-oxo-5-thia-1-aza-bicyclo [4.2.0] oct-2-ene-2-carboxylic acid. (Cefotaxime free acid form)* (1)**



A suspension of 7-ACA 62.9 g (231 mmol) in 755 mL of DCM was chilled to 5-10°C and 71.0 mL (513 mmol) of TEA were added with stirring. The mixture was heated to r.t. and 89.6 g (256 mmol) of MAEM was added. The resulting mixture was stirred for 1 h. After that the mixture was

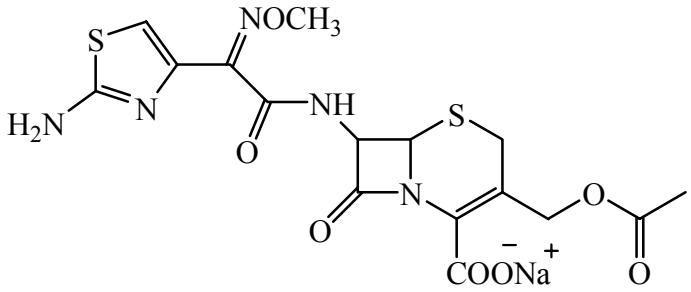
extracted twice with 320 and 160 mL of water. The combined aqueous extracts were adjusted to pH= 2.9 by adding 47 mL of 6 M hydrochloric acid with continuous stirring. The suspension was chilled to 0-5°C, the precipitate was isolated by vacuum filtration and washed successively with water (60 mL), ethanol (60 mL) and ethyl ether (2 x 80 mL). The solid was dried for 4 h at 40°C, affording 100 g (95%) of **1**.

TLC: Ethyl acetate-ethanol-water-formic acid (65:25:15:1 v:v).
m.p: 205°C (decomp).
^{1}H NMR (DMSO-d_6): 1.99 (s, 3H, $OCOCH_3$), 3.23 and 3.45 (Abq, 1H, H-2), 3.84 (s, 3H, $NOCH_3$), 4.76 and 4.98 (Abq, 1H, CH_2O), 5.01 (d, 1H, H-6), 5.6 (dd, 1H, H-7), 6.73 (s, 1H, thiazol), 7.28 (s, 2H, NH_2), 9.61 (d, 1H, NHCO).

^{13}C NMR (DMSO-d_6): 25.37 (C1), 112.17 (C2), 64.50 (C3), 134.87 (C4), 163.06 (C5), 57.34 (C-6), 58.07 (C7), 164.00 (C8), 162.21 (C9), 149.06 (C10), 61.88 (C11), 142.57 (C12), 109.02 (C13), 168.47 (C14), 170.55 (C15), 20.74 (C16).

1.1.3.2 *Preparation of cefotaxime sodium salt*

***Sodium 3-acetoxymethyl-7-[2-(2-amino-thiazol-4-yl)-2-methoxyimino-acetylamino]-8-oxo-5-thia -1-aza-bicyclo [4.2.0] oct-2-ene-2-carboxylate* (2)**



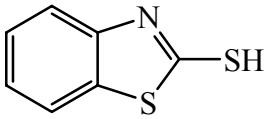
To a suspension of 50.0 g (110 mmol) of **1** in a mixture of 110 mL of water and 90 mL of ethanol was added 8.78 g (105 mmol) sodium hydrogen carbonate suspended in 25 mL of ethanol. The resulting solution was treated with 5 g of activated charcoal and stirred during 15 min. The mixture was vacuum filtered, the residue was washed successively with 250 mL of ethanol and 100 mL of water and filtrates were combined and evaporated to dryness. The residue was dissolved in 110 mL of methanol and poured into 2.2 L of diethyl ether under stirring. The

precipitate was filtered, washed with diethyl ether (2 x 50 mL) and vacuum dried during 3-4 h at 35-40 °C affording 50 g (95.4% yield) of **2**.

TLC: Ethyl acetate-ethanol-water-formic acid (65:25:15:1, v:v)[24].
pH (solution 10% w:v): 5.0 [17], HPLC: purity: 98%, retention time: 6.42 min [25].
^{1}H-NMR (DMSO-d_6): 9.61 (d, 1H, NH), 7.25 (s, 2H, NH_2), 6.75 (s, 1H, thiazole ring), 5.80 (dd, 1H, H-7), 5.16 (d, 1H, H-6), 5.0 (d, 2H, CH_2O), 4.70 (d, 2H, H-2), 3.86 (s, 3H, OCH_3), 2.07 (s, 3H, CH_3COO) [26].

1.1.3.3 *Recovery of 2-mercaptobenzothiazole*

***Procedure for the recovery of benzothiazole-2-thiol (2-mercaptobenzothiazole)* (3).**



The residual organic layer obtained, after extraction of cefotaxime with water, was washed with 140 mL of 2 M NaOH solution, the aqueous layer was then acidified with 43 mL of 6 M hydrochloric acid. The precipitate was filtered, washed with water (3 x 50 mL) and dried during 1 h at 100°C. Yield 34.6 g of **3**, (89.6%).

TLC: Ethyl acetate-*n*-hexane (1:1, v:v).
m.p: 176–178°C m.p:$_{lit}$: 174–178°C[27].
MS *m / z* 167 [M^+], 140, 122, 108, 95, 82, 76, 69, 63, 59, 50, 45, 38 and 32 [25].

1.1.4 CONCLUSIONS

Cefotaxime sodium salt has been synthesized in a two steps procedure from commercially available reagents. The reaction time has been reduced by lowering the time for acylation. The overall yield has been increased to 91% with good. Recovery of the byproduct (mercaptobenzothiazole)[22] was achieved to allow a greener process.

1.2 SYNTHESIS OF CEFPODOXIME PROXETIL

1.2.1 STATE OF THE ART

Cefpodoxime proxetil (Fig.2), developed during the eighties, is a prodrug, not active *in vitro*, with a wide activity spectra against Gram (-) bacteria and stable against the hydrolityc action of β-lactamases[28]. This product was approved by the FDA in 1992 and it is used in the treatment of pneumonia, pharyngitis and/or tonsilitis, uncomplicated gonorrhea and infections of the urinary tract. In children it is recomended in the theraphy of otitis, pharyngitis and amigdalitis[4]. This antibiotic is obtained from 7-ACA through of several steps of synthesis. Its structure is characterized by the presence of a methoxymethyl group linked to position three of the cephalosporanic nucleus as well as a fragment of 1-(isopropoxycarbonyloxy) ethyl attached to the acid function of the six member ring. Both of them are characteristics associate to its high oral absorption. Moreover, the 2-aminothiazole ring linked to position 7β by means of a (methoxyimino) acetamido system gives a high antimicrobial potency against Gram (-) germs and high resistance against β-lactamases. In this case, it is necessary to built three fundamental molecular fragments which should be coupled each other and together, so several strategies of synthesis have been made in order to obtain them.

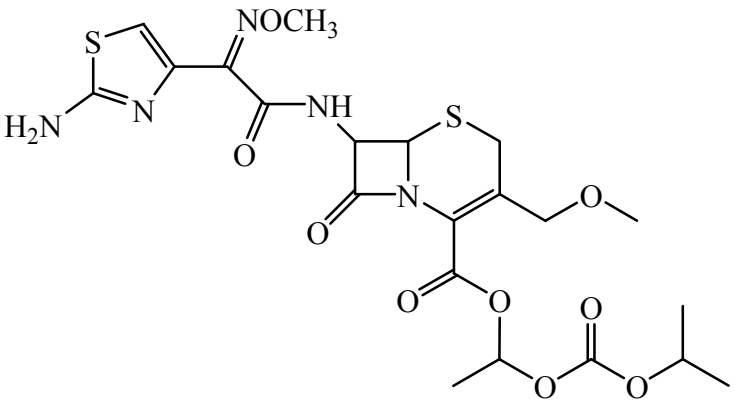
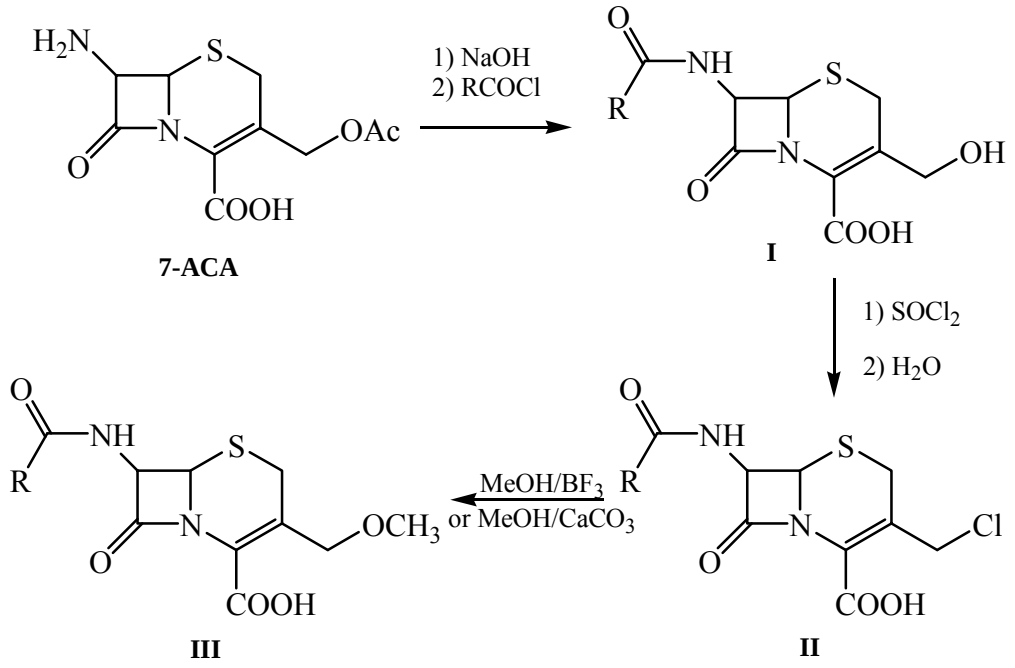


Fig.2 Cefpodoxime proxetil

1.2.1.1 *Procedures for obtaining the modified cephalosporanic nucleus (3-methoxymethylcephem)*

The first method[29] (Scheme 3) carries out the alkaline hydrolisys of 7-ACA with NaOH and subsequent acylation of with either phenylacetyl chloride, phenoxyacetlyl chloride or bencyloxyacetyl chloride to obtain **I**. This compound is halogenated with tionyl chloride using

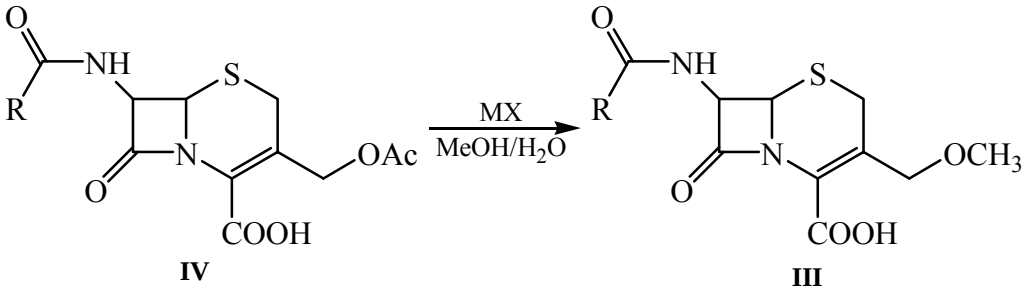
pyridine as base to yield **II** which is submitted to methanolysis in presence of either boron trifluoride or calcium carbonate to prepare **III**.



R = PhAc or PhOAc or BnOAc

Scheme 3

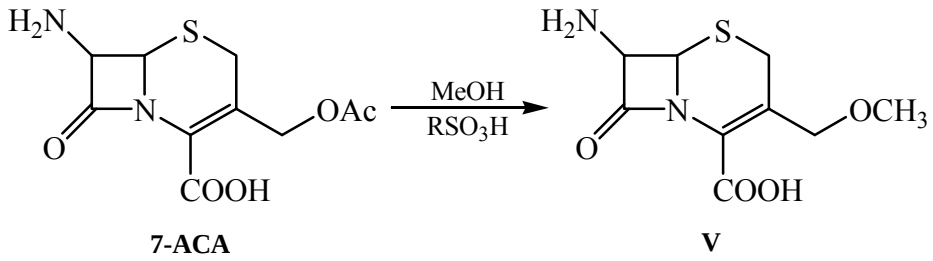
The second procedure[5,30] (Scheme 4) is based on the direct replacement of the acetoxy group by nucleophils. In this case, the amino protected 7-ACA derivative **IV** is treated with a salt of alkaline or earth-alkaline metal in aquous methanol at temperature next to 70 °C. The best yields are obtained by using calcium chloride in combination with a derivative of **IV** who has an acyl group with an electrowithdrawing group in the α position[31].



MX = alkaline or alkaline-earth salts

Scheme 4

Another method for synthesizing the modified nucleus with the non protected amino function is showed in the Scheme 5[32]. This process takes place by reaction of 7-ACA with stoichiometric quantities of methanol in presence of a sulfonic acid, particularly, the methanosulfonic acid.



7-ACA **V**

R = Me, Ph

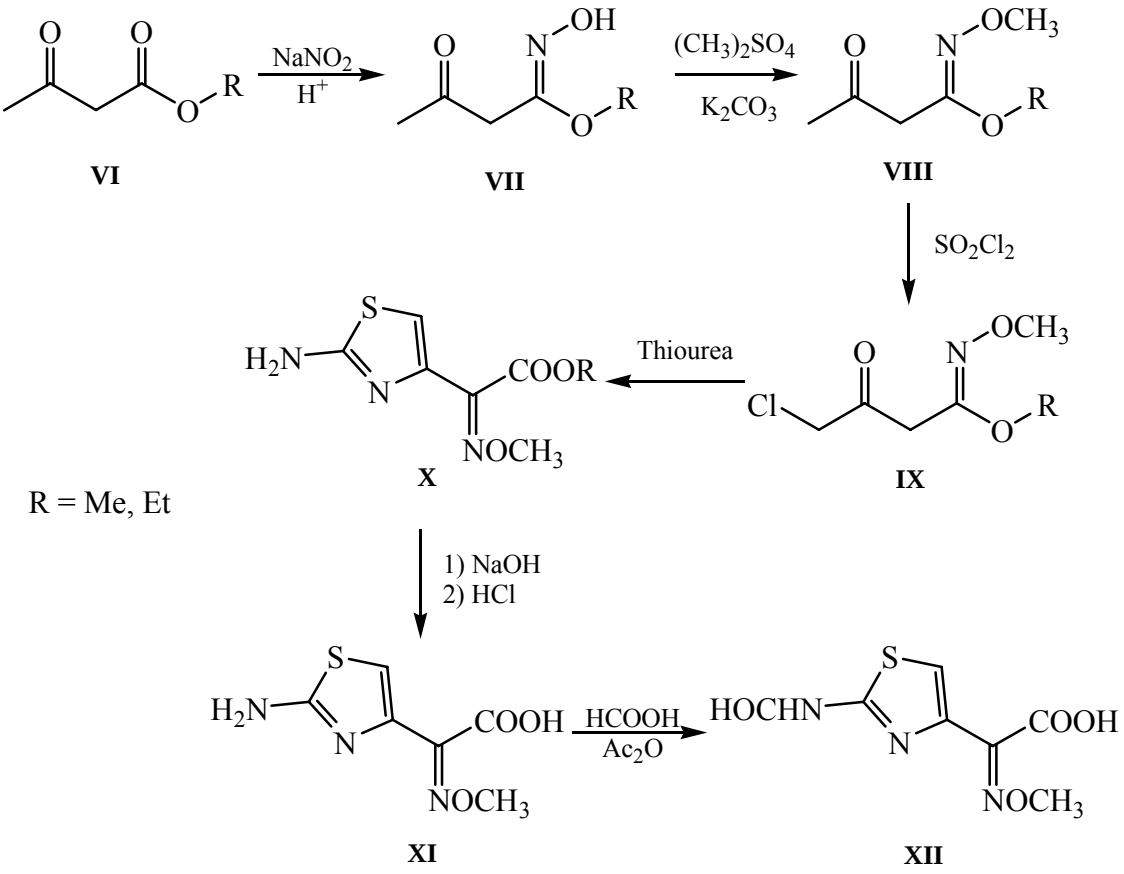
Scheme 5

A comparative analysis shows to have the procedures refered in the schemes 2 and 3 as the more advantageous. In the case of the second procedure (Scheme 3), transformation of **IV** into **III** gave maximum yields up to 65 %. Its main disadvantage was the need of deprotecting the amino group introduced at the begining in order to protect the amino function of 7-ACA. On the other hand, the third method gave **V** in a direct way with yields up to 54 %, although sulfonic acids are quite expensive.

1.2.1.2 *Procedures for preparing the 2-(2-aminothiazol-4-yl)-2-(Z)-methoxyimino acetic acid group*.

This acid was coupled to the modified cephalosporanic nucleus by means of acylation of the amino group of 7-ACA with a derivative of the 2-(2-amino-thiazol-4-yl)-2-(Z)-methoxyimino acetic acid. The common procedure is showed in scheme 6[33].

In the first step of the process, either ethyl or methy acetoacetate (**VI**) is treated with sodium nitrite in acetic acid or sulfuric acid to obtain **VII**. Subsequent methylation with dimethyl sulphate gives the methoxyimino derivative **VIII**. Halogenation with sulfuryl chloride in acetic acid yield **IX** which reacts with thiourea in presence of sodium acetate as base in a Hantzsch's cyclization reaction[34]. Hydrolysis of the resulting ester in alkaline media and acidification with hydrochloric acid yield the target compound **XI** [33]. In order to avoid side reactions in the step of coupling **XII** to the modified cephalosporanic nucleus the amino group is protected by formylation with a mixture of formic acid-acetic anhydride [33].



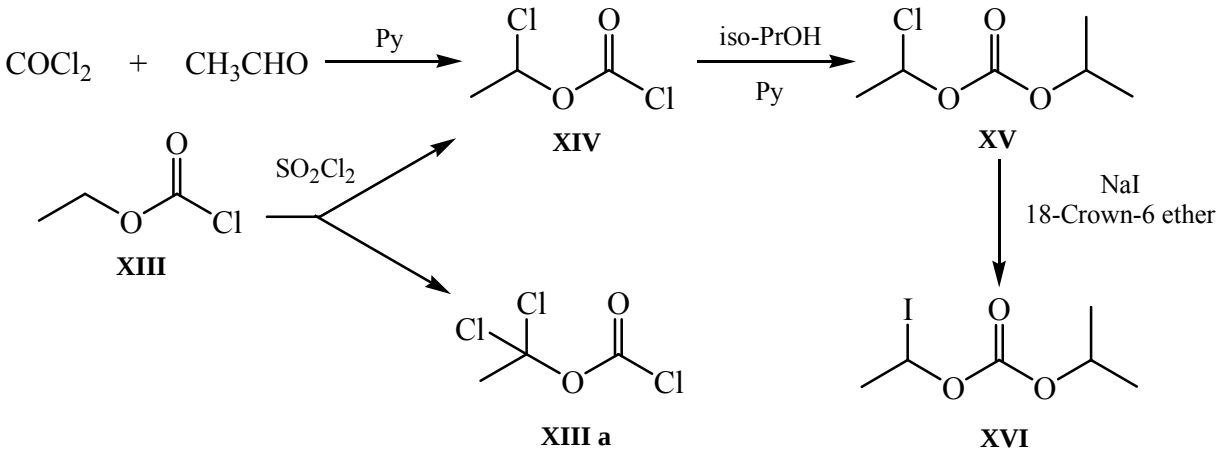
VI **VII** **VIII** **IX** **X** **XI** **XII**

Scheme 6

The 1-iodo ethyl isopropyl carbonate is the entity used in order to obtain the corresponding ester by coupling to the cephalosporin nucleus. The ways to prepare this intermediary are showed in scheme 6.

1.2.1.3 *Procedures for obtaining the iodo ethyl (isopropoxycarbonyloxy) group*.

The usually used way started from ethyl chloroformate (**XIII**) with subsequent steps of halogenation and alcoholysis with isopropanol and pyridin as base to obtain **XV**. The final step of the process carry out the halogen exchange with sodium iodide in benzene and 18-crown-6 ether as catalyst[5,31] (Scheme 7). As a disadvantage of this process considerable quantities of trichloro derivative (**XIII a**) is formed giving low yields of **XIV**. For that reason the alternative way starting from phosgene and acetaldehyde is used, obtaining high yields of **XIV** without formation of the trichloride derivative. Nevertheless, due to the toxicity of phosgene, its use is a limiting factor for the process[31].

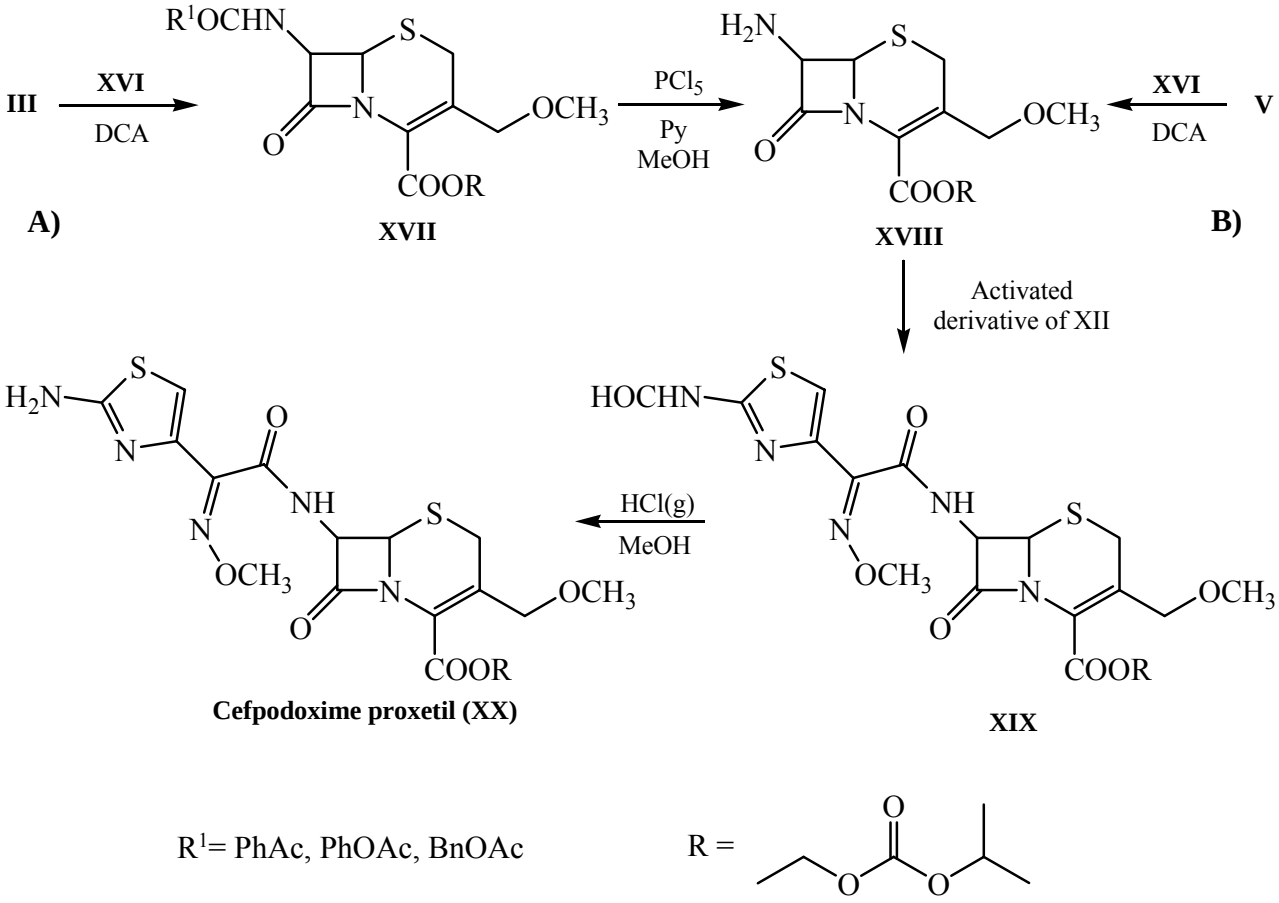


Scheme 7

1.2.1.4 *Procedures for condensing the 2-(2-aminothiazol-4-yl)-2-methoxyimino acetylamino acetic acid and ethyl (isopropoxycarbonyloxy) groups to the modified cephalosporanic nucleus (3-methoxymethylcephem).*

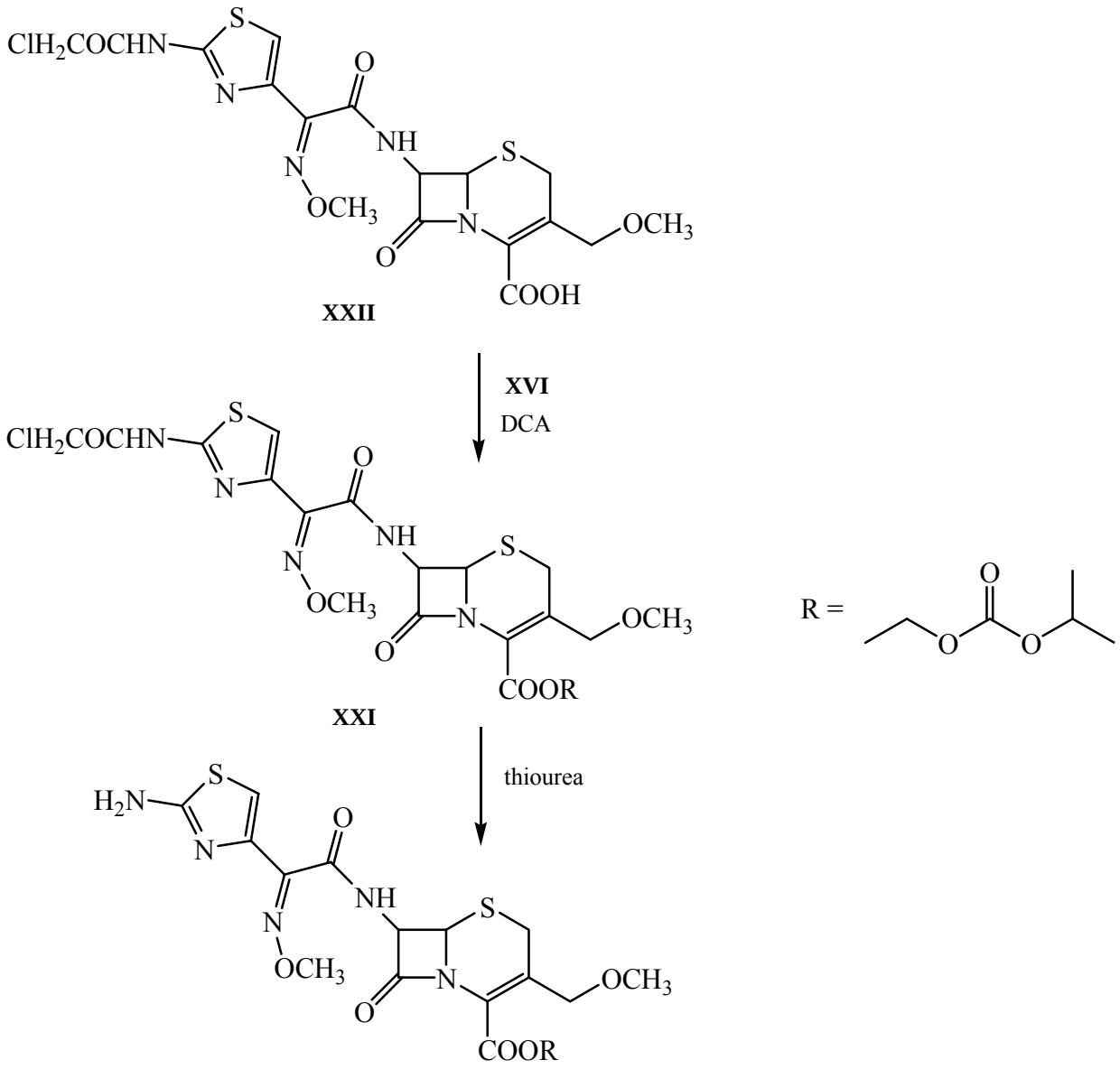
The condensation of the different fragments to the central cephalosporanic nucleus has been done by different ways. In the first procedure[31] (Scheme 8, pathway **A**), derivative **III** was condensed with the alkyl iodide (**XVI**) in presence of dicyclohexylamine to produce **XVII**. The amino cephalosporanic protecting group in position 7 was cleaved by the iminochloride method[35]. The obtained product (**XVIII**) was acylated with an active derivative of **XII**. Subsequent deprotection of the amino group yield cefpodoxime proxetil[1].

From a practical point of view a second method (Scheme 8, pathway **B**) gave best results[36,37]. It performed the modifications with the derivative **V** as raw material without protecting the amino group of the cephalosporanic nucleus and non iminochloride method is needed.



Scheme 8

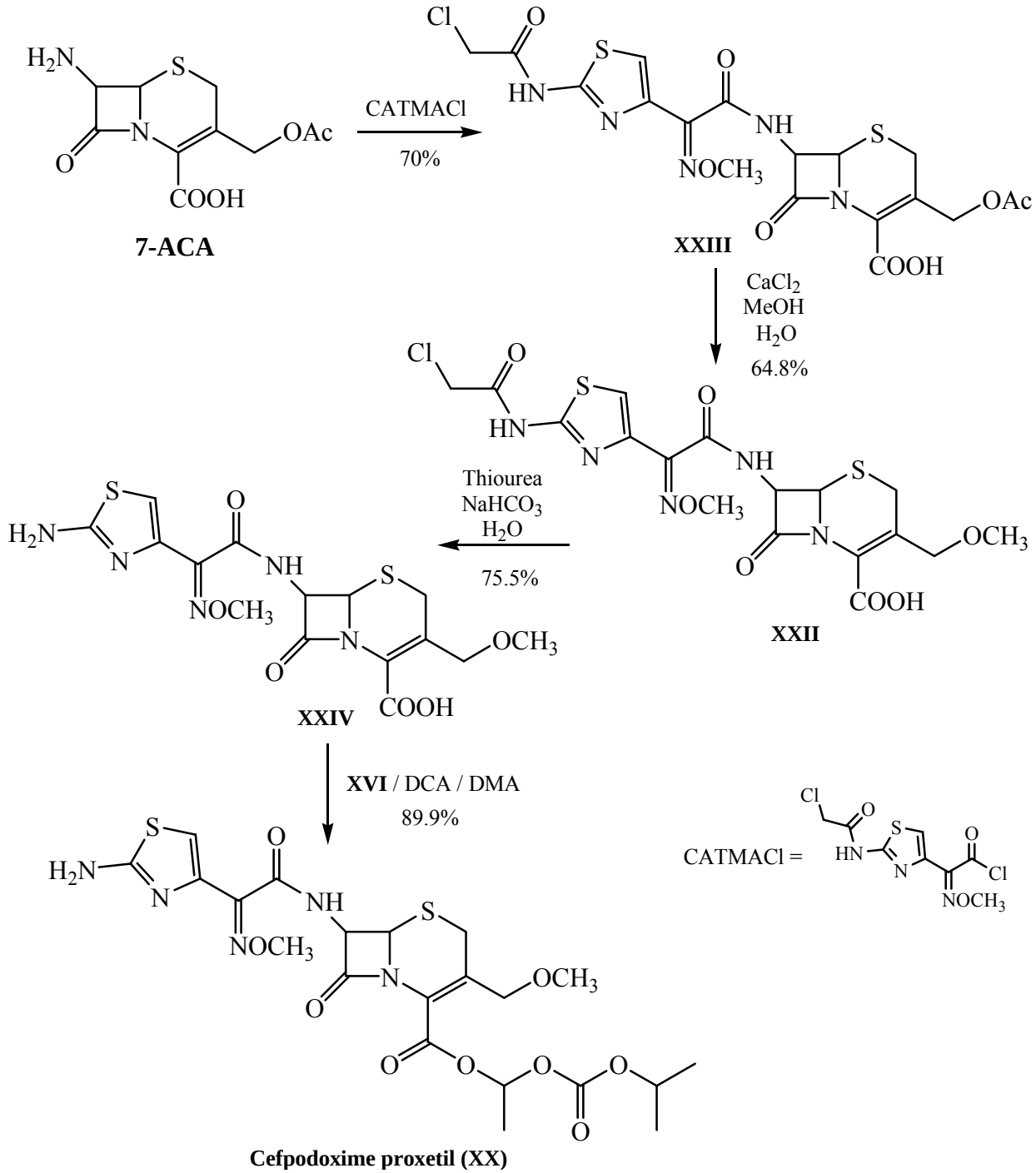
Another strategy for obtaining cefpodoxime proxetil could be considered as a particular case of the first procedure[5,31]. This way starts from the derivative **XXII** in which has been already attached the radical 2-(2-aminothiazol-4-yl)-2-(Z)-methoxyimino acetyl to the 7 position of the cephalosporanic nucleus. This compound (**XXII**) is esterified with **XVI** in presence of DCA to obtain **XXI** which is treated with thiourea yielding the cefpodoxime proxetil (Scheme 9). Moreover, it have been demonstrated that the inversion of these sequences does not affect the final yield.



Cefpodoxime proxetil (XX)

Scheme 9

The best current synthetic method to prepare cefpodoxime proxetil is outlined in Scheme 10. The 3-acetoxymethyl derivative **XXIII**, prepared by acylation of 7-aminocephalosporanic acid (7-ACA) with the acid chloride of (Z)-2-(2-chloroacetamido-4-thiazolyl)-2-(methoxyimino)acetic acid (CATMA), was treated with aqueous methanol in the presence of calcium chloride to give the corresponding 3-methoxymethyl derivative **XXII**. The chloroacetyl group of **XXII** was removed by treatment with thiourea in aqueous solution to afford compound **XXIV**. Finally, esterification of **XXIV** with 1-iodoethyl isopropyl carbonate (**XVI**) gaves the corresponding ester **XX** (cefpodoxime proxetil)[5,30,36,37,39] in good yields.



Scheme 10

1.2.2 RESULTS AND DISCUSSION

The better reported synthetic pathway [5,30,36,37,39] allows to get good overall yields (ca. 31% from 7-ACA) but it has some drawbacks:

a) The use of CATMA to introduce the (Z)-2-(2-aminothiazol-4-yl)(methoxyimino)acetamido moiety linked to the C-7 position increases the cost of the final product (cefpodoxime proxetil), though CATMA is a commercial raw material used in cephalosporin chemistry, it has a high price on the market (similar to that of 7-ACA).

b) It is necessary to prepare the acylating agent (the acid chloride of CATMA) by reaction of CATMA with phosphorous pentachloride or thionyl chloride, and it is known that acyl chlorides are difficult to be purified because they are water sensitive.

c) The yield of 7-ACA acylation reaction with the acid chloride of CATMA is relatively low (70%). As a consequence, the yield of cefpodoxime proxetil and the production cost of this antibiotic become negatively affected. Additionally, the acylation with the acid chloride of CATMA must be carried out at low temperatures (≈ -20 °C), an important handicap from a technological point of view.

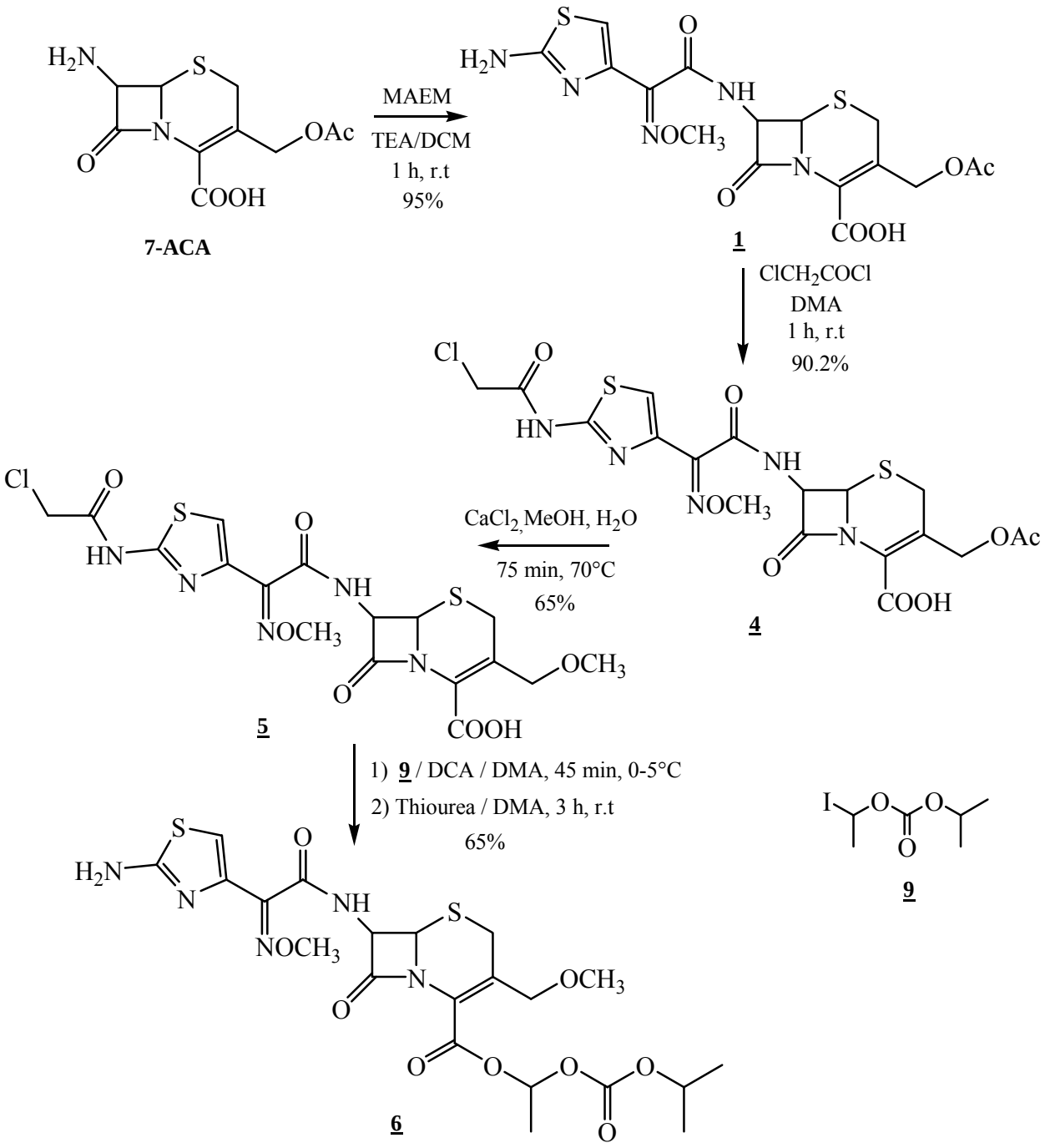
d) Removal of the chloroacetyl group in aqueous solution causes a lot of troubles, especially the absolute need of purifying the obtained cefpodoxime proxetil by column chromatography when the procedure is scaled up.

In order to overcome the drawbacks of the reported procedures we propose a synthetic methodology for obtaining cefpodoxime proxetil (Scheme 11). In this method, the CATMA used during the acylation of 7-ACA was replaced by S-benzothiazol-2-yl (2-amino-4-thiazolyl) (methoxyimino) thioacetate (MAEM, also a commercial raw material used in cephalosporin chemistry) and cefotaxime (**1**) was obtained in very good yield (95%) through a rapid and efficient procedure previously developed in our laboratory[22]. In the following step, it was necessary to block the free amino group of **1** in order to carry out further replacement of acetoxy group linked to C-3 position by the methoxy group without side reactions.

After evaluating some options, it was decided to effect the protection of amino function with a chloroacetyl group. With this objective, a rapid and efficient procedure was developed for chloroacetylation of **1** by treatment with chloroacetyl chloride in DMA.

The chloroacetylated derivative **4** was obtained with high purity and over 90% yields. Although it was necessary to introduce an additional synthetic step, compound **4** was prepared in

85.5% yield calculated from 7-ACA (Scheme.11), that is to say, a yield 15.5% higher than that of the previously reported procedures[5,30,36,37,39].



Scheme 11

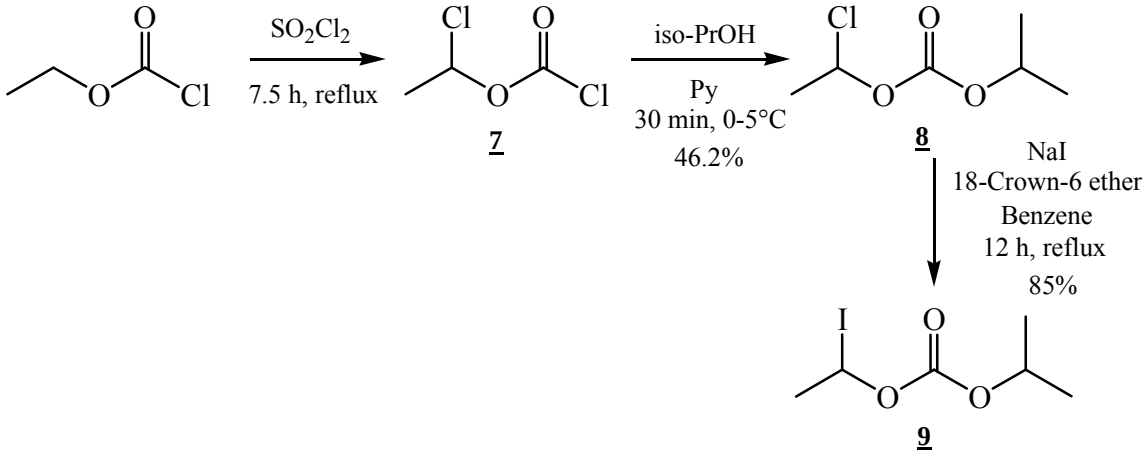
It is known that in cephalosporin synthesis, the cost of the final product (in this case cefpodoxime proxetil) depends almost exclusively on the mass relationship between 7-ACA and the final product, because the 7-ACA price is many times higher than the price of the auxiliary

reagents used during the synthetic procedures. Thus, whereas less 7-ACA quantity is needed to obtain a mass unity of the final product, more economic is the synthetic method.

The use of MAEM in place of CATMA during the acylation step provided an additional economic benefit, because MAEM price is two or three times less than the price of CATMA. From the technological point of view, using of MAEM to prepare cefpodoxime proxetil improves the synthetic process because it is not necessary to obtain and purify the acylating agent, and the acylation step can be carried out at r.t., thus avoiding the necessity of using low temperatures (as in the case of CATMA).

Following the developed synthetic procedure[38], in the next step the 3-methoxymethyl derivative **5** was obtained from **4** in 65% yield by reaction with aqueous methanol in the presence of calcium chloride as catalyst, according to the method reported in the literature[30,40].

The 1-iodoethyl isopropyl carbonate (**9**) was synthesized from ethyl chloroformate through the following sequence of reactions (Scheme 12).



Scheme 12

Radical chlorination with sulfuryl chloride, alcoholysis of the 1-chloroethyl chloroformate formed with isopropyl alcohol to obtain **8** and final halogen exchange by treatment of **8** with sodium iodide in the presence of a catalytic amount of 18-crown-6 ether. The resulting yield was the same as reported and no changes were introduced to the described method[39,40].

Another objective of this work was to overcome the problems found during the elimination of the chloroacetyl protective group of compound **5**. In the reported procedure[39,40], this process was

effected before the esterification of the acid function with **9**. Although relatively high yields are reported (75.5%), we found that it is very difficult to isolate the resulting compound **6**.

During the purification procedure, it is necessary to dissolve **6** by treatment with concentrated hydrochloric acid in order to remove reaction by-products. Further addition of sodium hydrogen carbonate (to precipitate **6**) produces a vigorous evolution of CO_2 and the formation of a gummy precipitate difficult to handle. In consequence, poor yields were obtained and at the end of the process an additional purification step by column chromatography was necessary to obtain cefpodoxime proxetil with the suitable purity.

In the present work[38], the reaction sequence was reversed and compound **5** was esterified by treatment with **9** in DMA in the presence of DCA. The low polarity of the resulting ester allowed the extraction of this compound from reaction mixture with ethyl acetate and the elimination of the impurities (dicyclohexylammonium iodide and dicyclohexylamine in excess) by washing with water and diluted hydrochloric acid, respectively.

In consequence, it was not necessary to isolate the ester and after removing the organic solvent, it was possible to effect the cleavage of the chloroacetyl protective group by reaction with thiourea in DMA. The use of an organic solvent to effect the deprotection allowed to overcome all the problems found when we carry out this reaction in aqueous solution (as reported in the literature).

The cefpodoxime proxetil formed was extracted from the reaction mixture with ethyl acetate and after evaporation of the solvent; the residue was crystallized from isopropyl ether to give the pure antibiotic as was demonstrated by ^{1}H NMR and ^{13}C NMR spectroscopy. This result made an additional purification of the product by column chromatography unnecessarily, which was an advantage in comparison with the reported method. On the other hand, the overall yield calculated from 7-ACA was 36.2%. It means that from 100 g of 7-ACA, it was possible to obtain 11 g more of cefpodoxime proxetil in comparison with the synthetic pathway reported in the literature consulted[5,30,36,37,39].

It is also possible to affirm that the different procedure used in the first step (synthesis of compound **1** from 7-ACA) and the reversing of the reaction sequence (synthesis of compound **6**

from **5**) improved the process for the preparation of this antibiotic, both from the economic and technological point of view.

Another advantage that results from the present research is the possibility to use the same raw materials (7-ACA and MAEM) in the synthesis of both antibiotics (cefotaxime (**1**) and cefpodoxime proxetil (**6**)).

1.2.3 EXPERIMENTAL PART

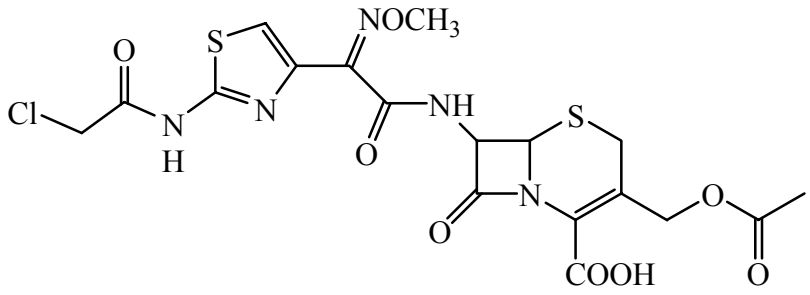
1.2.3.1 *Preparation of the modified cephalosporanic nucleus 3-methoxymethylcephem including the 2-(2-aminothiazol-4-yl)-2-(Z) methoxyimino acetyl group*.

General methods

Melting points were determined using the Gallenkamp capillary apparatus with a system of measurement and temperature control. ^{1}H NMR and ^{13}C NMR spectra were recorded at 250 and 62.5 MHz on a Bruker AC 250F spectrometer, using either deuterated dimethylsulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$) as solvent and tetramethylsilane (TMS) as an internal standard. The MS were recorded in a quadrupolar mass spectrometer TRIO 1000 (Fisons Instruments) based on the electronic impact technique with EI = 70 eV and DMK 400 V. Thin-layer chromatography (TLC) was performed on pre-coated plates of silica gel GF-254 (Merck). The chromatograms were visualized in a Camag UV-Vis lamp with a wavelength of 254 nm. The synthesis of each compound was confirmed by comparison of the recorded ^{1}H NMR spectra with the ^{1}H NMR data reported in the literature consulted[40].

The first step in the synthesis of the cephalosporanic nucleus of cefpodoxime proxetil was carried out from the 7β-[2-(2-Aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid) (cefotaxime acid form) (**1**).

***3-Acetoxymethyl-7-{2-[2-(2-chloro-acetylamino)-thiazol-4-yl]-2-methoxyimino-acetylamino} -8-oxo-5-thia-1-aza-bicyclo [4.2.0] oct-2-ene-2-carboxylic acid* (4)**.

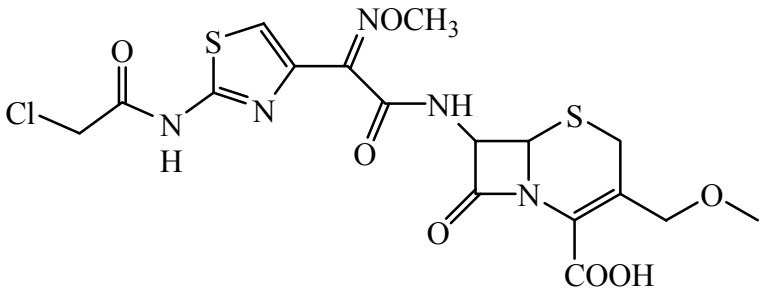


To a solution of **1** (58.5 g, 128 mmol) in DMA (295 mL), chloroacetyl chloride (15.4 mL, 193 mmol) was added, keeping the temperature between 5 and 10 °C. The mixture was stirred for 1 h at r.t. and then poured into ice water. The resulting precipitate was collected by filtration and washed successively with water (30 mL), ethanol (30 mL), diethyl ether (2 x 30 mL) and dried to obtain **4** (61.6 g, 90.2%).

TLC: Ethyl acetate-ethanol-water-formic acid (65:25:15:1, v:v).
m.p: 177-178 °C.
^{1}H-NMR (DMSO-d_6): 12.95 (s, 1H, NHCOchloroacetyl), 9.74 (d, 1H, NHCO), 7.48 (s, 1H, thiazol), 5.85 (dd, 1H, H-7), 5.19 (d, 1H, H-6), 5.0 and 4.6 (Abq, 2H, CH_2O), 4.40 (s, 2H, CH_2Cl), 3.92 (s, 3H, $NOCH_3$), 3.65 and 3.51 (Abq, 2H, H2), 2.05 (s, 3H, CH_3COO).

^{13}C-NMR (DMSO-d_6): 170.18 (C15), 165.34 (C17), 163.71 (C8), 162.78 (C5), 162.52 (C9), 157.89 (C14), 148.49 (C10), 141.62 (C12), 126.33 (C4), 123.51 (C2), 114.57 (C13), 62.66 (C3), 62.14 (C11), 58.57 (C7), 57.41 (C6), 42.16 (C18), 25.73 (C1), 20.53 (C16).

***7-{2-[2-(2-Chloro-acetylamino)-thiazol-4-yl]-2-methoxyimino-acetylamino}-3-methoxy methyl-8-oxo-5-thia-1-aza-bicyclo [4.2.0] oct-2-ene-2-carboxylic acid* (5).**



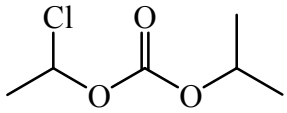
To a solution of **4** (26.0 g, 49 mmol) and sodium hydrogen carbonate (4.1 g, 49 mmol) in water (80 mL), methanol (170 mL, 4.2 mol) and calcium chloride dihydrate (375 g, 2.55 mol) were added. The mixture was stirred for 75 min at 70 °C and then poured onto 500 mL of ice water. The mixture was acidified with 37% hydrochloric acid (10 mL) and extracted with ethyl acetate (2 x 500 mL). The extracts were combined and the organic layer was extracted with 10% potassium hydrogen phosphate aqueous solution (350, 150 and 100 mL). The aqueous layers were combined and extracted with ethyl acetate (2 x 500 mL) after acidification with concentrated hydrochloric acid. The organic extracts were combined, washed with brine (100 mL) and dried over anhydrous sodium sulphate. The mixture was filtered and the filtrate was concentrated under reduced pressure to about 1/5 of the initial volume and left to stand at r.t. for 3 h. The resulting precipitate was separated by filtration, washed with ethyl acetate (30 mL) and dried to give **5** (16 g, 65% yield).

TLC: Ethyl acetate-ethanol-water-formic acid (65:25:15:1, v:v).
m.p: 208-210 °C.
^{1}H-NMR (DMSO-d_6): 12.95 (s, 1H, NHCOchloroacetyl), 9.75 (d, 1H, NHCO), 7.48 (s, 1H, thiazol), 5.84 (dd, 1H, H-7), 5.20 (d, 1H, H-6), 4.40 (s, 2H, CH_2O), 4.20 (s, 2H, CH_2Cl), 3.92 (s, 3H, $NOCH_3$), 3.62 and 3.51 (Abq, 2H, H1), 3.25 (s, 3H, CH_3O).

^{13}C-NMR (DMSO-d_6): 165.32 (C17), 163.62 (C8), 163.00 (C5), 162.52 (C9), 157.85 (C14), 148.48 (C10), 141.64 (C12), 126.14 (C4), 125.39 (C2), 114.57 (C13), 69.95 (C3), 62.13 (C11), 58.51 (C7), 57.59 (C6), 57.40 (C16), 42.15 (C18), 25.53 (C1).

1.2.3.2 *Preparation of the 1-iodo ethyl (isopropoxycarbonyloxy) group*

***1-Chloroethyl isopropyl carbonate* (8).**



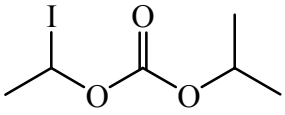
To a solution of ethyl chloroformate (140 mL, 1.47 mol) and sulfuryl chloride (130 mL, 1.61 mol) was added benzoyl peroxide (0.5 g, 2 mmol). The mixture was refluxed for 7.5 h and it was distilled at atmospheric pressure to give 1-chloroethyl chloroformate (**7**) (boiling range 119-140

°C). To a solution of the resulting 1-chloroethyl chloroformate in DCM (675 mL), isopropyl alcohol (134 mL, 1.74 mol) was added under cooling (0-5 °C) and with stirring and dried pyridine (78 mL, 0.96 mol) was added dropwise to the solution within 20 min. Once the addition finished the mixture was stirred for 30 min at the same temperature. The reaction mixture was washed successively with water (170 mL), brine (170 mL) and 5% potassium hydrogen sulphate aqueous solution (170 mL), and the organic layer dried over anhydrous sodium sulphate. The solvent was removed and the resulting liquid distilled under reduced pressure at 55 mmHg to give **8** (fraction boiling between 92 °C and 94 °C) (113.2 g, 46.2%).

^{1}H-NMR ($CDCl_3$): 6.44 (q, 1H, OCHCl), 4.95 (sept, 1H, CHO), 1.84 (d, 3H, CH_3), 1.34 (m, 6H, $(CH_3)_2$).

^{13}C-NMR ($CDCl_3$): 152.22 (C21), 84.30 (C20), 73.26 (C22), 25.16 (C23) and (C24), 21.60 and 21.55 (C19).

***1-Iodoethyl isopropyl carbonate* (9).**

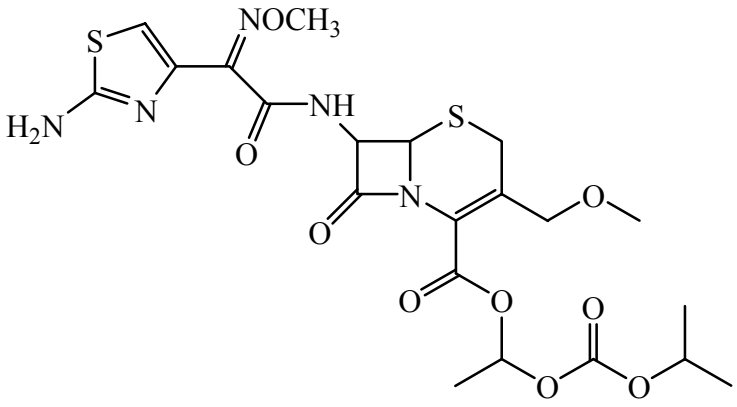


To a solution of **8** (5.1 g, 30.6 mmol) in benzene (50 mL) were added, at r.t., sodium iodide (10 g, 66.7 mmol) and 18-crown-6 ether (0.25 g, 0.95 mmol) and the mixture was refluxed with stirring during 12 h. The mixture was washed with water (3 x 15 mL) followed by 5% sodium thiosulphate aqueous solution (10 mL). The organic layer was dried over anhydrous sodium sulphate and after filtration, the filtrate was concentrated in vacuo to give **9** (6.7 g, 85%).

^{1}H-NMR ($CDCl_3$): 6.70 (q, 1H, OCHCl), 4.85 (sept, 1H, CHO), 2.15 (d, 3H, CH_3), 1.32 (m, 6H, $(CH_3)_2$).

1.2.3.3 *Condensation of the 2-(2-aminothiazol-4-yl)-2-(Z)-methoxyimino acetyl group and ethyl (isopropoxycarbonyloxy) group with the modified cephalosporanic nucleus (3-methoxymethylcephem)*

***7-[2-(2-Amino-thiazol-4-yl)-2-methoxyimino-acetylamino]-3-methoxymethyl-8-oxo-5-thia-1-aza -bicyclo [4.2.0] oct-2-ene-2-carboxylic acid 1-isopropoxycarbonyloxy-ethyl ester*(<u>6</u>).**



To a solution of **<u>5</u>** (2.0 g, 3.97 mmol) in DMA (10 mL), DCA (0.9 mL, 4.52 mmol) was added, followed by **<u>9</u>** (1.3 g, 5.04 mmol) under cooling (0-5 °C). The mixture was stirred for 45 min at the same temperature and ethyl acetate (50 mL) was added. The mixture was washed successively with water (15 mL), 1 M hydrochloric acid aqueous solution (15 mL) and brine (15 mL). The organic layer was dried over anhydrous sodium sulphate and the solvent was removed by vacuum distillation to give an oil (2.4 g). The oil was dissolved in DMA (20 mL) and thiourea (0.61 g, 1.97 mmol) was added. The resulting solution was stirred for 3 h at r.t.. The mixture was poured into 5% sodium hydrogen carbonate aqueous solution (50 mL) and extracted with ethyl acetate (75 mL). The organic layer was washed successively with 10% potassium bisulphate aqueous solution (25 mL) and brine (25 mL), and dried over anhydrous sodium sulphate. After filtration, the solvent was removed under reduced pressure and the residue was stirred with isopropyl ether (25 mL). The resulting precipitate was separated by filtration, washed with isopropyl ether (10 mL) and dried to give **<u>6</u>** (1.44 g, 65%).

TLC: Ethyl acetate-ethanol-water-formic acid (65:25:15:1, v:v).

m.p: 98-103 °C.

^{1}H-NMR (DMSO-d_6): 9.62 (m, 1H, NHCO), 7.25 (s, 2H, NH_2), 6.87 and 6.81 (2q, 1H, $OCH(CH_3)O$), 6.74 (s, 1H, thiazol), 5.85 (m, 1H, H-7), 5.20 (m, 1H, H-6), 4.82 (m, 1H,

OCH(CH_3)), 4.15 (s, 2H, CH_3OCH_2), 3.83 (s, 3H, $NOCH_3$), 3.65 and 3.52 (AB, q, 2H, H-2), 3.20 (s, 3H, CH_2OCH_3), 1.49 (d, 3H, CH_3CH), 1.25 (m, 6H, $(CH_3)_2$).

^{13}C-NMR (DMSO-d_6): 168.32 (C14), 163.97 (C8), 162.89 (C9), 159.48 (C5), 152.76 (C21), 148.89 (C10), 142.45 (C12), 128.82 and 128.48 (C4), 123.57 (C2), 108.89 (C13), 91.94 and 91.58 (C20), 72.53 (C22), 69.68 (C3), 61.82 (C11), 58.70 (C7), 57.72 (C6), 57.38 (C16), 25.71 (C1), 21.25 (C23 and C-24), 19.03 and 18.87 (C19).

1.2.4 CONCLUSION

The use of MAEM as starting material for preparing cefpodoxime proxetil allowed to obtain better yields for the synthesis of this antibiotic. Previous esterification of the chloroacetylated derivative **5**, followed by cleavage of the chloroacetyl protective group, allowed to eliminate the drawbacks of the classic pathways of synthesis, especially the final purification of cefpodoxime proxetil by column chromatography. Moreover, the utilization of MAEM allows to diminish the production cost of the final product.

1.3 **SYNTHESIS OF CEFDINIR**.

1.3.1 STATE OF THE ART

Cefdinir is an oral semisynthetic cephalosporanic antibiotic with an extended antibacterial spectrum, which was found to be active against Gram-positive and Gram-negative bacteria and demonstrated advantages in the antimicrobial activity over the available oral cephalosporins cefixime, cefpodoxime proxetil, cefaclor and cephalexin and very stable against the action of β-lactamases[41,42,43,44]. It was introduced on the market in the year 1991 by the Fujisawa Pharmaceutical Company from Japan[45].

This antibiotic is used against the infections of the respiratory tract like bronchitis, otitis[46], pneumonias[47], pharyngitis[48] and sinusitis[49], as well as in infections of the skin[50] and of the urinary tract[51].

From a structural point of view, one of the main characteristics of cefdinir associated to its good oral absorption is the presence of a vinyl group linked to the position three of the cephem

nucleus[52,53] (Fig. 3). Another particularity of this antibiotic is the presence of the radical 2-aminothiazol-4-yl as side chain linked to the amino group of the cephalosporanic nucleus through of a hydroxyimino acetamido system which result in a marked increase in its antimicrobial activity against Gram-positive and Gram-negative bacteria and also enhance its pharmacokinetic properties[54].

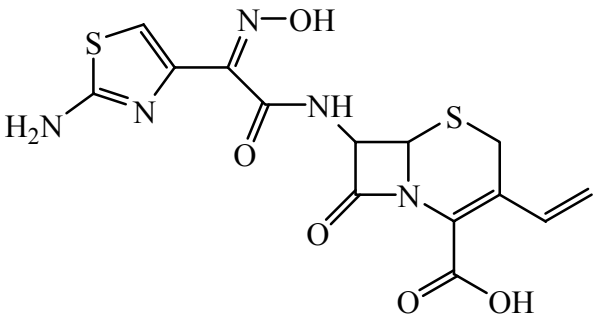
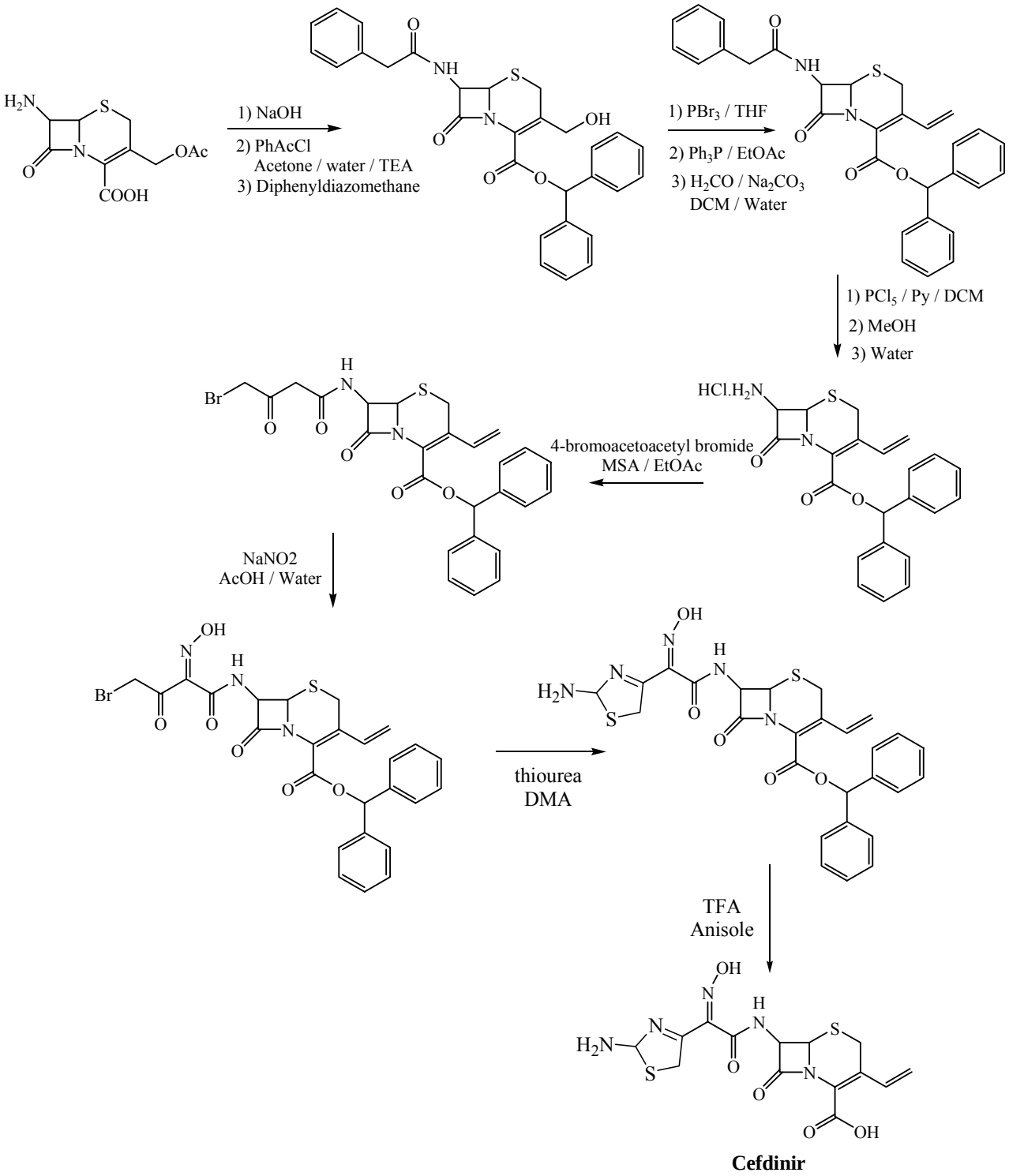


Fig. 3 Cefdinir

The best current synthetic method to prepare cefdinir is outlined in Scheme 13. This method is characterized by the fact that (Z)-2-(2-aminothiazol-4-yl)-2-(hydroxyimino) acetyl moiety is constructed directly over the cephalosporanic nucleus[55,56].



Cefdinir

Scheme 13

This synthetic pathway has few reaction steps and allows to obtain relatively good overall yields (ca. 11.3% from 7-ACA). However, it has a relevant drawback: the necessity of preparing the 4-bromoacetoacetyl bromide by reaction of bromine with diketene, a highly toxic and difficult to handle reagent.

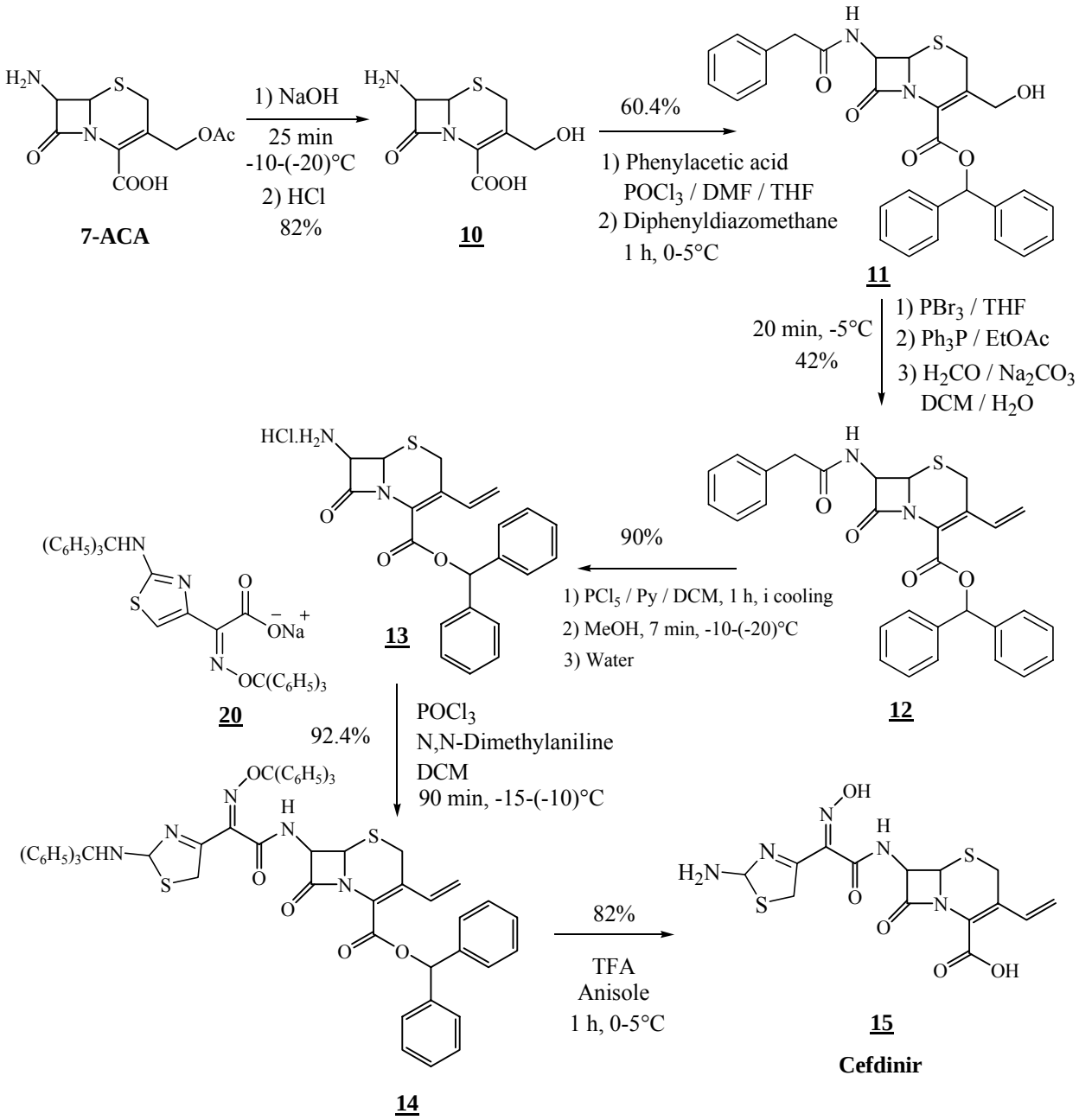
There is another general synthetic route to prepare (Z)-2-(2-aminothiazol-4-yl)-2-(hydroxyimino) acetamido-3-acetoxymethyl or 3-propenyl cephalosporins, not used to prepare cefdinir, which consists in synthesizing an intermediary carrier of the (Z)-2-(2-aminothiazol-4-yl)-2-(hydroxyimino) acetyl moiety, which is further coupled to the 3-acetoxymethyl or 3-propenyl cephem nucleus[8,57].

The objective of this part is to show the results obtained during the synthesis of cefdinir through this method. This procedure allows overcoming the drawbacks of the use of diketene during the synthesis of cefdinir by the first described method (Scheme 1).

1.3.2 RESULTS AND DISCUSSION

The compound, carrier of the 3-vinyl modified cephem nucleus (**12**), was synthesized in three steps from 7-ACA (Scheme 14).

The most critical step is the one corresponding to the synthesis of derivative **11**. According to the method reported in the literature[58], basic hydrolysis of 7-ACA is effected with NaOH in aqueous media and the 3-hydroxymethyl derivative formed (**10**) is not isolated from the reaction mixture. The 7β-amino group is then protected by acylation with phenylacetyl chloride in aqueous acetone according to Schotten-Baumann's procedure[59,60] and by keeping an almost neutral pH with TEA. Finally, the acid function of cephem nucleus is blocked by treating with diphenyldiazomethane to afford **11**. When this process was repeated, we found that only 32% yield of **11** can be obtained. TLC analysis at the end of the phenylacetylation reaction showed the presence of three spots, one of them corresponding to the desired phenylacetamido derivative and the others probably to the phenylacetyl ester and diphenylacetyl derivative of **10**. This fact, together with the partial decomposition of phenylacetyl chloride in aqueous media, may be the causes of the low yields obtained.



Scheme 14

To overcome the problems cited above, two methods were developed for preparing **11** characterized by the fact that the protection of 7β-amino group was achieved in totally organic media and the 3-hydroxymethyl group was blocked in order to avoid the formation of the phenylacetylated by-products. In consequence, it was necessary to isolate the 3-hydroxymethyl derivative (**10**) after basic hydrolysis of the acetoxy group of 7-ACA. This process was performed by treating 7-ACA with NaOH in a mixture of methanol-water at temperatures between -10 °C and -20 °C, followed by adjusting the reaction mixture to pH=3 in order to precipitate **10** in good yields (82%).

In the first developed method, **10** was dissolved in DMA by treatment with BSA and the phenylacetylation of the 7β-amino group was achieved by reaction with phenylacetyl chloride.

In the second procedure, **10** was dissolved in THF by reaction with BSA and phenylacetylation was performed with the phenylacetic acid activated by the Vilsmeier reagent. In both cases, TLC analysis of the reaction mixture revealed the presence of only one spot corresponding to the desired phenylacetamido derivative.

It was evident that the protection of the 3-hydroxymethyl group as the corresponding trimethylsilyl ether with BSA avoided the formation of the phenylacetyl ester. On the other hand, the absence of water in the reaction media reduced the decomposition of the acylating agents and allowed to obtain better yields, as can be observed in Table 2.

Table 2. Yields of 11 obtained through of the developed procedures.

Acylating agent	Catalyst	Solvent	From 10 (%)	From 7-ACA (%)
Phenylacetyl chloride	TEA	Acetone-water		32
Phenylacetyl chloride	BSA	DMA	51.9	42.6
Phenylacetic acid (Vilsmeier)	BSA	THF	60.4	49.6

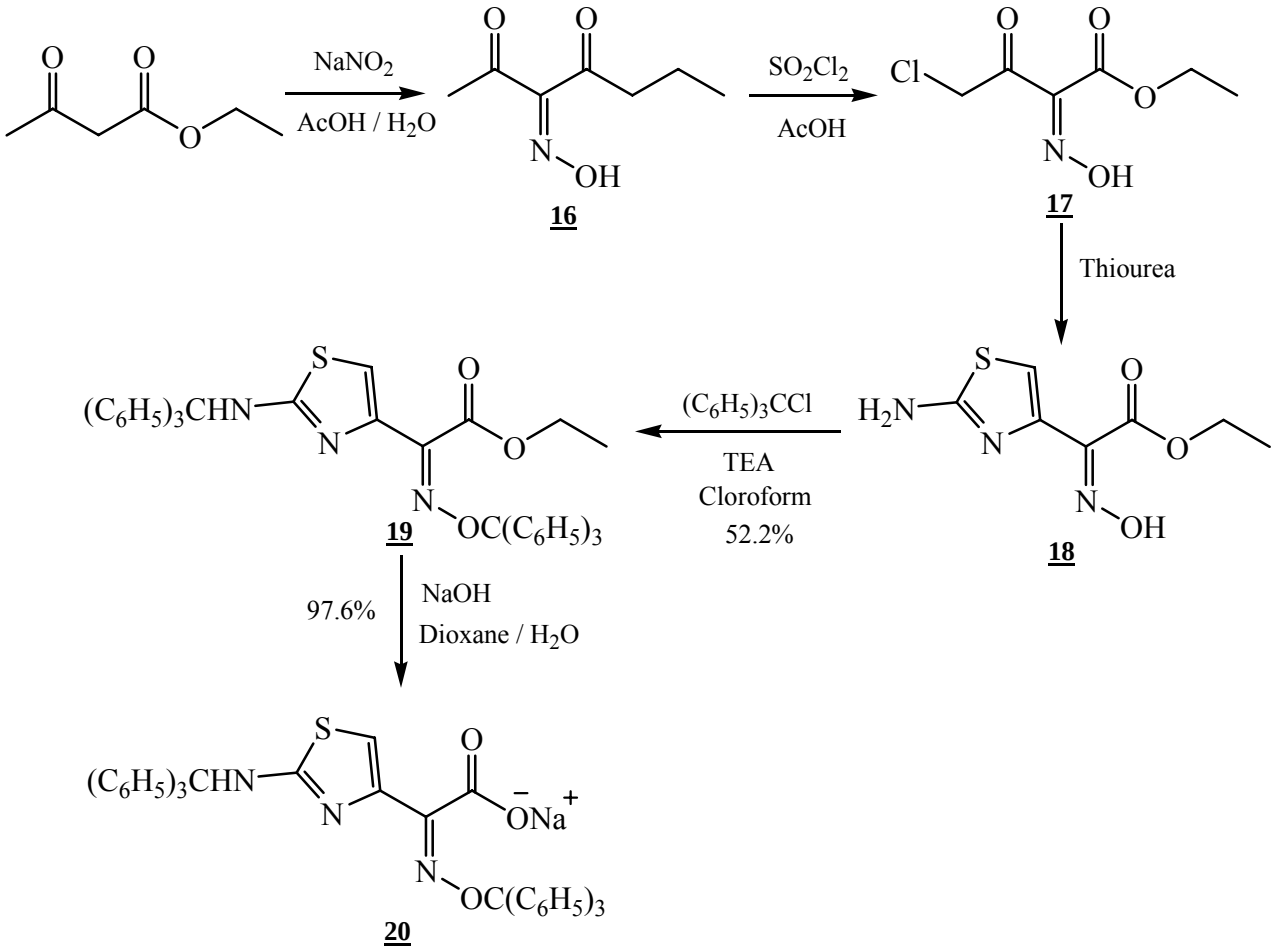
The best results were obtained when phenylacetylation was effected by activating phenylacetic acid with Vilsmeier reagent and in consequence this procedure was selected for the preparation of **11**.

Compound **12** was synthesized by means of the Wittig reaction according to the method reported in the literature[61], where **11** reacted with phosphorous tribromide in THF to obtain the 3-bromomethyl derivative, followed by treatment with triphenylphosphine in ethyl acetate to afford the corresponding phosphonium salt. Further reaction of this salt with aqueous formaldehyde in the presence of sodium carbonate as a base allowed to obtain **12** with 42% yield.

The phenylacetamido protective group of **12** was cleaved by the known iminochloride method[61,62,63]. Treatment of **12** with a phosphorous pentachloride-pyridine mixture in DCM, followed by a reaction with methanol and a final hydrolysis of the imino ether, afforded **13** in very good yields (90%). Although it was necessary to introduce an additional step during the synthesis

of **13**, the overall yield obtained from 7-ACA was 18.9%, that is to say a yield ca. 9% higher than the reported in the literature (11.8%) when the preparation of **11** was performed without isolating the 3-hydroxymethyl derivative (**10**).

The second part of the proposed synthetic pathway to prepare cefdinir, was the synthesis of the protected intermediary **20** which carries the (Z)-2-(2-aminothiazol-4-yl)-2-(hydroxyimino) acetyl moiety. The method for preparing **20** has been previously described[18,12,64] while obtaining other cephalosporins and is outlined in Scheme 15.



Scheme 15

The oxime **16** is obtained from ethyl acetoacetate by treatment with sodium nitrite in acetic acid. The methyl group is then halogenated with sulfuryl chloride to prepare **17**. Reaction of the derivative **17** with thiourea afforded the 2-aminothiazole ring (**18**) according to the conditions of Hantzch's method[34]. In the next step the amino and the hydroximino groups of **18** are simultaneously protected by treatment with trityl chloride to give compound **19**. Finally, the basic

hydrolysis of the ester group of **19** takes place in presence of NaOH to obtain **20**. The most critical step in the sequence of reactions is the formation of the 2-aminothiazole ring during the preparation of compound **18**. In the literature two methods are reported to prepare **18** from **17**. In the first one, **17** is treated with thiourea in ethanol in presence of N,N-dimethylaniline as base[64]. In the second one, no base is used and the solvent employed is a mixture of ethanol-water[12].

Although the formation of oxime **16** and synthesis of the halogenated derivative **17** occur in almost quantitative yields when both procedures were used, poorly overall yields of **18** were obtained.

To overcome the drawback cited above, two methods were developed in the present work in order to synthesize **18** from **17** by reaction with thiourea. In the first procedure developed, **17** was treated with thiourea by using a mixture of THF-water as solvent and sodium acetate as basic catalyst, while in the second one, DMA was used as solvent without addition of any catalyst. Both synthetic variants allowed to obtain better yields than the previously reported as can be observed from Table 3.

Table 3. Obtained results for preparing 18

Solvent	Catalyst	Yield (%) of 18 from ethyl acetoacetate
Ethanol	N,N-dimethylaniline	15.1
Ethanol-Water	None	17.2
THF-Water	Sodium acetate	26.1
DMA	none	36.9

The best results were obtained when the reaction was effected in DMA as solvent without using any catalyst. In consequence, this procedure was selected for the preparation of **18**.

In the following step, the amino function and the hydroxyimino group of **18** were simultaneously blocked by treatment with trityl chloride in chloroform in presence of TEA as acceptor of the hydrogen chloride formed. The protection of both functional groups is necessary in order to avoid side reactions during the further acylation of the cephalosporanic nucleus.

The protected intermediary **19** was obtained in 52.2% yield as reported in the literature. Finally, the ester group of **19** was hydrolyzed by treatment with NaOH in a mixture of dioxane-water as solvent affording the corresponding sodium salt **20** in 97.6% yield.

In the last part of the synthetic pathway used, the compound carrier of the (Z)-2-(2-aminothiazol-4-yl)-2-(hydroxyimino) acetyl moiety (**20**) was coupled to the modified cephem nucleus (**13**) to obtain the protected derivative **14**. In the next step all the protective groups were removed using TFA in anisole to prepare cefdinir (**15**).

Usually (during the synthesis of other cephalosporins) the acylation of the cephem nucleus is performed with the compound **20** in its free acid form, and by formation of reactive derivatives such as acid chlorides, active esters or mixed anhydrides. It means that sodium salt **20** has to be transformed into the corresponding free acid by treatment with dilute hydrochloric acid in the presence of an organic solvent such as ethyl acetate[65] or DCM[8,12].

We found that this apparently simple step causes a lot of trouble because partial deprotection of the amino function of **20** takes place. In consequence emulsions are formed and reduced yields of the free acid are obtained.

In order to overcome the problems cited above, in the present work was developed a method which allowed performing the acylation reaction directly with the sodium salt **20**.

It is known that a reaction of carboxylic acid salts with phosphorous oxychloride produces the corresponding acid chloride. In this case a mixture of the cephem nucleus (**13**) and compound **20** in the presence of N,N-dimethylaniline was treated with phosphorus oxychloride to generate in situ the acid chloride of **20** as acylating agent. The acylation of **13** occurred in a short time and the protected derivative **14** was obtained in very good yields (92.4%). The final step to prepare cefdinir was the cleavage of the protective groups of **14**. The trityl protective group of the amine and the diphenylmethyl group were removed by treating **14** with trifluoroacetic acid (TFA) in the presence of anisol as cation scavenger. The trityl protective group of oxyimino moiety was further cleaved with 90% formic acid to afford cefdinir (**15**) in 82% yield (calculated from **14**). Although the procedure used in the present work[66] has more synthetic steps, the overall yield of cefdinir (calculated from 7-ACA) was 14.3%, higher than when the best current procedure is employed (11.3%).

1.3.3 EXPERIMENTAL PART

General Methods

Melting points were determined using the Gallenkamp capillary apparatus with a system of measurement and temperature control. ^{1}H NMR and ^{13}C NMR spectra were recorded at 250 and 62.5 MHz, respectively, on a Bruker AC 250F spectrometer, using deuterated dimethylsulfoxide (DMSO-d_6) as solvent and TMS as an internal standard. TLC was performed on pre-coated plates of silica gel GF-254 (Merck). The chromatograms were visualized in a Camag UV-Vis lamp with a wavelength of 254 nm. The synthesis of each compound was confirmed by comparison of registered ^{1}H NMR spectra with the ^{1}H NMR data reported in the literature consulted.

1.3.3.1 *Obtaining of diphenyldiazomethane*

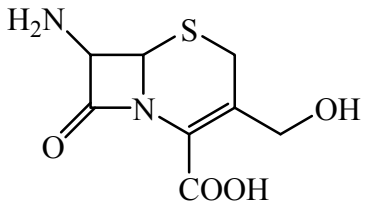
Benzophenone hydrazone (10.78 g, 55 mmol) and iodine (2.2 mL, 1% w/v) were dissolved in DMA (55 mL) and water (5 mL). A solution of chloramine T (15.5 g, 55 mmol) in DMA (55 mL) and water (5 mL) was then added slowly over 30 min at 20°C. The mixture was stirred for 15 min before partition between DCM (110 mL) and 5 % NaOH aqueous solution (275 mL). The organic layer was washed with water (1 x 100 mL and 3 x 50 mL), dried over anhydrous sodium sulphate and made up to 200 mL in a volumetric flask with DCM.

1.3.3.2 *Quantitative determination of diphenyldiazomethane*

Diphenyldiazomethane solution (10 mL) was exactly measured and the solvent was evaporated until dryness at reduced pressure. The obtained residue was dissolved with 10 mL of 1,2-dichloroethane, cooled down to 0-5 °C and glacial acetic acid was added until the total fading of the solution. The content of diphenyldiazomethane was determined by measurement of the nitrogen volume that came off during the reaction to afford 8.70 g (81.5%) of diphenyldiazomethane.

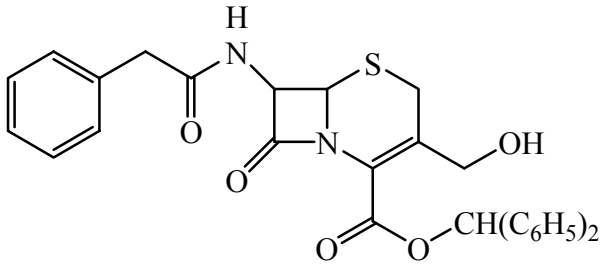
1.3.3.3 *Preparation of 7-Amino-2-diphenylacetyl-3-vinyl-5-thia-1-aza-bicyclo [4.2.0] oct-2-en-8-one hydrochloride*.

***7-Amino-3-hydroxymethyl-8-oxo-5-thia-1-aza-bicyclo [4.2.0] oct-2-ene-2-carboxylic acid* (<u>10</u>)**



7-ACA (9.0 g, 33 mmol) was suspended in methanol (60 mL), water (60 mL) was added and the mixture was cooled to -20°C. A 10 M NaOH aqueous solution (7 mL) was then added slowly and the resulting solution was stirred for 25 min between -10 and -20°C. The solution was adjusted to pH= 3 by addition of concentrated hydrochloric acid at 0-5°C. The precipitated solid was separated by vacuum filtration, washed successively with methanol (30 mL), acetone (30 mL) and diethyl ether (2 x 30 mL), and dried under vacuum to r.t to obtain **<u>10</u>** (6.24 g, 82%).

***N- (2- Diphenylacetyl- 3 -hydroxymethyl- 8- oxo-5-thia-1-aza-bicyclo [4.2.0] oct-2-en-7-yl) - 2-phenyl-acetamide* (<u>11</u>)**



- *Acylation with phenylacetyl chloride in aqueous-organic media (method reported in the literature)*

A solution of NaOH (5.2 g, 130 mmol) in water (26 mL) was added to a stirred suspension of 7-ACA (16.0 g, 59 mmol) in water (64 mL) during 5 min to keep the reaction temperature between 2 and 5°C under cooling with an ice bath. After stirring for 5 min at this temperature, the reaction solution was adjusted to pH= 8.5 with glacial acetic acid and diluted with acetone (48 mL). A solution of phenylacetyl chloride (11.0 g, 71 mmol) in acetone (11 mL) at 0-5°C was dropwise

added to the solution, keeping the pH between 7.5 and 8.5 with TEA (≈13 mL). The reaction mixture was stirred for 1 h at the same temperature and then concentrated in vacuo to remove the organic solvent. Ethyl acetate (220 mL) was added to the resulting solution and the stirred mixture was acidified to pH= 3.5 with 6 M hydrochloric acid (≈10 mL). The aqueous layer was further extracted with ethyl acetate (100 mL). The combined organic layers were washed with brine (80 mL), dried over anhydrous sodium sulphate and filtered. A solution of diphenyldiazomethane (13.77 g, 71 mmol) in ethyl acetate (50 mL) was added to the filtrate and the mixture was stirred for 1 h at r.t. (r.t.). The reaction solution was concentrated to a volume of ca. 50 mL and then cooled to 0-5°C during 12 h. The resulting white precipitate was collected by vacuum filtration, washed successively with ethyl acetate (3 x 25 mL), n-hexane (3 x 25 mL) and dried to give **11** (9.7 g, 32%).

TLC: Ethyl acetate-n-hexane, (4:1, v:v)
m.p: 178-180 °C.
^{1}H-NMR (DMSO-d_6) δ: 9.14 (d, 1H, NH), 7.20-7.55 (m, 15H, aromatics), 6.91 (s, 1H, $CHPh_2$), 5.73 (dd, 1H, H-7), 5.18 (t, 1H, OH); 5.12 (d, 1H, H-6), 4.23 (d, 2H, CH_2OH), 3.63 (s, 2H, H-2), 3.57 and 3.52 (Abq, 2H, CH_2CO).

^{13}C NMR (DMSO-d_6) δ: 170.92 (C-8), 165.17 (C-7), 160.83 (C-4), 135.75 (C-10), 128.95 (C-11), 128.95 (C-15), 128.16 (C-13), 126.71 (C-12), 126.71 (C-14), 121.95 (C-3), 134.40 (C-2), 78.29 (C-16), 59.72 (C-17), 58.86 (C-6), 57.66 (C-5), 41.54 (C-9), 25.51 (C-1).

- Acylation with phenylacetyl chloride in organic media

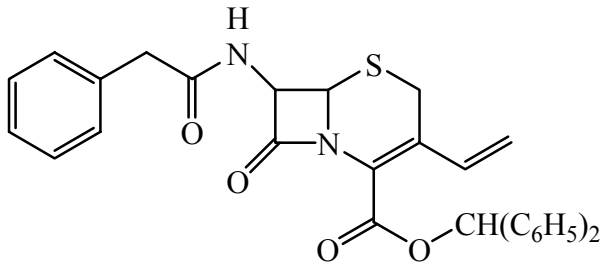
BSA (16 mL, 57.8 mmol) was added to a suspension of **10** (5.0 g, 21.7 mmol) in DMA (50 mL) and the mixture was stirred for 30 min at r.t. The resulting solution was cooled to -30°C, phenylacetyl chloride (3.5 mL, 26.4 mmol) was added over 10 min and the mixture was stirred for 90 min between -10 and -20°C. The reaction mixture was poured into ice-water (200 mL) and extracted with ethyl acetate (100 mL). The aqueous layer was further extracted with ethyl acetate (45 mL). The combined organic layers were washed with brine (35 mL), dried over anhydrous sodium sulphate and filtered. A solution of diphenyldiazomethane (5.12 g, 26.4 mmol) in ethyl acetate (25 mL) was added to the filtrate and the mixture was stirred for 1 h at r.t. The reaction solution was concentrated to a volume of ca. 25 mL and then cooled to 0-5°C during 12 h. The

resulting white precipitate was collected by vacuum filtration, washed successively with ethyl acetate (3 x 15 mL), n-hexane (3 x 15 mL) and dried to give **11** (5.81 g, 42.6% from 7-ACA).

- Acylation with phenylacetic acid activated by Vilsmeier reagent

Phosphorous oxychloride (3.3 mL, 36 mmol) at 0-5°C was dropwise added to a mixture of DMF (2.7 mL, 35 mmol) and THF (30 mL) under stirring, and the mixture was stirred at this temperature for 30 min to prepare the Vilsmeier reagent. Phenylacetic acid (4.33 g, 31.8 mmol) was added to the above solution under ice-cooling, and the reaction mixture was stirred at the same temperature for 1 h to prepare an activated solution of phenylacetic acid. BSA (24 mL, 98.16 mmol) was added to a suspension of I (7.33 g, 31.87 mmol) in THF (70 mL) and the mixture was stirred for 15 min at 40 °C. The above activated phenylacetic acid solution was added to the obtained solution (cooled to -30 °C) and the mixture was stirred at -20°C for 1 h. The reaction mixture was poured into ice-water (200 mL) and extracted with ethyl acetate (145 mL). The aqueous layer was further extracted with ethyl acetate (65 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous sodium sulphate and filtered. A solution of diphenyldiazomethane (7.42 g, 26.4 mmol) in ethyl acetate (35 mL) was added to the filtrate and the mixture was stirred for 1 h at r.t. The reaction solution was concentrated to a volume of ca. 35 mL and then cooled to 0-5 °C during 12 h. The resulting white precipitate was collected by vacuum filtration, washed successively with ethyl acetate (3 x 25 mL), n-hexane (3 x 25 mL) and dried to give **11** (6.76 g, 49.6% from 7-ACA).

***N-(2-Diphenylacetyl -8- oxo -3- vinyl-5-thia-1-aza-bicyclo [4.2.0] oct -2- en -7-yl)-2- phenyl acetamide* (12)**



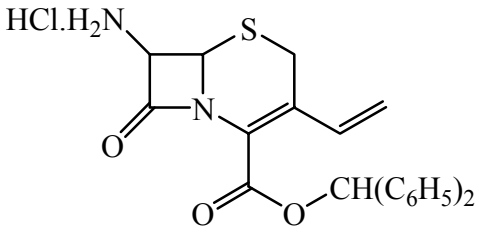
Phosphorus tribromide (1.88 g, 6.98 mmol) was dropwise added to a suspension of **11** (9.7 g, 18.9 mmol) in THF (38 mL) at -5°C with stirring. After stirred at this temperature for 20 min, the reaction mixture was poured into ice-water (56 mL), and extracted with ethyl acetate (38 mL). The

separated organic layer was washed with brine (10 mL), dried over anhydrous sodium sulphate, filtered and evaporated in vacuo. The residue was dissolved in ethyl acetate (38 mL) and triphenylphosphine (5.94 g, 22.56 mmol) was added. After stirring at r.t. for 5 h, the precipitated phosphonium salt was collected by filtration, washed with ethyl acetate (3 x 15 mL) and dried. 37% percent aqueous formaldehyde (62.7 mL, 752 mmol) and a solution of sodium carbonate (7.97 g, 75.2 mmol) in water (30 mL) were added to a solution of the phosphonium salt in DCM (90 mL) at r.t. After stirred at this temperature for 90 min, the reaction mixture was neutralized with 20% sulfuric acid (≈32 mL). The separated organic layer was washed with brine (25 mL), dried over anhydrous sodium sulphate, filtered and evaporated in vacuo until dryness. The residue was left to stir with methanol (30 mL) for 1 h and the obtained solid was separated by filtration, washed with methanol (3 x 10mL) and dried to afford **12** (4.04 g, 42%).

TLC: Ethyl acetate-ethanol-water-formic acid, (60:25:15:1, v:v)
m.p: 188-189 °C.
^{1}H-NMR (DMSO-d_6) δ: 9.21 (d, 1H, NHCO), 7.20-7.50 (m, 15H, aromatics), 6.96 (s, 1H, $CHPh_2$), 6.72 (dd, 1H, CH=), 5.77 (dd, 1H, H-7), 5.66 (d, 1H, $=CH_2$ (trans)), 5.30 (d, 1H, $=CH_2$ (cis)), 5.20 (d, 1H, H-6), 3.94 and 3.61 (ABq, 2H, H-2); 3.58 and 3.52 (Abq, 2H, $PhCH_2CO$).

^{13}C-NMR (DMSO-d_6) δ: 170.90 (C-8), 165.09 (C-7), 160.89 (C-4), 135.75 (C-10), 131.24 (C-17), 128.94 (C-11), 128.94 (C-15), 128.49 (C-13), 127.84 (C-2), 126.75 (C-12), 126.75 (C-14), 123.77 (C-3), 118.61 (C-18), 78.46 (C-16), 59.13 (C-6), 57.86 (C-5), 41.52 (C-9), 23.24 (C-1).

***7-Amino-2-diphenylacetyl -3- vinyl-5-thia-1-aza-bicyclo[4.2.0]oct-2-en-8-one hydrochloride* (13)**



Pyridine (1.9 g, 24 mmol) was added to a suspension of phosphorous pentachloride (4.95 g, 23.8 mmol) in DCM (48 mL) under ice-cooling, and the suspension was stirred at this temperature for 1 h. Then, compound **12** (4.04 g, 7.9 mmol) was added and the reaction mixture was stirred for 90 min keeping the temperature between 8°C and 10°C. The mixture was cooled to -35°C,

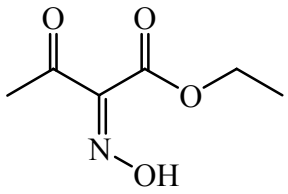
methanol (31.7 mL, 782 mmol) was added and the resulting solution was stirred between –10°C and -20°C for 75 min. The temperature was raised to -5°C and water (6.3 mL) was added. After removing the solvent in vacuo, the residue was stirred with a mixture of water (1.6 mL) and diethyl ether (16 mL). The resulting precipitate was collected by vacuum filtration, washed successively with water (10 mL) and diethyl ether (10 mL), and dried to give **13** (3.05 g, 90%).

TLC: Ethyl acetate-n-hexane, (4:1, v:v).
m.p: 170-171 °C.
^{1}H-NMR (DMSO-d_6) δ: 7.25-7.52 (m, 10H, aromatics), 6.95 (s, 1H, $CHPh_2$), 6.93 (dd, 1H, CH=), 5.81 (d, 1H, $=CH_2$ (trans)), 5.44 (d, 1H, $=CH_2$ (cis)), 5.33 (d, 1H, H-7), 5.24 (d, 1H, H-6), 4.00 and 3.75 (ABq, 2H, H-2),

^{13}C-NMR (DMSO-d_6) δ: 160.58 (C-7), 160.37 (C-4), 132.67 (C-2), 130.68 (C-8), 123.77 (C-3), 120.74 (C-9), 78.41 (C-10), 57.94 (C-6), 54.80 (C-5), 23.71 (C-1).

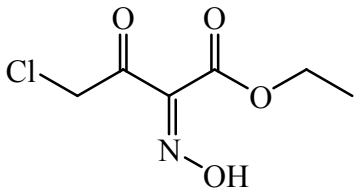
1.3.3.4 *Preparation of the 2-(2-aminothiazol-4-yl)-2-(Z)–hydroxyimino acetyl group.*

***Oxime preparation. 2-hydroxyimino-3-oxo-butyric acid ethyl ester* (16)**



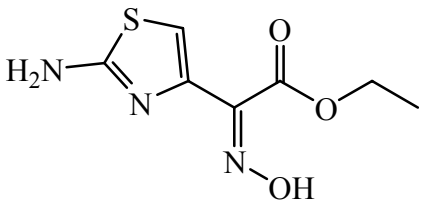
A solution of sodium nitrite (18 g, 308 mmol) in water (40 mL) was added to an ice-cooled solution of ethyl acetoacetate (29.2 g, 225 mmol) in glacial acetic acid (29.6 mL) keeping the temperature below 10°C. The obtained solution was stirred for 30 min at 10°C and the solvents were evaporated under reduced pressure until dryness. The residue was dissolved in ethyl acetate (50 mL) and the solution was washed with a 5% sodium hydrogen carbonate aqueous solution (2 x 50 mL). The separated organic layer was dried over anhydrous sodium sulphate, filtered and evaporated until dryness to give **16** as an oil and was used in the next step without further purification.

***Oxime halogenation. 4-chloro-2-hydroxyimino-3-oxo-butyric acid ethyl ester* (17)**



Compound **16** was dissolved in glacial acetic acid (23 mL), the solution was heated to 58-60°C and sulfuryl chloride (23.12 g, 171 mmol) were added slowly over 3.5 h. The mixture was heated for an additional hour at the same temperature and then it was evaporated under reduced pressure until dryness. The residue was dissolved in ethyl acetate (90 mL) and was washed with brine (3 x 30 mL). The separated organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuo until dryness to give **17** as an oil and was used in the next step without further purification.

Formation of 2-aminothiazol ring. (2-Amino-thiazol-4-yl)-hydroxyimino-acetic acid ethyl ester (<u>18</u>)



- Using ethanol as the reaction solvent and N,Ndimethylaniline as base.

N,N-Dimethylaniline (7.7 mL, 59.3 mmol) and thiourea (4.2 g, 55.2 mmol) were added successively to a solution of **<u>17</u>** (36 g, 186 mmol) in ethanol (50 mL) and the mixture was stirred for 2 h at r.t. The precipitated solid was collected by filtration, washed with ethanol (20 mL), acetone (20 mL) and diethyl ether (20 mL) and dried to give **<u>18</u>** (7.3 g, 15.1% from ethyl acetoacetate).

TLC: Ethyl acetate-methanol, (8:1, v:v).
m.p: 191-193°C.
^{1}H-NMR (DMSO-d_6) δ: 11.65 (s, 1H, NOH), 7.20 (s, 2H, NH_2), 6.83 (s, 1H, thiazol), 4.24 (q , 2H, OCH_2), 1.25 (t, 3H, CH_3).

^{13}C-NMR (DMSO-d_6) δ: 168.57 (C-5), 163.35 (C-1), 146.74 (C-2), 142.09 (C-3), 106.14 (C-4), 60.96 (C-6), 13.96 (C-7).

- Using an ethanol-water mixture as the reaction solvent without any base.

A solution of **<u>17</u>** (36 g, 186 mmol) in water (42 mL) was added to a solution of 14.0 g (184 mmol) of thiourea in a mixture of ethanol (42 mL) and water (84 mL). The mixture was stirred for 1 h at r.t., the resulting solution was concentrated to about a half of the initial volume and it was adjusted to pH= 6 with a 5% sodium hydrogen carbonate aqueous solution. The precipitated was collected by filtration, was washed successively with diethyl ether (50 mL) and acetone (50 mL) and dried in vacuum to afford **<u>18</u>** (8.3 g, 17.2% from ethyl acetoacetate).

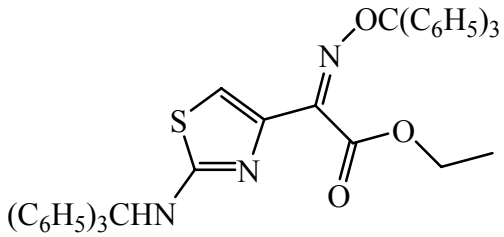
- Using a mixture of THF-water as a reaction solvent and sodium acetate as base.

Water (48 mL), thiourea (8.5 g, 111.6 mmol) and anhydrous sodium acetate (15.0 g, 182.8 mmol) were added successively to a solution of **17** (18 g, 93 mmol) in THF (48 mL). The mixture was stirred for 4 h at r.t., adjusted to pH= 6.7-6.8 with sodium hydrogen carbonate (5.2 g, 61.8 mmol) and extracted with ethyl acetate (2 x 100 mL). The organic layer was discarded and the precipitated formed in the aqueous phase was collected by filtration, washed with a mixture of ethyl acetate-water (1:1) (2 x 20 mL) and vacuum dried to afford **18** (6.3 g, 26.1% from ethyl acetoacetate).

- Using DMA as reaction solvent without any base

Thiourea (4.3 g, 56.5 mmol) was added to a solution of **17** (18 g, 93 mmol) in DMA (40 mL) and the mixture was stirred for 3 h at r.t. The reaction solution was poured onto ice-water (200 mL), ethyl acetate (200 mL) was added and the resulting mixture was adjusted to pH= 6.1-6.2 with sodium hydrogen carbonate (4.3 g, 51.1 mmol). The organic layer was discarded and the precipitated formed in the aqueous phase was collected by vacuum filtration, washed with a mixture of ethyl acetate-water (1:1) (2 x 20 mL) and vacuum dried to afford **18** (8.9 g, 36.9% from ethyl acetoacetate).

***[2-(Trityl-amino)-thiazol-4-yl]-trityloxyimino-acetic acid ethyl ester* (19)**



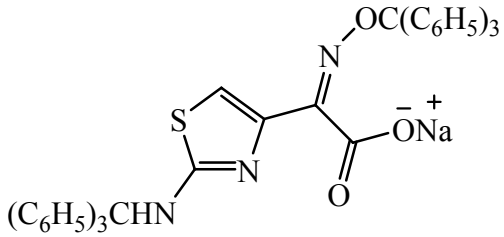
A suspension of **18** (8.3 g, 38.6 mmol) in chloroform (60 mL) was cooled to 0-5°C and then TEA (11.5 mL, 82.7 mmol) was added. A solution of trityl chloride (23.2 g, 83 mmol) in chloroform (46 mL) was added to the resulting mixture over 40 min keeping the temperature between 0-5°C. The reaction mixture was stirred for 90 min at r.t. and the obtained solution was washed successively with water (140 mL), diluted hydrochloric acid (60 mL) and water (3 x 150 mL). The separated organic layer was dried over anhydrous sodium sulphate, filtered and

evaporated under reduced pressure until dryness. The residue was stirred with isopropyl alcohol (60 mL) and the white solid formed was collected by filtration, washed with cold isopropyl alcohol (3 x 10 mL) and dried to give **19** (14.1g, 52.2%).

TLC: n-Hexane-ethyl acetate, (3:1, v:v).
m.p: 126-127°C.
^{1}H-NMR (DMSO-d_6) δ: 8.80 (s, 1H, $HNCPh_3$), 7.40-7.10 (m, 15H, aromatics), 6.74 (s, 1H, thiazol), 4.13 (q, 2H, OCH_2), 1.18 (t, 3H, CH_3).

^{13}C-NMR (DMSO-d_6) δ: 166.42 (C-5), 162.54 (C-1), 147.49 (C-2), 140.31 (C-3), 109.66 (C-4), 90.81 (C-8), 71.72 (C-9), 61.26 (C-6), 13.90 (C-7).

***Sodium, [2-(trityl-amino)-thiazol-4-yl]-trityloxyimino-acetate* (20)**



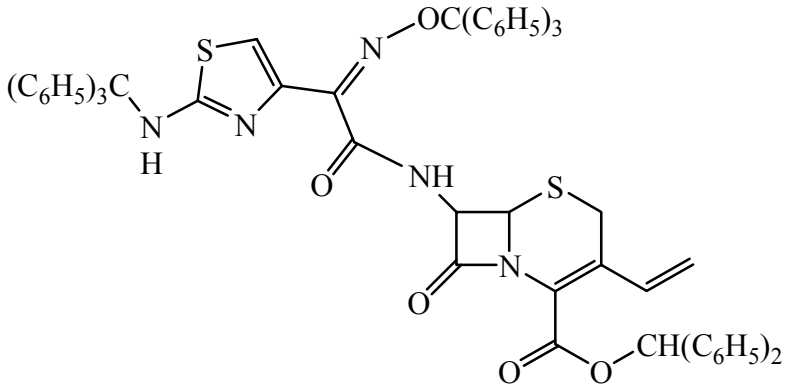
A 2 M NaOH aqueous solution (17 mL) was added to a suspension of **19** (12.2 g, 17.4 mmol) in dioxane (62 mL) over 5-10 min and the mixture was stirred for 2 h at 100-110°C. The reaction mixture was cooled to 0-5°C and the precipitated solid was collected by filtration, washed successively with a mixture of dioxane-water (3:1) (3 x 20 mL), diethyl ether (2 x 20 mL) and dried to afford **20** (11.8 g, 97.6%).

TLC: Ethyl acetate-methanol, (8:1, v:v)
m.p: 248-249°C.
^{1}H NMR (DMSO-d_6) δ: 8.50 (s, 1H, $HNCPh_3$), 7.40-7.10 (m, 15H, aromatics), 6.47 (s, 1H, thiazol).

^{13}C-NMR (DMSO-d_6) δ: 166.38 (C-5), 165.95 (C-1), 155.33 (C-2), 144.28 (C-3), 109.67 (C-4), 88.56 (C-8), 71.17 (C-9).

1.3.3.5 *Coupling of the 2-(2-aminothiazol-4-yl)-2-(Z) –hydroxyimino acetyl group to the cephem nucleus (Final preparation of cefdinir).*

***8-Oxo-7-{2-[2-(trityl-amino)-thiazol-4-yl]-2-tritylimino-acetylamino}-3-vinyl-5-thia-1-aza-bicyclo [4.2.0] oct-2-ene-2-carboxylic acid benzhydryl ester* (<u>14</u>)**



A solution of **<u>13</u>** (3.05 g, 4.67 mmol) in DCM (40 mL) was cooled to -20°C and then N,N-dimethylaniline (2 mL, 15.65 mmol) followed by **<u>20</u>** (5.19 g, 7.49 mmol) were added. Phosphorous oxychloride (0.69 mL, 7.5 mmol) was added to the resulting suspension and the formed solution was stirred for 90 min at temperatures between -15 and -10°C. The temperature was raised to 25-30°C and the solvent was evaporated under reduced pressure. Ethyl acetate (75 mL) and diluted hydrochloric acid (30 mL) were added to the residue and the mixture was stirred for 10 min at r.t. The separated organic layer was washed successively with water (30 mL), 5% sodium hydrogen carbonate aqueous solution (30 mL) and brine (30 mL), dried over anhydrous sodium sulphate, filtered and evaporated in vacuo until dryness. The residue was stirred with methanol (30 mL), the formed precipitate was collected by filtration, washed with methanol (3 x 15 mL) and dried to give **<u>14</u>** (6.71 g, 92.4%).

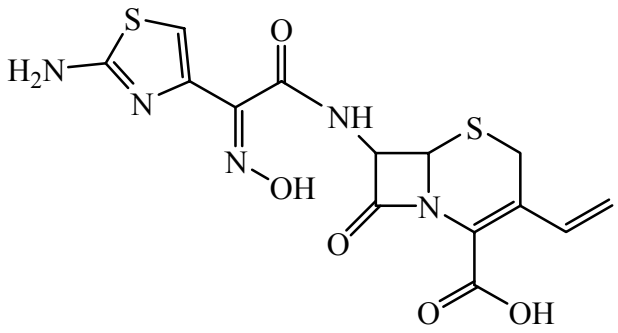
TLC: n-Hexane-ethyl acetate, (3:1, v:v).

m.p: 124-129 °C.

^{1}H-NMR (DMSO-d_6) δ: 9.95 (s, 1H, HNCO), 8.80 (s, 1H, $HNCPh_3$), 7.55-7.10 (m, 40H, aromatics), 6.95 (s, 1H, $CHPh_2$), 6.82 (dd, 1H, CH=), 6.63 (s, 1H, thiazol), 5.94 (dd, 1H, H-7), 5.66 (d, 1H, $=CH_2$ (trans)), 5.32 (d, 1H, $=CH_2$ (cis)), 5.30 (d, 1H, H-6), 3.88 and 3.62 (Abq, 2H, H-2).

^{13}C-NMR (DMSO-d_6) δ: 166.64 (C-14), 164.31 (C-9), 163.75 (C-10), 160.86 (C-4), 149.29 (C-11), 142.07 (C-12), 131.20 (C-5), 127.86 (C-2), 123.75 (C-3), 119.07 (C-6), 111.70 (C-13), 90.43 (C-16), 78.47 (C-15), 71.45 (C-17), 58.90 (C-8), 57.95 (C-7), 23.52 (C-1).

***7-[2-(2-Amino-thiazol-4-yl)-2-hydroxyimino-acetylamino]-8-oxo-3-vinyl-5-thia-1-aza-bicyclo [4.2.0] oct-2-ene-2-carboxylic acid (cefdinir)* (15)**



Anisole (10 mL) and TFA (22 mL, 288 mmol) were added successively to an ice-cooled solution of **14** (6.72 g, 6.43 mmol) in DCM (9 mL). The resulting solution was stirred for 1 h at 0-5°C and the solvent was evaporated under reduced pressure. The residue was stirred with iPE (130 mL), the precipitate formed was separated by filtration, washed with iPE (3 x 20 mL) and dried. 90% formic acid (34 mL) was added to the obtained solid and the mixture was stirred for 3 h at r.t. The reaction mixture was concentrated in vacuo and the residue was stirred with iPE (100 mL) for 10 min at r.t. The resulting solid was collected by filtration, washed with iPE (3 x 10 mL) and dried to afford **15** (2.54 g, 82%).

TLC: Ethyl acetate-ethanol-water-formic acid, (60:25:15:1, v:v:v).
^{1}H-NMR (DMSO-d_6) δ: 11.37 (s, 1H, NOH), 9.50 (d, 1H, NHCO), 7.21 (s, 2H, NH_2), 6.92 (dd, 1H, CH=), 6.69 (s, 1H, thiazol), 5.80 (dd, 1H, H7), 5.60 (d, 1H, $=CH_2$ (trans)), 5.34 (d, 1H, $=CH_2$ (cis)), 5.20 (d, 1H, H-6), 3.83 and 3.60 (ABq, 2H, H-2).

^{13}C-NMR (DMSO-d_6) δ: 168.23 (C-14), 163.80 (C-9), 163.76 (C-10), 163.18 (C-4), 148.30 (C-11), 143.16 (C-12), 131.95 (C-5), 125.45 (C-3), 124.28 (C-2), 117.21 (C-6), 106.80 (C-13), 58.73 (C-8), 57.83 (C-7), 23.18 (C-1).

1.3.4 CONCLUSIONS

Cefdinir (**15**) was prepared by an alternative synthetic route which allowed to avoid the use of diketene and to obtain better yields than in the method reported in literature. In the present part of the work, two procedures were developed in order to protect the cephem amino function by phenylacetylation in the synthesis of compound **11**. In both cases, yields were higher than those described in the literature method. Moreover, it was demonstrated that using phenylacetic acid as acylating agent activated by Vilsmeier reagent, allowed to obtain the best yields of **11**.

On the other hand, two methods were developed in order to form the 2-aminothiazol ring during the synthesis of compound **18** demonstrating that using DMA as solvent was the best choice and allowed to obtain better yields of **18** than those described in literature.

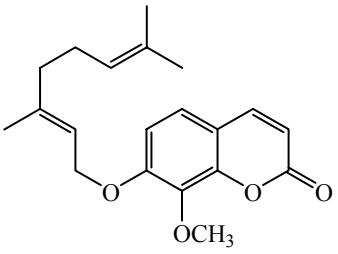
Finally, it was developed a method to acylate compound **13** directly with the sodium salt **20** in presence of $POCl_3$. This procedure allowed overcoming the drawback corresponding to the conversion of **14** into its free acid form [Cefdinir (**15**)].

CHAPTER II

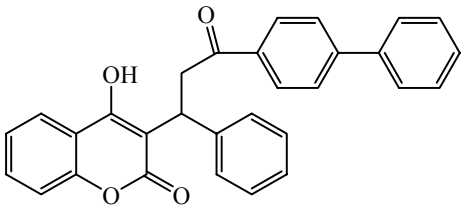
SYNTHESIS OF BENZOPYRAN-2-ONES AND 3-ONES (COUMARINS)

2. INTRODUCTION

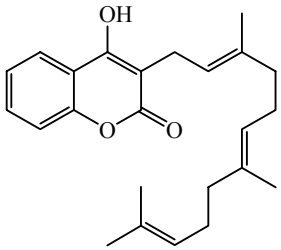
Benzopyran-2-ones and 3-ones (coumarins) are widely distributed in nature in form of hydroxylated and alkoxylated derivatives, as well as part of different glycosides, among others[67]. These kind of compounds have been studied for years because their possibility of being used as additives in food and cosmetics[68] and their wide spectra of biological activities; in that sence, they have been used with valuable applications in laboratory investigations due their good antiimflammatory activity[69,70,71], as well as anticoagulants[72,73], acetylcholinesterase inhibitors[74], inhibitory activity against cytochromes P 450[75], antibacterial[76] and fungicidal properties[77], antioxidant potency[78,79] and antitumoral[80] and anticancer activities[81], among others[82,83,84] (Fig. 4).



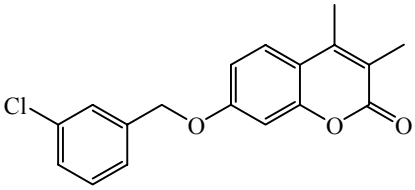
Antiinflammatory activity [70]



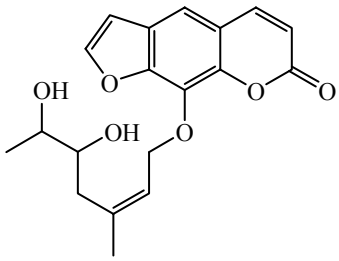
Anticoagulant activity [72]



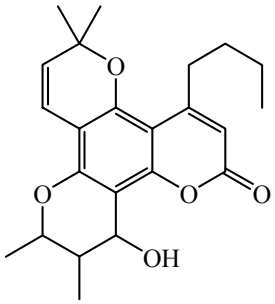
Antibacterial activity [76]



Acetylcholinesterase inhibitor [74]



Inhibitory activity against cytochromes P450 [75]



Anticancer activity [81]

Fig. 4

Nevertheless, nowadays they continue to focuse the attention of researchers and industry and extensive works have been done directed to increase their biological activity through the structural modifications carried out by chemical synthesis. In that case we have the reactions with reactive oxygen species, such as peroxyls, with biological molecules *in vivo*; they have been focused due their implication in various degenerative diseases[85], such as cancer, heart disease, inflammation, and the aging process[86].

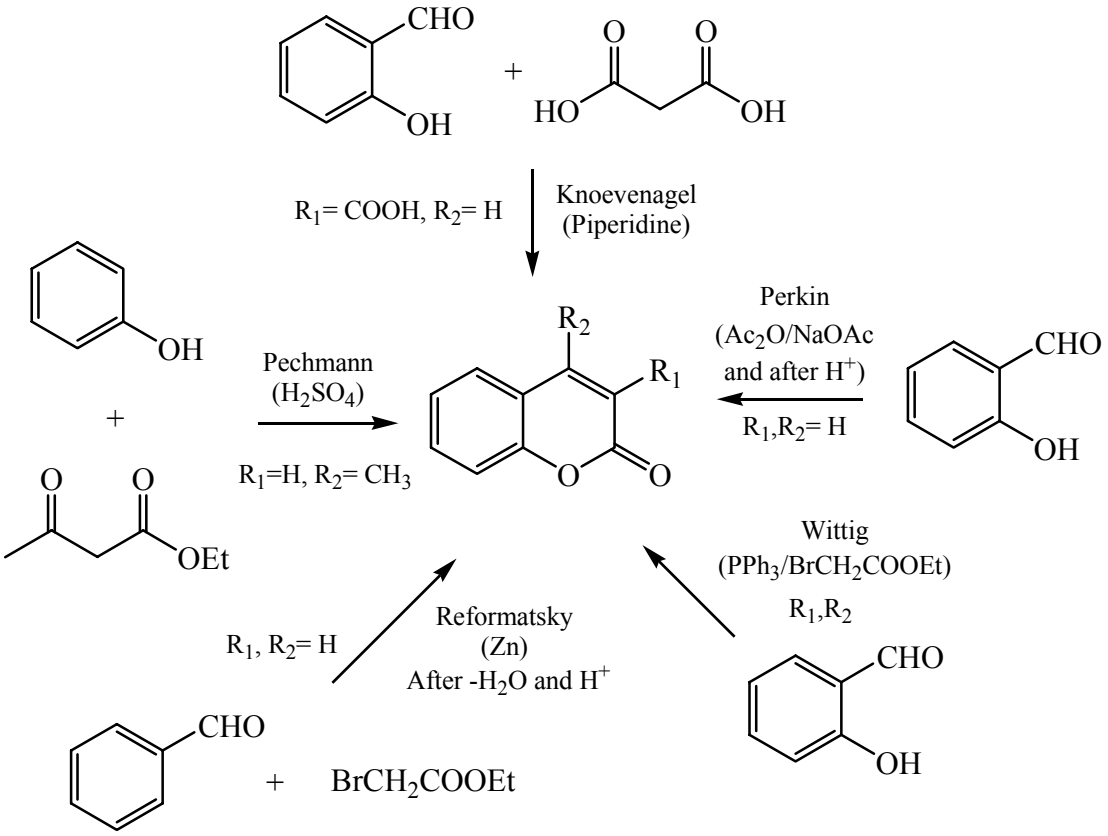
Relationship between cytotoxicity and antioxidant effect has been narrowly related with those diseases. The effects of antioxidants in animal studies are complex, and however, also include tumor promotion, carcinogenic, and co-carcinogenic activities[87]. The different cytotoxic values found for the coumarins could be related to the presence and positions of the hydroxyls in their structures. Generally the *in vitro* structure-activity relationship, studies have shown that cytotoxicity and antioxidant effect are found with derivatives containing ortho-dihydroxy substituents[88,89,90]. Also, the chemical-structure/biological activity study of coumarins showed, that the addition of a cathecolic group to the basic structure induces an increase in the cytotoxic activity in tumor cell lines[88].

The benzopyran-2-ones and 3-ones (coumarins) molecules have been shown to possess unique antiedema and antiinflammatory activities, and these make it particularly effective in the treatment of all high-protein edemas[91,92]. Several natural or synthetic coumarins with various hydroxy and other substituents were found to inhibit lipid peroxidation and to scavenge hydroxyl radicals, superoxide radicals and hypochlorous acid[93]. The dihydroxylated coumarins with neighboring hydroxy groups are all also actives[94]. Furthermore some newly synthesized coumarins condensed with an heteroaromatic ring were found to act as antiinflammatories or antioxidants[95,96,97]. In this case we find naphthalene derivatives, from of which it has been recently isolated a new antioxidant naphthalene glycoside from plants[98]. Some synthetic structures have been reported involving the naphthol aromatic system, producing an antioxidant effect 10 times higher than vitamin E[99], besides of other compounds reported as potential anti HIV agents[100,101].

In this work the attention has been focused mainly on hydroxycoumarins as they are presents as valuable compounds with associated biological activities[102,103,104,105] and naphtopyrones which have not been significately explored as biologically active compounds, and of which some biological activities have been recently reported[106] that are not significately explored.

2.1 STATE OF THE ART

The known Knoevenagel condensation[107,108,109], methods of Perkin[110], Reformatsky[111], Wittig[112,113] and Pechmann's reaction[114] have been the most commonly used for obtaining coumarins. However, is the last one, the most widely applied in the course of the years due to its importance in the obtaining either tri or tetra substituted hydroxy coumarins or benzopyran-(2 or 3)-ones[115,116,117] (Scheme 16).



Scheme 16

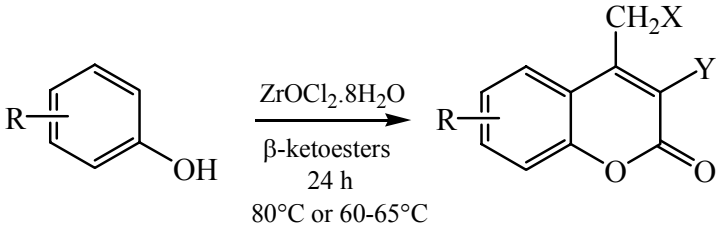
The Pechmann's method is based on the simple condensation of phenols with β-ketoesters in presence of an acidic catalyst in good yields[118]. Catalyst such as sulfuric acid[114], phosphorus pentoxide[119,120], aluminium chloride[121], zinc chloride[122], trifluoroacetic acid[123], zeolites[124] as a modification of the method, chloroaluminated ionic liquids[125], indium chloride[126], gallium triiodide[117], sulfamic acid[127], titanium tetrachloride[128] and recently zirconium tetrachloride[129] have been used.

2.2 RESULTS AND DISCUSSION

The classical Pechmann synthesis of coumarins requires significant quantities of sulfuric acid as both catalyst and solvent. Since this procedure inherently leads to large quantities of acidic waste, alternative, environmentally friendly reagents and catalysts (as mentioned before) have been sought in order to reduce the amount of acidic effluent that has to be treated, thus diminishing the total cost of the process. However, the catalyst used in quantities of 10% mol continues to give strongly acidic waste. Only in case of using a combination of acrilyc acid instead of β-ketoesters,

and zeolites as catalyst[124] not acidic waste is obtained, although this is a great advantage, the scope of the method is quite limited and it has been only applicable to a reduced number of phenols, high temperatures are used and byproducts are formed.

With this work it is wish to report that catalytic amounts (1% w/w) of zirconyl chloride octahydrate either in neat or in alcoholic solution enables coumarins to be prepared in good yields without the need of acid solvents (Scheme 17).



R= OH, OCH_3, COOH X or Y= H or Cl

Scheme 17

In order to obtain the wished coumarins we started to use the zirconyl chloride octahydrate as catalyst in a quantity of 10% (w/w) in relation with resorcin, and ethyl acetoacetate as β-ketoester. In a first experience, reaction was carried out at r.t. showing not much progress after 24 hours. A second experience was carried out under the same reagents and quantity of the catalyst, this time increasing the temperature up to 80°C and results were promissings with total consumption of the starting phenol but isolation of the final product from the mixture was not possible by crystallization and gummy products were obtained.

With all these results was settled a third experience keeping similar the conditions of reaction and concentration of reagents, this time bringing the catalyst concentration down to 1% (w/w). The reaction was left overnight and after 24 hours we observed by TLC a total transformation of the starting phenol into the final coumarin without formation of byproducts. This time the product was easily isolated and cristallized in good yield.

The experience was extended to pyrogallol, phloroglucinol, 2,6-dihydroxy benzoic acid and 4-hydroxyanisole obtaining similar results, only in case of 4-hydroxyanisole the yield was lower as usually (13%). Moreover, reaction was possible only in case of having a strong activating group in the ring because when we carried out the same reaction on phenol, 2-bromophenol, 4-ethylphenol,

4-*tert*-butyl-2-methylphenol, 3-chlorophenol, 4-chlororesorcin and 6-bromo-2-naphthol, the starting materials were quantitatively recovered. It should also be noted as exception of the rule that catechol and *tert*-butyl catechol do not react under these conditions.

Once the conditions of reaction were settled and knowing the limitations of the method we changed the β-ketoester by using 2 and 4-chloroketoesters. Under those parameters we obtained resinification of the starting materials and final products were difficulted to extract from the compact resin formed. Having these results, we decided to diminish the temperature until a range of 60 to 65°C keeping constants the others conditions. With this change, significant results were obtained and final products (coumarins) were easily isolated and cristallized. Furthermore, yields were not affected by longer reaction time or increases in the amount of catalyst. The obtained results with zirconyl chloride octahydrate are shown in table 4 in comparison with some of the most communly used catalysts in the Pechmann synthesis of coumarins.

Table 4. Comparison of obtained results with 1% (w/w) zirconyl chloride octahydrate as catalyst with the most communly used catalysts.

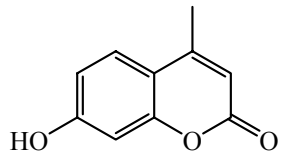
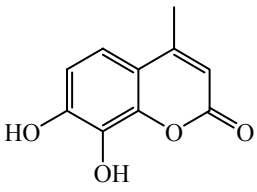
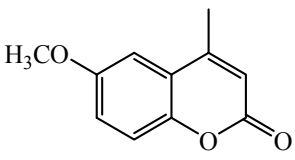
Obtained compounds	Catalysts					
	H_2SO_4[a] Yield (%)	Montm. K-10[b] Yield (%)	$InCl_3$[c] Yield (%)	$TiCl_4$[d] Yield (%)	$ZrCl_4$[e] Yield (%)	$ZrOCl_2.8H_2O$ Yield (%)
 **21**	82-90[130]	96[131]	98[126]	97[128]	92[129] 95[132]	90.6
 **22**	69[133]	66[131]	-	-	96[129]	64
 f * **23**[134]	-	-	-	-	-	13

Table 4. *Continuation.*

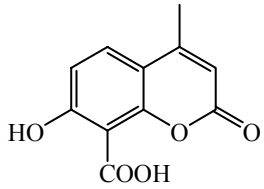
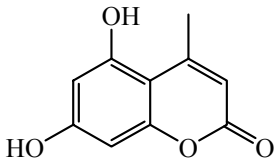
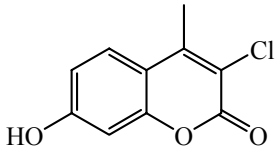
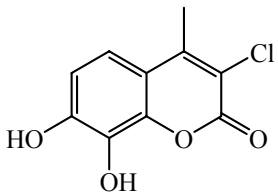
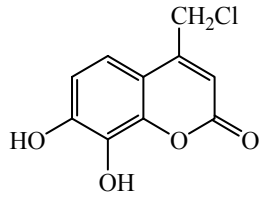
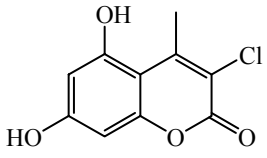
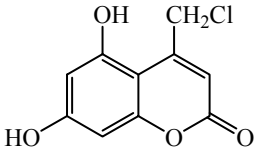
Obtained compounds	Catalysts					
	H_2SO_4[a] Yield (%)[130]	**Montm. K-10[b] Yield (%)**	**$InCl_3$[c] Yield (%)**	**$TiCl_4$[d] Yield (%)**	**$ZrCl_4$[e] Yield (%)**	**$ZrOCl_2.8H_2O$ Yield (%)**
 g * **24**[135]	-	-	-	-	-	58.2
 **25**	Not rep.[136]	88[131]	92[126]	96[128]	93[129]	47.6
 **26**	Not rep.[137]	-	-	-	-	98.3
 **27**	Not rep.[137]	-	-	-	-	85.7
 **28**	65[138]	-	-	-	98[129]	91.6
 **29**	Not rep.[139]	-	-	-	-	77

Table 4. *Continuation.*

Obtained compounds	Catalysts					
	H_2SO_4[a] Yield (%)[130]	Montm. K-10[b] Yield (%)	$InCl_3$[c] Yield (%)	$TiCl_4$[d] Yield (%)	$ZrCl_4$[e] Yield (%)	$ZrOCl_2.8H_2O$ Yield (%)
 **30**	-	-	-	95[128]	97[129]	93.8

a: Concentrated sulphuric acid was used both as solvent and catalyst at r.t.

b: Toluene was used as solvent at reflux.

c: 10 mol % used in relation with the starting phenol without solvent, reflux (65°C).

d: 50 mol % used in relation with the starting phenol without solvent, r.t.

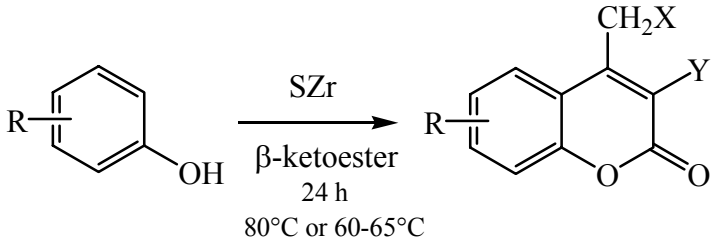
e: 10 mol % used in relation with the starting phenol without solvent, r.t.

f: Silica chloride (SiO_2Cl) was used at 85°C without solvent, 81% yield.

g: Synthesized by carboxylation of **21**, 47% yield.

*: The only reference in literature.

These results encorageous us to deepen in the use of zirconium catalysts as alternatives of using strongly acidic catalysts and another derivative was tested, the sulfated zirconia (SZr) (Scheme 18).



R= OH X or Y= H or Cl

Scheme 18

Sulfated zirconia is an acidic catalyst, which is not as strong as inorganic acids. It has been employed in the nitration of chlorobenzene[140], dehydration of alcohols[141], synthesis of benzodiazepine derivatives[142], alkane isomerizations[143] and acylation of aromatic rings[144]. This catalyst was prepared from zirconyl chloride octahydrate and in the same way that Sun *et. al*.[145].

Taking as reference the settled conditons when we worked with zirconyl chloride octahydrate, we carried out the same reaction using SZr (1% w/w) as catalyst and similar results were obtained when we work with ethyl acetoacetate and chloro-β-ketoesters. Moreover, reactions where SZr was used did neither elapse when not strongly activating groups are not presents nor in case of catechol and *tert*-butyl catechol.

Yields are not affected by longer reaction time or increases in the amount of catalyst when SZr is used. Only for obtaining 7,8-dihydroxy-4-methyl coumarin (**22**) and 3-chloro-7-hydroxy-4-methyl coumarin (**26**), a greater amount of SZr (10% w/w) had to be used in order to increase yields.

As difference between both catalysts, the activity of SZr was slightly lower than zirconyl chloride. This affirmation was possible when comparing results they show almost similar behavior which are reflected in the similar yields of reactions and only in the case of 4-hydroxyanisole (13% yield is obtained when zirconyl chloride is used), reaction did not takes place when using SZr (see Table 5). Moreover, it was neither possible to obtain compounds **23** and **24** using SZr.

Table 5. Comparison of obtained results with sulfated zirconia and zirconyl chloride octahydrate.

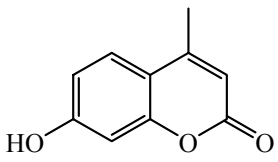
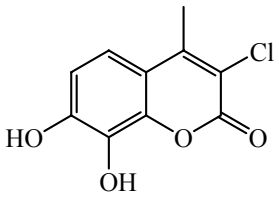
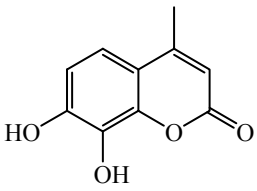
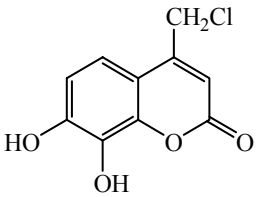
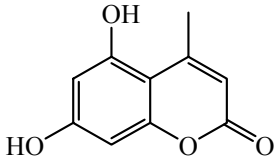
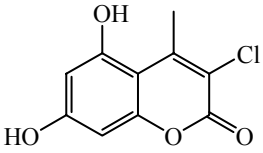
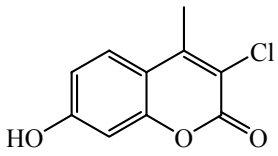
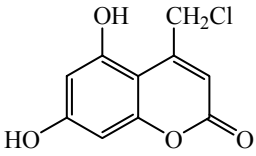
Obtained compounds	Catalysts		Obtained compounds	Catalysts	
	$ZrOCl_2.8H_2O$ Yield (%)	SZr Yield (%)		$ZrOCl_2.8H_2O$ Yield (%)	SZr Yield (%)
 **21**	90.6	90.6	 **27**	85.7	83.5
 **22**	64	92.3*	 **28**	91.6	85.1

Table 5. *Continuation.*

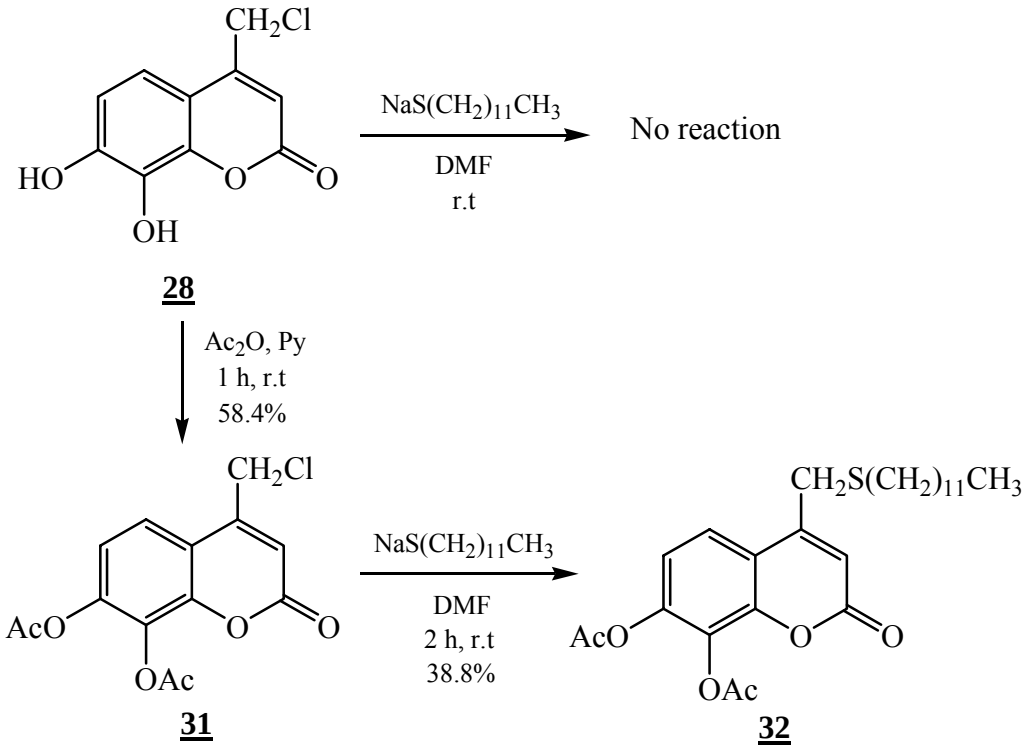
Obtained compounds	Catalysts		Obtained compounds	Catalysts	
	$ZrOCl_2.8H_2O$ Yield (%)	SZr Yield (%)		$ZrOCl_2.8H_2O$ Yield (%)	SZr Yield (%)
 **25**	47.6	52	 **29**	77	73.5
 **26**	98.3	87*	 **30**	93.8	93

* 10% of SZr was used.

Having the already synthesised hydroxycoumarins as starting point and knowing the antioxidative effect of some of them in a preliminary test and some literature reports[146,147,148], it was decided to carry out some structural modifications in order to increase their antioxidant activity depending of the substituent.

Different attempts were developed to carry out the structural modifications over the most promissing compound, the 4-chloromethyl-7,8-dihydroxy pyran-3-one (4-chloromethyl-7,8-dihydroxy coumarin) (**28**). In that way the strategy was to exchange the chlorine atom by other nucleophiles and the catecholic hydroxy functions by fused six member ring derivatives, more exactly in order to obtain naphtho pyran-2-ones and 3-ones.

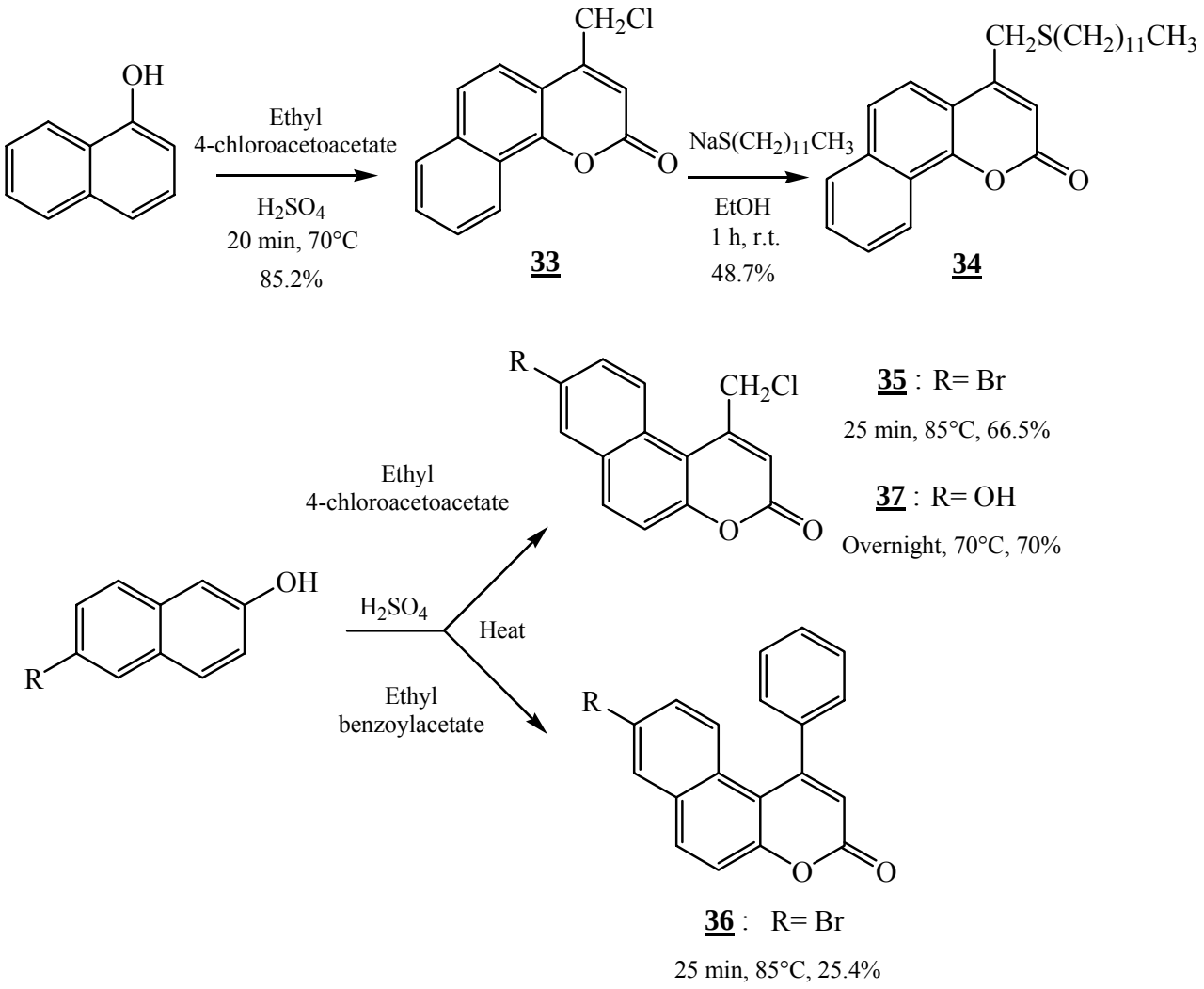
Taking the vast experience of the work group in the synthesis of sulfur containing compounds and the importance of having molecules with good lipophilicity, the first attempt of structural modification consisted in susbstituting the chlorine atom by the dodecylsulfanyl radical. This was done by reaction of the thiolate with compound **28** keeping the hydroxy functions not protected. Despite of the reactivity of the alkyl halide **28**, the reaction did not takes place and starting coumarin and dodecylmercaptan were recovered after acidifying with hydrochloric acid (Scheme 19).



Scheme 19

A second experience was carried out over the di-O-acetylated derivative **31**, which was previously obtained by reaction of **28** with a mixture acetic anhydride-pyridine. The alkylation reaction of **31** over the corresponding thiolate tooks place in two hours and desired product (**32**) is recovered after acidifying, in good purity with yields next to 39%.

In the other hand, it was not possible to apply the zirconium catalysts to the synthesis of compounds **33**-**37**, so in these cases the classical Pechmann procedure was used but with only 2 equivalents of sulfuric acid instead of large volumens as both solvent and catalyst (Scheme 20). In these cases, sensitive variations in yields were obtained as consecuence of the different reactivities of the β ketoesters and used naphtols.

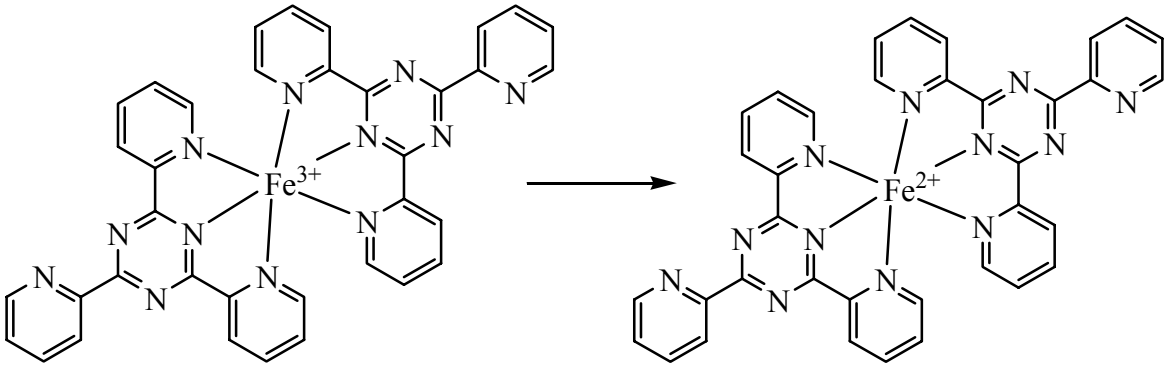


Scheme 20

Compound **34** was synthesized by attack of the previously formed thiolate over the weak electronically charged methylene group of **33** (Scheme 20).

2.2.1 *Antioxidant properties of synthesized coumarins.*

As a way of complementig the synthesis work we have tested the antioxidant properties of different cumarines by measuring their capacity of reducing the Fe^{3+} according the method of Benzie and Strain[149], called FRAP method (Ferric Reducing Ability of Plasma). According to this procedure, reduction of the yellowish colored Fe^{3+}-tripyridiltriazine complex (Fe^{3+}-TPTZ) in acidic media produce a Fe^{2+} dark blue colored complex (Scheme 21).



Fe^{3+}-TPTZ + Reducing agent ⟶ Fe^{2+}-TPTZ Dark bleu coloration at 593 nm

Scheme 21

The Fe^{3+}-TPTZ complex is prepared as a mixture of TPTZ and $FeCl_3$ in hydrochloric acid 40 mM and acetate tampon until pH= 3,6. The reducing agent is added to the previously prepared mixture and after a brief period of incubation (30 min) the absorbance measurement is carries out at 593 nm. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. The results of the FRAP method are showed in table 6.

Table 6. Evaluation of the antioxidant potential of some of the synthesized coumarins by the FRAP method.

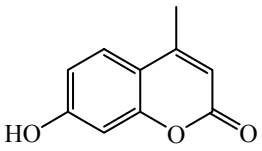
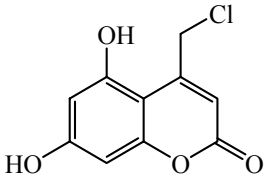
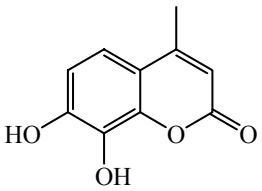
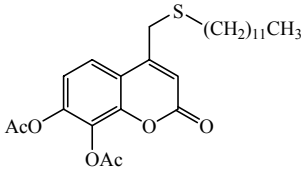
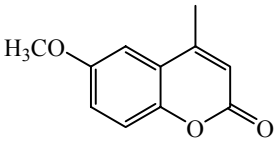
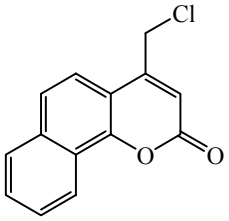
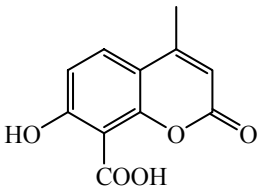
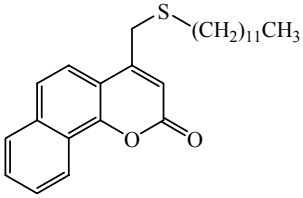
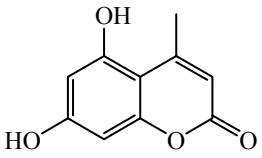
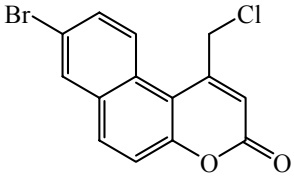
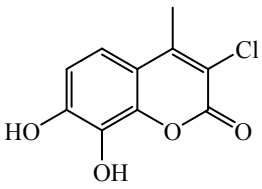
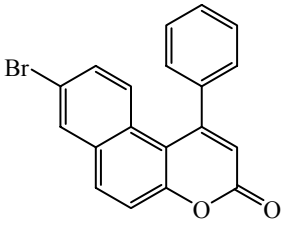
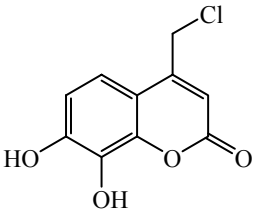
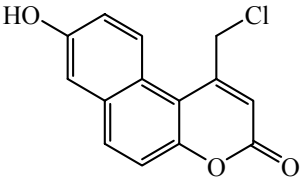
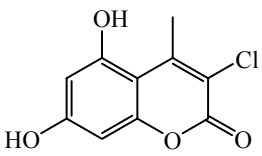
Compound	FRAP (mole Fe^{2+}/ mole compound)*	Compound	FRAP (mole Fe^{2+}/ mole compound)*
 **21**	0,18 ± 0,12	 **30**	0,71 ± 0,2
 **22**	**3,15 ± 0,22**	 **32**	0.12 ± 0.01

Table 6. *Continuation.*

Compound	FRAP (mole Fe^{2+}/ mole compound)*	Compound	FRAP (mole Fe^{2+}/ mole compound)*
 **23**	0.21 ± 0.14	 **33**	0.11 ± 0.01
 **24**	0,2 ± 0,12	 **34**	0.18 ± 0
 **25**	1,04 ± 0,22	 **35**	0.06 ± 0
 **27**	**3,38 ± 0,28**	 **36**	0.23 ± 0.01
 **28**	**3,66 ± 0,26**	 **37**	1.35 ± 0.04
 **29**	1,134 ± 0,25	* 5 μM of compound was used.	

The obtained results were as expected. Obtained values for compounds **22**, **27** and **28** shows an antioxidant activity increased when hydroxyl catecholic functions are presents. Another structural aspect to have into account, if we take the refered compounds as reference, is the presence of chlorine atoms in the estructure which makes possible a slight increment of the antioxidant activity.

These results were better than those obtained for commercial drugs as Trolox and Resveratrol and almost similar to those of Mangiferin, a polyhydroxylated xanthone, and as a manner of comparison, we show them in Fig. 5.

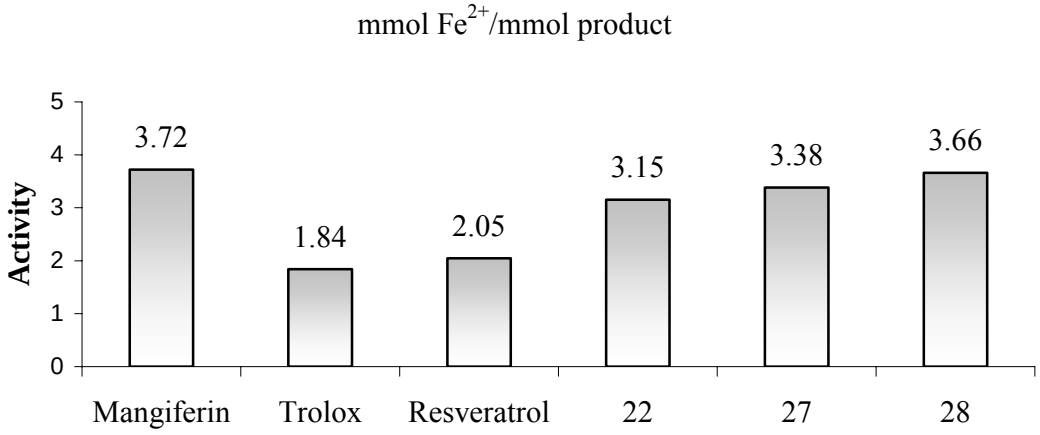


Fig. 5

2.3 EXPERIMENTAL PART

General Methods

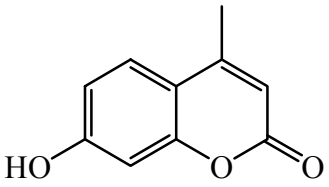
The reagents were purchased from Acros Organics. TLC was carried out using silica gel plates Alugram Sil G/UV 254. The ^{1}H and ^{13}C NMR spectra were recorded on an AC Bruker 250 MHz spectrometer using DMSO-d_6 as both solvent and internal standard. The chemical shifts are reported in ppm and coupling constants (*J*) in Hz. Melting points were determined on a Stuart Scientific SMP 3 capillary melting point apparatus and are uncorrected. MS were recorded on a HRMS Micromass Autospec 3F.

2.3.1 *Synthesis of hydroxy benzopyran-2-ones (hydroxycoumarins) by using zirconyl chlorid octahydrate as catalyst.*

a) Typical procedure for obtaining not halogenated hydroxycoumarins.

Zirconyl chloride octahydrate (1 mol%) was added to an equimolar mixture of phenol and acetoacetyl ester and stirred at 80°C for 24 h. When all the starting material was consumed (TLC), and while still hot, the mixture was poured into vigorously stirred cold water (20 mL/g of starting phenol). The precipitate was filtered, washed with cold water and dried at 50°C overnight, yielding the coumarin.

***7-Hydroxy-4-methyl-1-benzopyran-2-one (7-hydroxy-4-methyl coumarin)* (21)**



Yield: 7.25 g (90.6 %) of **21** from 5g (45 mmol) of resorcin and 5.74 mL (45 mmol) of ethyl acetoacetate.

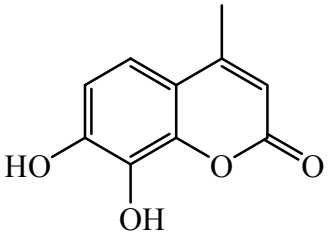
TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 186-189°C mp_{lit}: 185°C [130]

^{1}H-NMR (DMSO-d_6): δ= 10.53 (s, 1H, OH), 7.59 (d, $J_{8,6}$ = 8.73, 1H, H-8,), 6.81 (dd, $J_{6,5}$ = 2.03, $J_{6,8}$ = 2.3, 1H, H-6), 6.71 (d, $J_{5,6}$ = 2.22, 1H, H-5), 6.13 (s, 1H, H-3), 2.37 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 161.33, 160.47, 155.01, 153.72, 126.8, 113.03, 112.20, 110.43, 102.37, 18.3.

***7,8-Dihydroxy-4-methyl-1-benzopyran-2-one (7,8-dihydroxy-4-methyl coumarin)* (22).**



Yield: 4.87 g (64 %) of **22** from 5g (39.6 mmol) of pyrogallol and 5.01 mL (39.6 mmol) of ethyl acetoacetate.

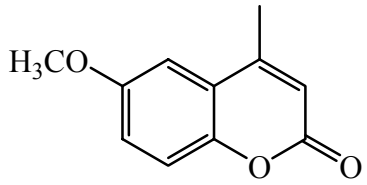
TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 242-244°C mp_{lit}: 241-243°C[150], 234-235°C [151]

^{1}H-NMR (DMSO-d_6): δ= 7.09 (d, $J_{6,5}$=8.6, 1H, H-6), 6.82 (d, $J_{5,6}$ = 8.61, 1H, H-5), 6.13 (s, 1H, H-3), 2.35 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 160.42, 154.14, 149.61, 143.53, 132.35, 115.69, 112.98, 112.32, 110.40, 18.45.

***6-Methoxy-4-methyl-1-benzopyran-2-one (6-methoxy-4-methyl coumarin)* (23).**



Yield: 1.0 g (13 %) of **23** from 5g (40.3 mmol) of 4-hydroxy anisol and 5.09 mL (40.3 mmol) of ethyl acetoacetate.

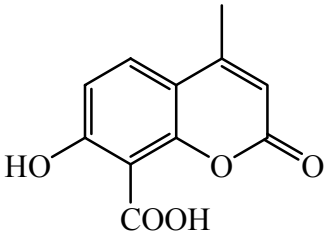
TLC: Chloroform-acetone, (10:1, v:v)

m.p: 163-165°C mp_{lit}: 169°C[134]

^{1}H-NMR (DMSO-d_6): δ= 7.31 (d, $J_{8,7}$ = 12.8, 1H, H-8), 7.23 (d, $J_{5,7}$ = 3.05, 1H, H-5), 7.17 (d, $J_{7,6}$ = 2.43, 1H, H-7), 6.4 (s, 1H, H-3), 3.84 (s, 3H, CH_3O), 2.44 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 160.04, 155.71, 153.12, 147.36, 120.23, 119.20, 117.62, 114.83, 108.24, 55.89, 18.32.

***8-Carboxy-7-hydroxy-4-methyl-1-benzopyran-2-one (8-carboxy-7-hydroxy-4-methyl coumarin)* (24).**



Yield: 4.16 g (58.2 %) of **24** from 5g (32.4 mmol) of 2,6-dihydroxy benzoic acid and 4.1 mL (32.4 mmol) of ethyl acetoacetate.

TLC: Chloroform-EtOAc-AcOH, (8:6:1, v:v)

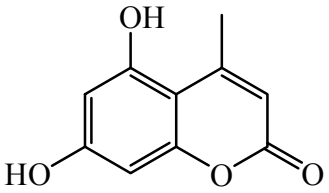
m.p: 266-268°C mp_{lit}: 282-284°C[135]

^{1}H-NMR (DMSO-d_6): δ= 7.67 (d, $J_{5,6}$ = 8.77, 1H, H-5), 6.92 (d, $J_{6,5}$ = 8.84, 1H, H-6), 6.24 (s, 1H, H-3), 2.38 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 169.3, 166.7, 159.97, 159.31, 127.8, 113.15, 112.2, 110.9, 110.6, 18.5.

HRMS: *m/z*$[M+H]^+$ calcd for $C_{11}H_8O_5$: 220.178; found: 220.037.

***5,7-Dihydroxy-4-methyl-1-benzopyran-2-one (5,7-dihydroxy-4-methyl coumarin)* (25)**



Yield: 2.82 g (47.6 %) of **25** from 5g (30.8 mmol) of phloroglucinol and 3.89 mL (30.8 mmol) of ethyl acetoacetate.

TLC: Chloroform-ethy acetate-AcOH, (8:6:1, v:v)

m.p: 289-290°C (decomp.) mp_{lit}: 292-293°C [152]

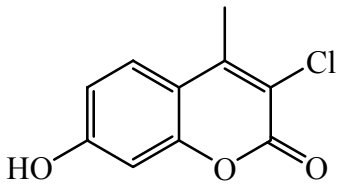
^{1}H-NMR (DMSO-d_6): δ= 10.53 (s, 1H, OH), 10.31 (s, 1H, OH), 6.26 (d, $J_{8,6}$ = 1.82, 1H, H-8), 6.16 (d, $J_{6,8}$ = 2.45, 1H, H-6), 5.85 (s, 1H, H-3), 2.48 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 161.33, 160.38, 158.20, 156.76, 155.24, 109.08, 102.35, 99.34, 94.77, 23.70.

b) Typical procedure for obtaining halogenated hydroxycoumarins.

Zirconyl chloride octahydrate (1 mol%) was added to an equimolar mixture of phenol and acetoacetyl ester and stirred at 60–65°C for 2 h before EtOH (1 mL/g of phenol) was added. The reaction was stirred for an additional 22 h. When all the starting material was consumed (TLC), the reaction was diluted with EtOH (1 mL/g of phenol) and poured into vigorously stirred cold water (20 mL/g of starting phenol). The precipitate was filtered, washed with cold water and dried at 50 °C overnight, yielding the coumarin.

***3-Chloro-7-hydroxy-4-methyl-1-benzopyran-2-one (3-chloro-7-hydroxy-4-methyl coumarin)*(<u>26</u>).**



Yield: 9.41 g (98.3 %) of **<u>26</u>** from 5g (45 mmol) of resorcin and 6.17 mL (45 mmol) of ethyl 2-chloroacetoacetate.

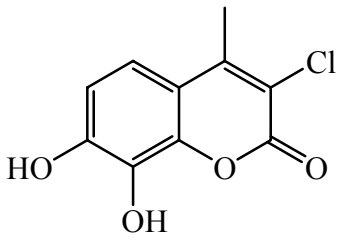
TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 236-240°C mp_{lit}: 242-243°C [153]

^{1}H-NMR (DMSO-d_6): δ= 7.65 (d, $J_{5,6}$ = 9.15, 1H, H-5), 6.84 (dd, $J_{6,5}$ = 2.45, $J_{6,8}$ = 2.43, 1H, H-6), 6.72 (d, $J_{8,6}$ = 1.83, 1H, H-8), 2.51 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 161.51, 156.78, 152.94, 149.23, 127.34, 115.52, 113.79, 111.85, 102.34, 16.21.

***3-Chloro-7,8-dihydroxy-4-methyl-1-benzopyran-2-one (3-chloro-7,8-dihydroxy-4-methyl coumarin)* (<u>27</u>).**



Yield: 7.7 g (85.7 %) of **27** from 5g (39.6 mmol) of pyrogallol and 5.4 mL (39.6 mmol) of ethyl 2-chloroacetoacetate.

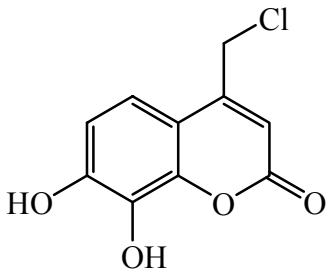
TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 267-268°C (decomp.) mp_{lit}: 265°C [154]

^{1}H-NMR (DMSO-d_6): δ= 7.17 (d, $J_{6,5}$ = 8.75, 1H, H-6), 6.86 (d, $J_{5,6}$ = 8.63, 1H, H-5), 2.49 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 156.76, 149.83, 149.73, 141.62, 132.44, 116.31, 115.52, 113.09, 112.74, 16.43.

***4-Chloromethyl-7,8-dihydroxy-1-benzopyran-2-one (4-chloromethyl-7,8-dihydroxy coumarin)* (28).**



Yield: 8.23 g (91.6 %) of **28** from 5g (39.6 mmol) of pyrogallol and 5.4 mL (39.6 mmol) of ethyl 4-chloroacetoacetate.

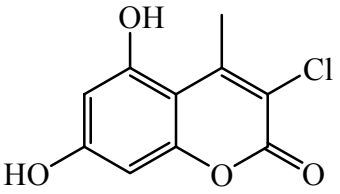
TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 200-201°C mp_{Lit}: 198-199 °C [155]

^{1}H-NMR (DMSO-d_6): δ= 7.18 (d, $J_{6,5}$ = 8.65, 1H, H-6); 6.86 (d, $J_{5,6}$ = 8.78, 1H, H-5), 6.42 (s, 1H, H-3), 4.94 (s, 2H, CH_2Cl).

^{13}C-NMR (DMSO-d_6): δ= 160.37, 151.64, 150.02, 143.93, 132.73, 115.72, 112.59, 111.21, 110.38, 41.75.

***3-Chloro-5,7-dihydroxy-4-methyl-1-benzopyran-2-one (3-chloro-5,7-dihydroxy-4-methyl coumarin)* (29).**



Yield: 5.38 g (77 %) of **29** from 5g (30.8 mmol) of phloroglucinol and 4.2 mL (30.8 mmol) of ethyl 2-chloroacetoacetate.

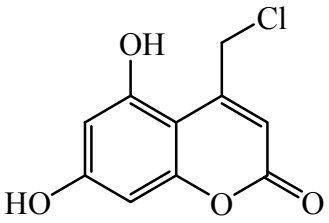
TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 317-319°C mp_{lit}: 306-308°C [156]

^{1}H-NMR (DMSO-d_6): δ= 10.79 (s, 1H, OH), 10.46 (s, 1H, OH), 6.32 (d, $J_{8,6}$ = 2.42, 1H, H-8), 6.21 (d, $J_{6,8}$ = 1.82, 1H, H-6), 2.70 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 161.35, 157.88, 156.55, 154.32, 150.53, 113.86, 102.06, 99.94, 94.68, 20.18.

***4-Chloromethyl-5,7-dihydroxy-1-benzopyran-2-one (4-chloromethyl-5,7-dihydroxy coumarin)* (30).**



Yield: 6.56 g (93.8 %) of **30** from 5g (30.8 mmol) of phloroglucinol and 4.2 mL (30.8 mmol) of ethyl 4-chloroacetoacetate.

TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 246-248°C mp_{lit}: 243-245°C [150]

^{1}H-NMR (DMSO-d_6): δ= 10.94 (s, 1H, OH), 10.42 (s, 1H, OH), 6.28 (d, $J_{8,6}$ = 2.42, 1H, H-8), 6.22-6.21 (m, 2H, H-6 and H-3), 5.03 (s, 2H, CH_2), 2.48 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 161.72, 160.33, 157.34, 156.69, 152.22, 108.94, 100.02, 99.46, 95.02, 45.21.

2.3.2 *Synthesis of hydroxy benzopyran-2-ones by using sulphated zirconia as catalyst*

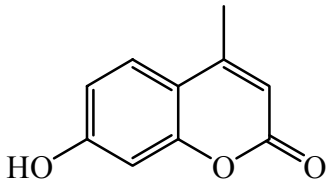
a) Preparation of the catalyst (Sulfated zirconia).

1 g (3.1 mmol) of zirconyl chloride octahydrate and 2.46 g (18.6 mmol) of ammonium sulphate were grounded in a mortar for 20 min at r.t.. The mixture was left to r.t. for 18 hours and calcined for 5 h at 600 °C[145].

b) Typical procedure for obtaining not halogenated hydroxycoumarins.

Sulphated zirconia (1% weight of the phenol) was added to a mixture of equimolar quantities of phenols and acetoacetyl esters and left at 80°C with rapid stirring for 24 h. The reaction was monitored by TLC. When all the starting materials were consumed and while hot the mixture was poured into cold water (20 mL/g of starting phenol) with rapid stirring. The precipitate was filtered, washed with cold water and dried at 50°C overnight, yielding the hydroxycoumarins.

***7-Hydroxy-4-methyl-1-benzopyran-2-one (7-hydroxy-4-methyl coumarin)* (21)**



Yield: 7.25 g (90.6 %) of **21** from 5g (45 mmol) of resorcin and 6.17 mL (45 mmol) of ethyl 2-chloroacetoacetate.

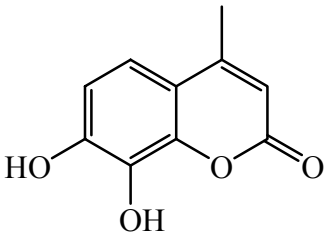
TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 186-189°C mp_{lit}: 188°C[157]

^{1}H-NMR (DMSO-d_6): δ= 10.53 (s, 1H, OH), 7.59 (d, $J_{8,6}$ = 8.73, 1H, H-8,), 6.81 (dd, $J_{6,5}$ = 2.03, $J_{6,8}$ = 2.3, 1H, H-6), 6.71 (d, $J_{5,6}$ = 2.22, 1H, H-5), 6.13 (s, 1H, H-3), 2.37 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 161.33, 160.47, 155.01, 153.72, 126.8, 113.03, 112.20, 110.43, 102.37, 18.3.

***7,8-Dihydroxy-4-methyl-1-benzopyran-2-one (7,8-dihydroxy-4-methyl coumarin)* (22).**



Yield: 7.03 g (92.3 %) of **22** from 5g (39.6 mmol) of pyrogallol and 5.01 mL (39.6 mmol) of ethyl acetoacetate. In this case 10% of SZr was used.

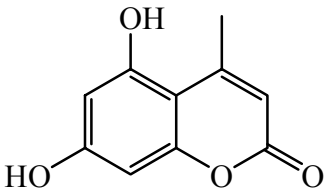
TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 242-244°C mp_{lit}: 241-243°C [150]

^{1}H-NMR (DMSO-d_6): δ= 7.09 (d, $J_{6,5}$=8.6, 1H, H-6), 6.82 (d, $J_{5,6}$ = 8.61, 1H, H-5), 6.13 (s, 1H, H-3), 2.35 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 160.42, 154.14, 149.61, 143.53, 132.35, 115.69, 112.98, 112.32, 110.40, 18.45.

***5,7-Dihydroxy-4-methyl-1-benzopyran-2-one (5,7-dihydroxy-4-methyl coumarin)* (25)**



Yield: 3.08 g (52 %) of **25** from 5g (30.8 mmol) of phloroglucinol and 3.89 mL (30.8 mmol) of ethyl acetoacetate.

TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 289-290°C (decomp.) mp_{lit}: 292-293°C [152]

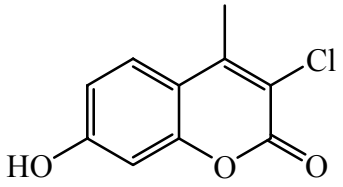
^{1}H-NMR (DMSO-d_6): δ= 10.53 (s, 1H, OH), 10.31 (s, 1H, OH), 6.26 (d, $J_{8,6}$ = 1.82, 1H, H-8), 6.16 (d, $J_{6,8}$ = 2.45, 1H, H-6), 5.85 (s, 1H, H-3), 2.48 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 161.33, 160.38, 158.20, 156.76, 155.24, 109.08, 102.35, 99.34, 94.77, 23.70.

c) Typical procedure for obtaining halogenated hydroxycoumarins.

Sulphated zirconia (1% weight of the phenol) was added to a mixture of equimolar quantities of phenols and acetoacetyl esters and left at 60-65°C with rapid stirring for 24 h. The reaction was monitored by TLC. When all the starting materials were consumed and while hot the mixture was poured into cold water (20 mL/g of starting phenol) with a rapid stirring. The precipitate was filtered, washed with cold water and dried at 50°C overnight, yielding the hydroxycoumarins.

***3-Chloro-7-hydroxy-4-methyl-1-benzopyran-2-one (3-chloro-7-hydroxy-4-methyl coumarin)*(<u>26</u>).**



Yield: 8.3 g (87 %) of **<u>26</u>** from 5g (45 mmol) of resorcin and 6.17 mL (45 mmol) of ethyl 2-chloroacetoacetate. 10% SZr was used in this case.

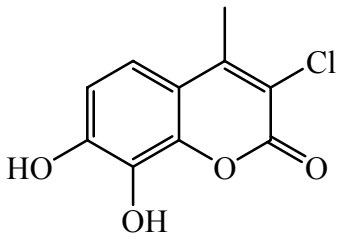
TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 236-240°C mp_{lit}: 236°C[137] , 240°C[158]

^{1}H-NMR (DMSO-d_6): δ= 7.65 (d, $J_{5,6}$ = 9.15, 1H, H-5), 6.84 (dd, $J_{6,5}$ = 2.45, $J_{6,8}$ = 2.43, 1H, H-6), 6.72 (d, $J_{8,6}$ = 1.83, 1H, H-8), 2.51 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 161.51, 156.78, 152.94, 149.23, 127.34, 115.52, 113.79, 111.85, 102.34, 16.21.

***3-Chloro-7,8-dihydroxy-4-methyl-1-benzopyran-2-one (3-chloro-7,8-dihydroxy-4-methyl coumarin)* (<u>27</u>).**



Yield: 7.5 g (83.5 %) of **27** from 5g (39.6 mmol) of pyrogallol and 5.4 mL (39.6 mmol) of ethyl 2-chloroacetoacetate.

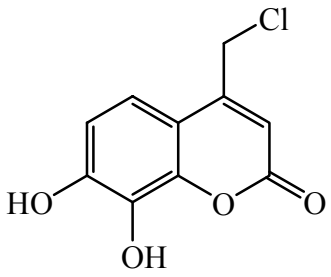
TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 267-268°C (decomposition) mp_{lit}: 265°C[154]

^{1}H-NMR (DMSO-d_6): δ= 7.17 (d, $J_{6,5}$ = 8.75, 1H, H-6), 6.86 (d, $J_{5,6}$ = 8.63, 1H, H-5), 2.49 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 156.76, 149.83, 149.73, 141.62, 132.44, 116.31, 115.52, 113.09, 112.74, 16.43.

***4-Chloromethyl-7,8-dihydroxy-1-benzopyran-2-one (4-chloromethyl-7,8-dihydroxy coumarin)* (28).**



Yield: 7.65 g (85.1 %) of **28** from 5g (39.6 mmol) of pyrogallol and 5.4 mL (39.6 mmol) of ethyl 4-chloroacetoacetate.

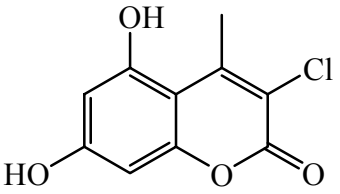
TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 200-201°C mp_{Lit}: 198-199 °C[155]

^{1}H-NMR (DMSO-d_6): δ= 7.18 (d, $J_{6,5}$ = 8.65, 1H, H6); 6.86 (d, $J_{5,6}$ = 8.78, 1H, H-5), 6.42 (s, 1H, H-3), 4.94 (s, 2H, CH_2Cl).

^{13}C-NMR (DMSO-d_6): δ= 160.37, 151.64, 150.02, 143.93, 132.73, 115.72, 112.59, 111.21, 110.38, 41.75.

***3-Chloro-5,7-dihydroxy-4-methyl-1-benzopyran-2-one (3-chloro-5,7-dihydroxy-4-methyl coumarin)* (29).**



Yield: 5.14 g (73.5 %) of **29** from 5g (30.8 mmol) of phloroglucinol and 4.2 mL (30.8 mmol) of ethyl 2-chloroacetoacetate.

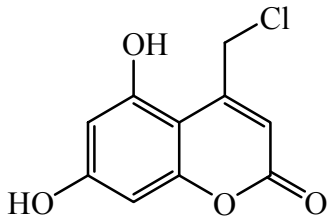
TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

m.p: 317-319°C mp_{lit}: 306-308°C[154]

^{1}H-NMR (DMSO-d_6): δ= 10.79 (s, 1H, OH), 10.46 (s, 1H, OH), 6.32 (d, $J_{8,6}$ = 2.42, 1H, H-8), 6.21 (d, $J_{6,8}$ = 1.82, 1H, H-6), 2.70 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ= 161.35, 157.88, 156.55, 154.32, 150.53, 113.86, 102.06, 99.94, 94.68, 20.18.

***4-Chloromethyl-5,7-dihydroxy-1-benzopyran-2-one (4-chloromethyl-5,7-dihydroxy coumarin)* (30).**



Yield: 6.5 g (93 %) of **30** from 5g (30.8 mmol) of phloroglucinol and 4.2 mL (30.8 mmol) of ethyl 4-chloroacetoacetate.

TLC: Chloroform-ethyl acetate-AcOH, (8:6:1, v:v)

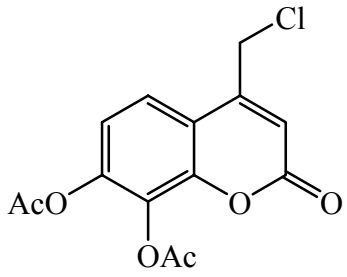
m.p: 246-248°C mp_{lit}: 243-245°C[132]

^{1}H-NMR (DMSO-d_6): δ: 10.94 (s, 1H, OH), 10.42 (s, 1H, OH), 6.28 (d, $J_{8,6}$ = 2.42, 1H, H-8), 6.22-6.21 (m, 2H, H-6 and H-3), 5.03 (s, 2H, CH_2), 2.48 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ: 161.72, 160.33, 157.34, 156.69, 152.22, 108.94, 100.02, 99.46, 95.02, 45.21.

2.3.3 *Preparation of other pyran-2 and 3-ones with potential biological activities.*

***4-Chloromethyl-7,8-diacetoxy-1-benzopyran-2-one (4-chloromethyl-7,8-diacetoxy coumarin)* (31)**



To a solution of 0.2 g (0.88 mmol) of **28** in 1 mL of pyridine were cooled and 1 mL of Ac_2O were dropped and left to r.t. for 1 hour. The mixture was poured onto cold water and left to stir for 30 min. The suspention was filtered and the solid was dried at r.t., protected from light, until constant weight to obtain 0.16g (58.4%) of **31** as a ligh-cream powder.

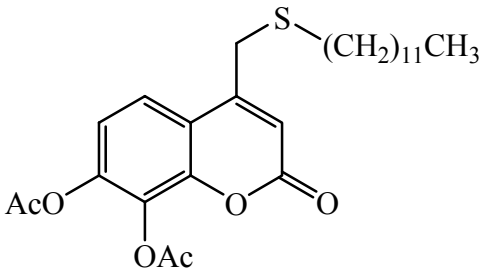
TLC: Chloroform-Acetone; (10:1, v:v).

m.p: 165-167°C (from water). mp_{lit}: 155-157°C[159]

^{1}H-NMR ($CDCl_3$): δ: 7.53 (d, 1H, H-Ph, *J*= 8.75), 7.17 (d, 1H, H-Ph, *J*= 8.75), 6.52 (s, 1H, H-3), 4.61 (s, 2H, CH_2), 2.38 (s, 3H, AcO), 2.32 (s, 3H, AcO).

^{13}C-NMR ($CDCl_3$): δ: 167.72, 167.34, 158.78, 149.26, 147.19, 145.68, 130.79, 121.51, 119.04, 116.25, 115.66, 41.14, 20.66, 20.28.

***7,8-Diacetoxy-4-dodecylsulfanylmethyl-1-benzopyran-2-one (7,8-diacetoxy-4-dodecylsulfanyl-methyl coumarin)* (32)**

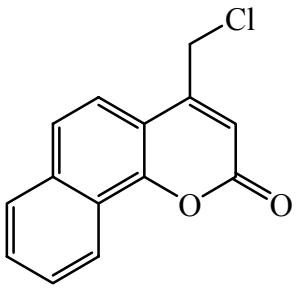


To a suspention of 016 g (6.76 mmol) of NaH, previously washed with petroleum ether, in 10 mL of dry DMF was cooled and 1.62 mL (6.75 mmol) of 1-dodecanthiol were added and left to stir for 2 hours to r.t.. The mixture was cooled and 2g (6.44 mmol) of compound **31** were added at once and mixture was left to stir at r.t. for 60 min. Once all **31** was consumed the mixture was poured onto a solution of HCl (6 mol/L) and extracted with DCM. The organic layer was washed with water and dried. The solvent was removed by reduced presion and the solid remaining was tritured with ethyl ether and filtered to obtain 1.19 g (38.8%) of **32**.

TLC: Petroleum ether-ethyl acetate; (3:1, v:v).
m.p: 98°C (from cyclohexane).
^{1}H-NMR (DMSO-d_6): δ: 7.82 (d, 1H, H-Ph, *J*= 10.0), 7.31 (d, 1H, H-Ph, *J*= 7.5), 6.49 (s, 1H, H-3), 3.95 (s, 2H, CH_2), 2.39 (s, 3H, AcO), 2.33 (s, 3H, AcO), 1.49 (m, 2H, CH_2), 1.20 (s, 20H, CH_2-chain), 0.83 (s, 3H, CH_3).

^{13}C-NMR (DMSO-d_6): δ: 162.36, 161.81, 157.34, 153.17, 146.57, 141.64, 139.75, 124.87, 117.62, 113.44, 111.68, 108.71, 73.75, 26.58, 26.31, 24.03, 24.00, 23.94, 23.73, 23.63, 23.46, 23.23, 17.11, 15.24, 14.85, 8.83.

***4-Chloromethyl-naphtho [1,2-b] pyran-2-one* (33)**



To a mixture of 5g (34.68 mmol) of 1-naphtol and 4.7 mL (34.68 mmol) of ethyl 4-chloroacetoacetate to temperature of 0-10°C were slowly dropped 3.69 mL (69.36 mmol) of concentrated sulfuric acid. Once the addition finished the mixture was kept to 70°C for 20 min. The reaction was controlled by TLC and once it was finished ice and iced water (50 mL) and ethanol (1 mL) were added and left to stir to r.t. for 30 min. The precipitate obtained was filtered, washed with ice water and dried until constant weight to obtain 7.23 g (85.2%). Recristalization from ethanol afforded 4.92 g of a yellow-creamy solid (**33**).

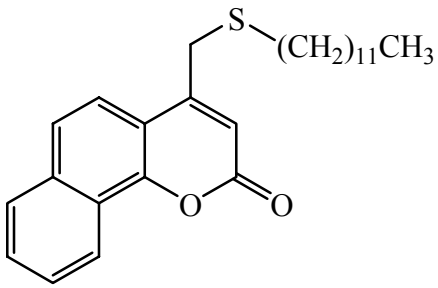
m.p: 171-174°C.

TLC: Petroleum ether-ethyl acetate; (3:1, v:v)

^{1}H-NMR (DMSO-d_6): δ: 8.33 (m, 1H), 8.03 (m, 1H), 7.85 (m, 2H), 7.71 (m, 2H), 6.77 (s, 1H, CH, H-3), 5.08 (s, 2H, CH_2).

^{13}C-NMR (DMSO-d_6): δ: 160.04, 151.99, 150.86, 134.89, 129.51, 128.51, 128.07, 124.61, 122.75, 122.13, 121.43, 115.37, 113.32, 42.17.

***4-Dodecylsulfanylmethyl-naphtho [1,2-b] pyran-2-one* (34)**



36 mg (0.899 mmol) of NaOH were dissolved in 1 mL of EtOH and 0.22 mL (0.889 mmol) of 1-dodecanethiol was added and left to stir to r.t. for 30 min. The mixture was colded and 220 mg (0.899 mmol) of compound **33** were added and left to stir for 1 hour to r.t. Reaction was monitored by TLC. After that time the mixture is poured onto cold water and concentrated HCL was dropped until pH= 1. The obtained precipitate was filtered, washed with cold water and dried for 24 hours at 60°C until constant weight to yield 180 mg (48.7%) of **34** as a yellow solid.

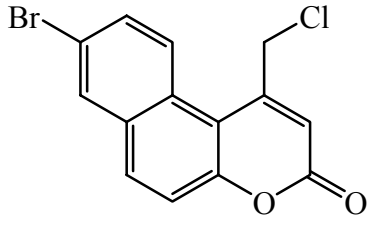
m.p: 75-76°C.

TLC: Petroleum ether-ethyl acetate; (3:1, v:v)

^{1}H-NMR ($CDCL_3$): δ: 8.57 (m, 1H), 7.88 (m, 1H), 7.71 (s, 2H), 7.65 (m, 2H), 6.42 (s, 1H, CH, H-3), 3.85 (s, 2H, CH_2), 2.54 (t, 2H, CH_2-chain), 1.60 (m, 4H, CH_2-chain), 1.13 (m, 16H, CH_2-chain), 0.88 (m, 3H, CH_3).

^{13}C-NMR ($CDCL_3$): δ: 160.69, 152.36, 151.51, 134.90, 128.86, 127.72, 127.24, 124.19, 123.42, 122.69, 122.47, 114.07, 113.69, 33.06, 32.28, 31.97, 29.68, 29.66, 29.61, 29.53, 29.39, 29.25, 29.03, 28.87, 22.74, 14.17.

***8-Bromo-1-chloromethyl-naphtho [2,1-b] pyran-3-one* (35)**

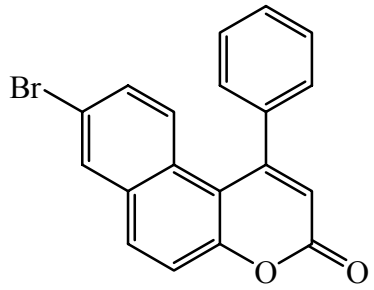


To a mixture of 5g (22.41 mmol) of 6-bromo-2-naphtol and 3.05 mL (22.41 mmol) of ethyl 4-chloroacetoacetate to temperature of 0-10°C were slowly dropped 2.39 mL (44.81 mmol) of concentrated sulfuric acid. Once the addition finished the mixture was kept to 85°C during 25 min. The reaction was monitored by TLC and once it was finished iced water (50 mL) was added and left to stir for 30 min. The obtained precipitate was filtered, washed with cold water and dried until constant weight. Recristalization from ethanol afforded 4.82 g (66.5%) of yellow crystals.

m.p: 238-240°C.
TLC: Petroleum ether-ethyl acetate; (3:1, v:v)
^{1}H-NMR (DMSO-d_6): δ: 8.45 (d, 1H, H-Ph, *J*= 9.25), 8.37 (d, 1H, H-Ph, *J*= 2.00), 8.22 (d, 1H, H-Ph, *J*= 9.00), 7.84 (dd, 1H, H-Ph, *J*= 9.25 and *J*= 9.25), 7.64 (d, 1H, H-Ph, *J*= 8.75), 6.88 (s, 1H, H-3), 5.37 (s, 2H, CH_2).

^{13}C-NMR (DMSO-d_6): δ: 159.46, 155.22, 151.93, 133.91, 132.86, 131.67, 131.36, 128.30, 127.47, 119.38, 119.31, 117.94, 112.54, 46.55.

***8-Bromo-1-phenyl-naphtho [2,1-b] pyran-3-one* (36)**



To a mixture of 5g (22.41 mmol) of 6-bromo-2-naphtol and 3.88 mL (22.41 mmol) of ethyl benzoylacetate to temperature of 0-10°C were slowly dropped 2.4 mL (44.81 mmol) of concentrated sulfuric acid. Once the addition ended the mixture was kept to 85°C during 25 min. The reaction was controlled by TLC and once it was finished iced water (50 mL) was added and left to stir for 30 min. The obtained precipitate was filtered, washed with cold water and dried at 60°C until constant weight. Recristalization from ethanol afforded 2 g (25.4 %) of green-yellowish crystals.

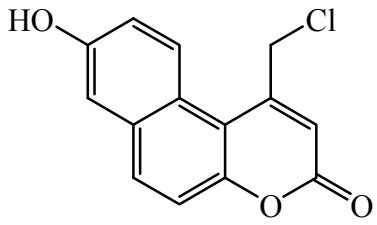
m.p: 166-168°C.

TLC: Petroleum ether-ethyl acetate; (3:1, v:v)

^{1}H-NMR (DMSO-d_6): δ: 8.08 (s, 1H, H-Ph), 7.85 (d, 1H, H-Ph, *J*= 7.5), 7.69 (d, 2H, H-Ph, *J*= 7.5), 7.47-7.11 (m, 6H, H-Ph), 5.47 (s, 1H, CH, H-3).

^{13}C-NMR (DMSO-d_6): δ: 185.76, 149.75, 149.02, 142.28, 133.50, 133.14, 131.17, 129.98, 129.37, 129.15, 129.02, 128.40, 127.32, 126.51, 124.62, 119.97, 119.33, 117.29, 109.31.

***1-Chloromethyl-8-hydroxy-naphtho[2,1-b] pyran-3-one* (37)**



To a mixture of 5g (31.22 mmol) of 1,5-dihydroxynaphthalene and 4.24 mL (31.22 mmol) of ethyl 4-chloroacetoacetate to temperature of 0-10°C were slowly dropped 3.33 mL (62.44 mmol) of concentrated sulfuric acid. Once the addition finished the mixture was kept to 70°C overnight. The reaction was monitored by TLC and once it was finished iced water (50 mL) was added and left to stir for 30 min. The obtained precipitate was filtered, washed with cold water and dried until constant weight yielding 5.68 g (70%) of a green powder.

m.p: 242-243°C.

TLC: Petroleum ether-ethyl acetate; (3:1, v:v)

^{1}H-NMR (DMSO-d_6): δ: 10.59 (s, 1H, OH), 8.02 (d, 1H, H-Ph, *J*= 7.5), 7.76-7.68 (m, 2H, H-Ph), 7.47 (t, 1H, H-Ph), 7.06 (d, 1H, H-Ph, *J*= 7.5), 6.72 (s, 1H, H-Pyr), 5.05 (s, 2H, CH_2).

^{13}C-NMR (DMSO-d_6): δ: 160.46, 150.50, 150.37, 134.93, 129.11, 127.79, 127.48, 125.21, 125.13, 124.36, 119.58, 115.35, 112.77, 42.03.

2.4 CONCLUSIONS

Despite of the methods related in this work are only applicable to phenols with at less a strong activating group in the ring, the fact of bringing the catalysts concentration down to 1% is a great

improvement over the large quantities of sulfuric acid employed in the classical Pechmann method and even the 10% catalyst in already improved procedures. These two catalysts bring alternative ways for obtaining hydroxy coumarins in good yields through environmentally safe procedures as they does not produce acidic and/or toxic waste[160,161].

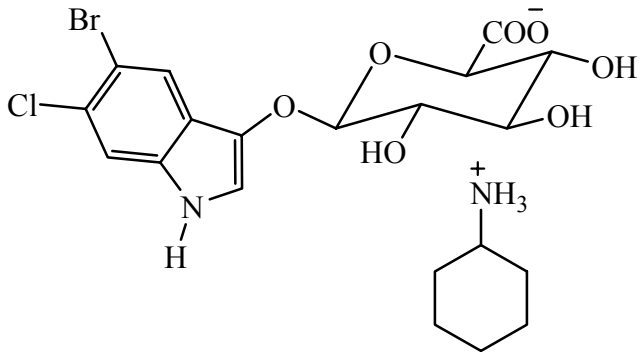
In the other hand, substituting the large volumes of sulfuric acid by only two equivalents of the same acid is a good improvement for obtaining of pyran-2- and -3-ones.

CHAPTER III

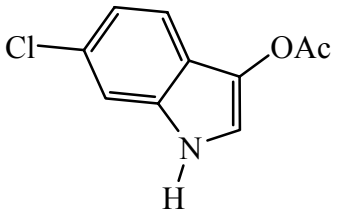
SYNTHESIS OF (1-ACETYL-INDOL-3-YL) ACETATES

3. **INTRODUCTION**.

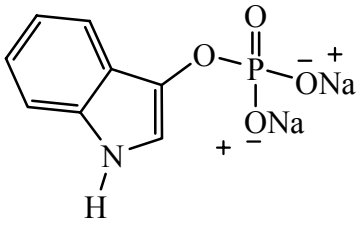
2,3-Dihydro-3-oxo-indole (indoxyl) is a very useful *in situ* intermediate in the synthesis of indigo and related dyes. Due to its unstable behavior it is commonly found in nature as the aglycon of indican glycoside. However, since some years its unstability has been taken into account to develop some chromogenic indicators. These compounds are very used in the histochemical demonstration of nonspecific stearases in mammalian tissue[162] and for quantitatively identifying and differentiating the presence of the members of coliform group and *Escherichia coli* in water, food and dairy products[163] (Fig. 6).



5-Bromo-6-chloro-3-indoxyl-β-D-glucuronic acid, cyclohexylammonium salt



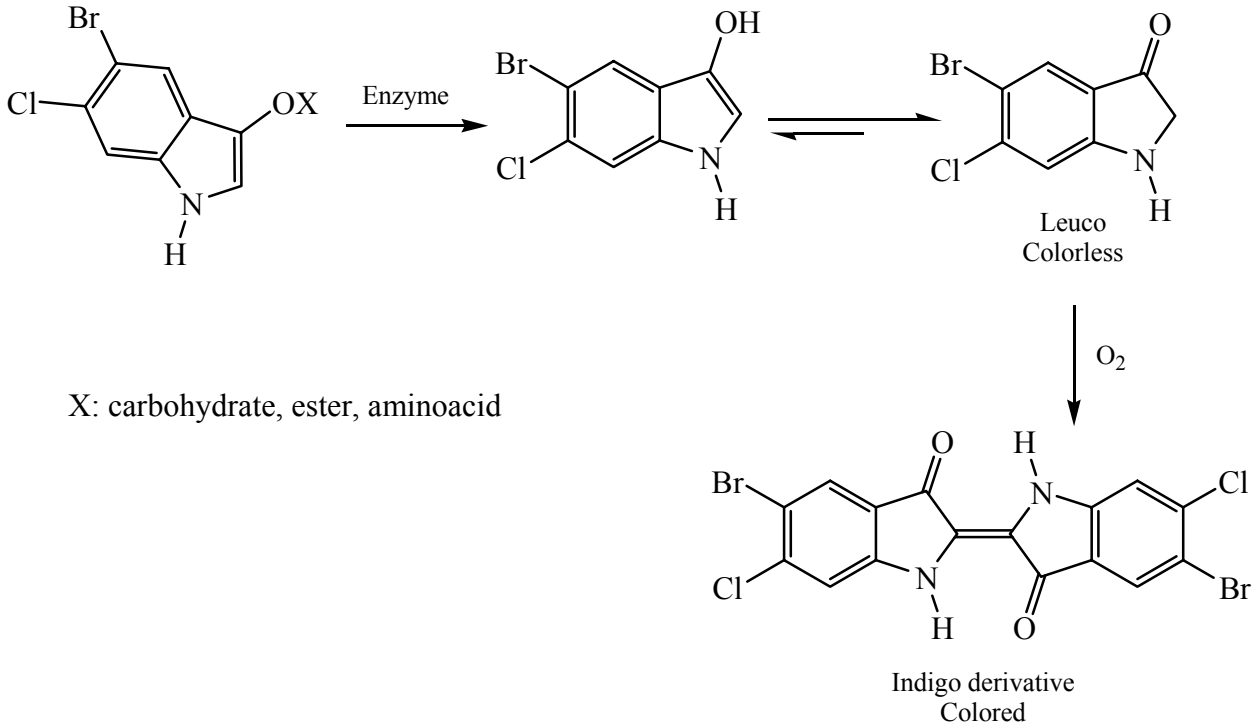
6-Chloro-3-indoxyl-3-acetate



3-Indoxyl phosphate, disodium salt

Fig. 6

These synthetic molecules are coupled to different entities in order to identify the presence of different microorganisms. These microorganisms excrete some specific enzymes which cut the glycosidic linkage of the chromogenic indoxyl derivative. As result of this enzymatic reaction and after some time, the indoxylic aglicon produce the dimer (indigo derivative) showing a characteristic coloration in the media (Scheme 22).

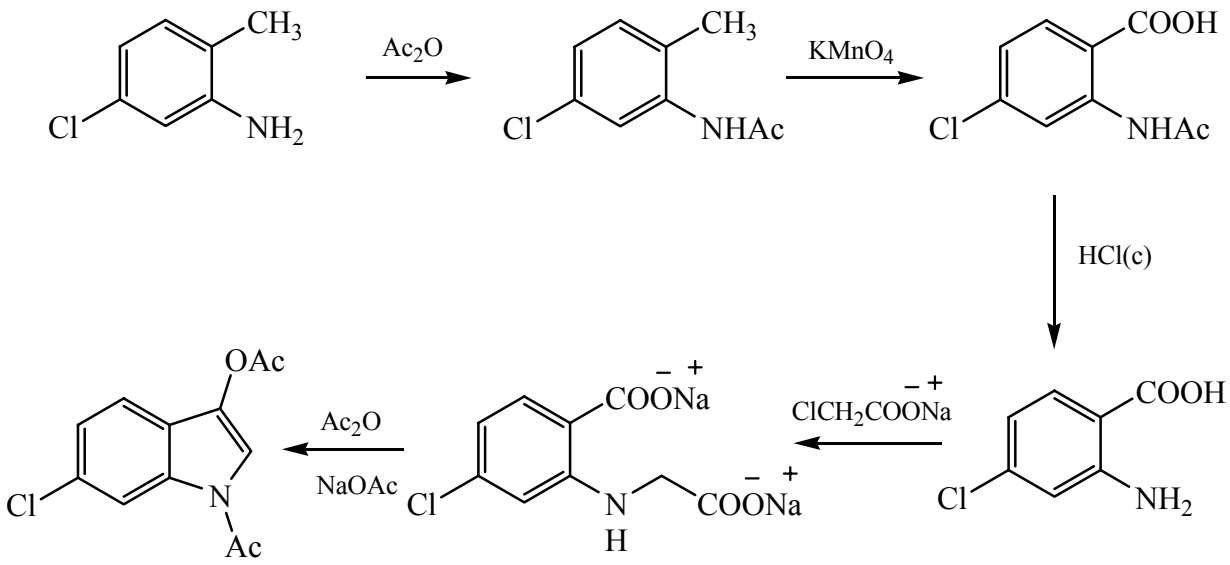


Scheme 22

Other very important aspects are the final color of the indoxyl derivative and its solubility in water. These two things are narrowly related depending of the indoxyl substituent. The commonly used substituents are halogens or nitro groups, which give a wide range of colors and poor solubility in the media depending of the type of group, how many times it is present in the molecule and combinations of them[164,165,166].

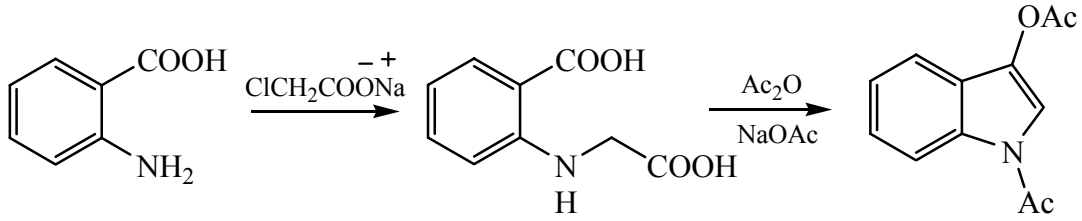
3.1 STATE OF THE ART

The literature procedure for the preparation of most of the chromogenic aglycons of the substituted indolxyl derivatives consists of five steps to obtain mono halogenated indoxyl derivatives starting from the commercially available toluidines or anthranilic acids (Scheme 23) [167].



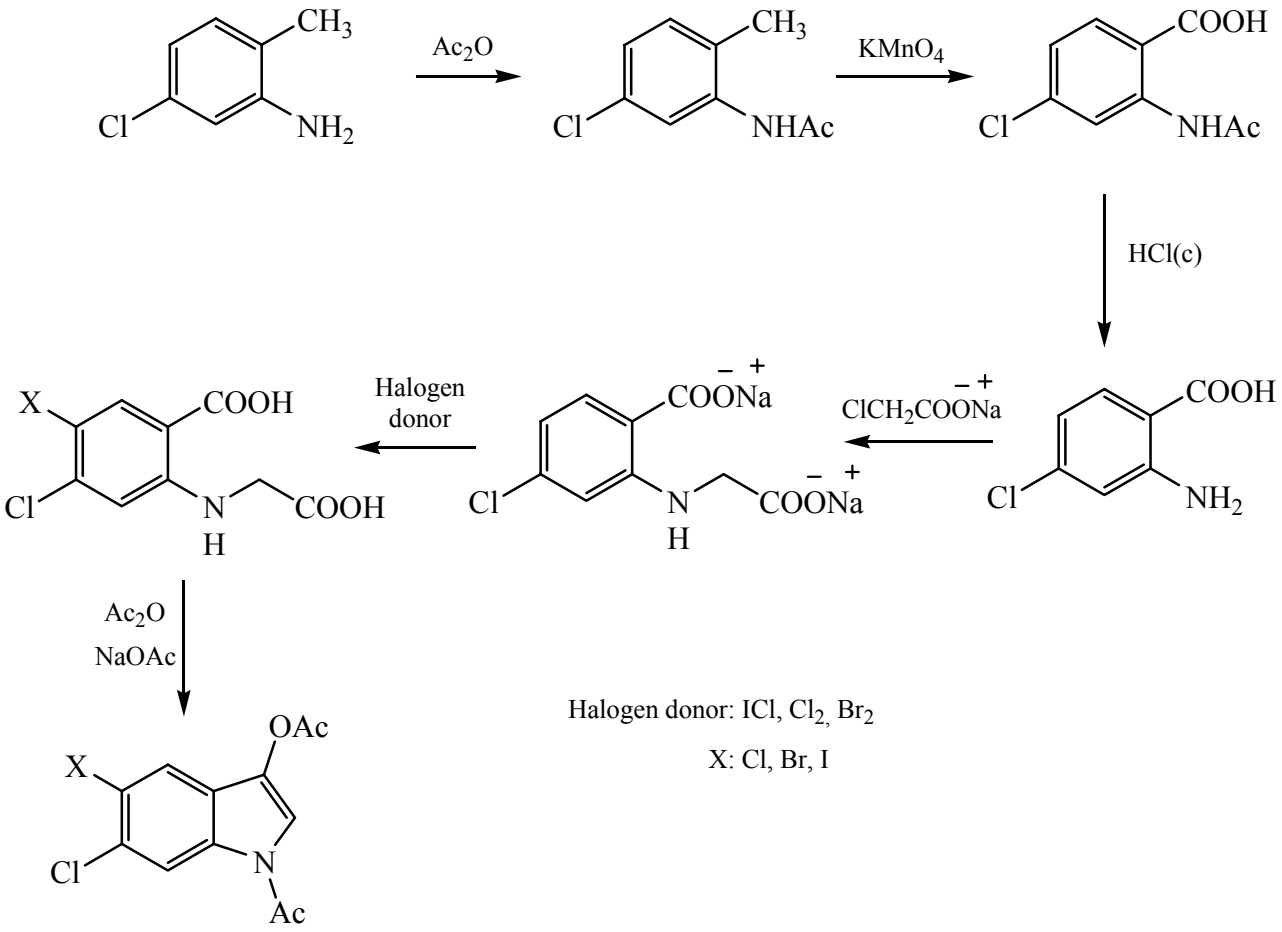
Scheme 23

For this procedure long reaction time is required (between 6 h to 72 h) for the aminoalkylation and poor yields are obtained[168]. It is also the case for the non substituted 1-acetyl-1*H*-indol-3-yl acetate prepared in only two steps from anthranilic acid (Scheme 24)[169,170].

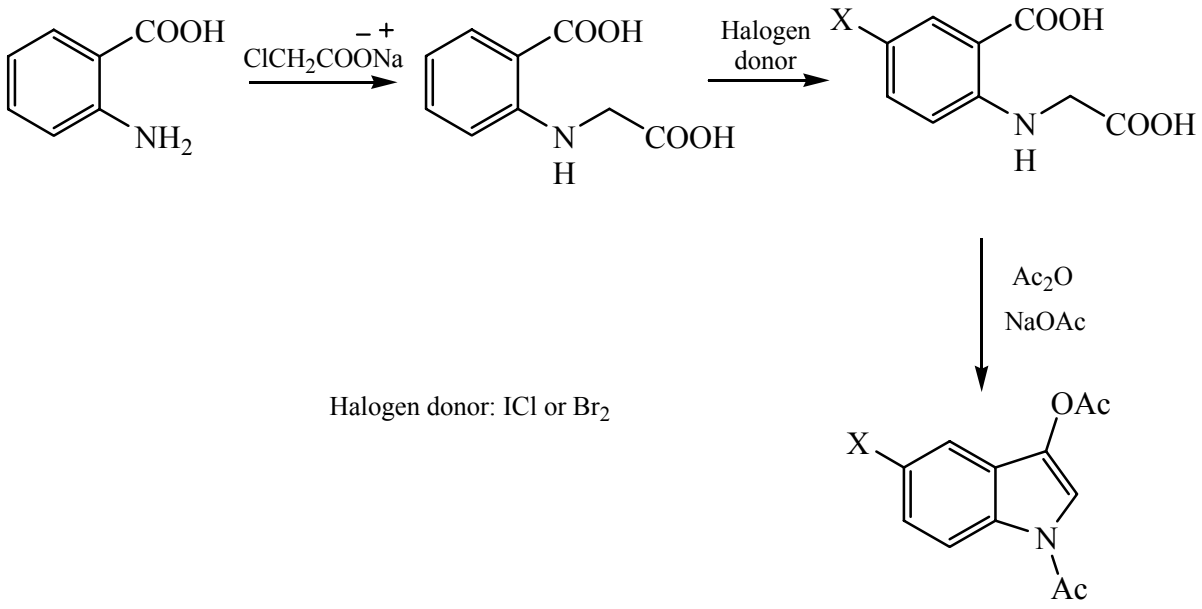


Scheme 24

In case of synthesising either di-substituted or poly-substituted indoxyl derivatives it is necessary to add an additional halogenation step (Scheme 25), making the process a little more expensive and shooting up the prices of the final products[171]. However, in order to obtain the substituted 2-[(carboxymethyl) amino] benzoic acids it has been combined the alternative of 2-[(carboxymethyl) amino] benzoic acids halogenation (Scheme 26) with the procedure showed in scheme 23[1677,172,173] but always with long reactions time to obtain 2-[(carboxymethyl) amino] benzoic acids. On the other hand, the synthesis of 2-[(carboxymethyl) amino]-4-nitrobenzoic acid has only been reported in 1905 by Schwarz[174] starting from 4-nitro-anthranilic acid and chloroacetic acid. In case of the 2-[(carboxymethyl) amino]-5-nitrobenzoic acid the Ullman condensation was reported using water as solvent[175].

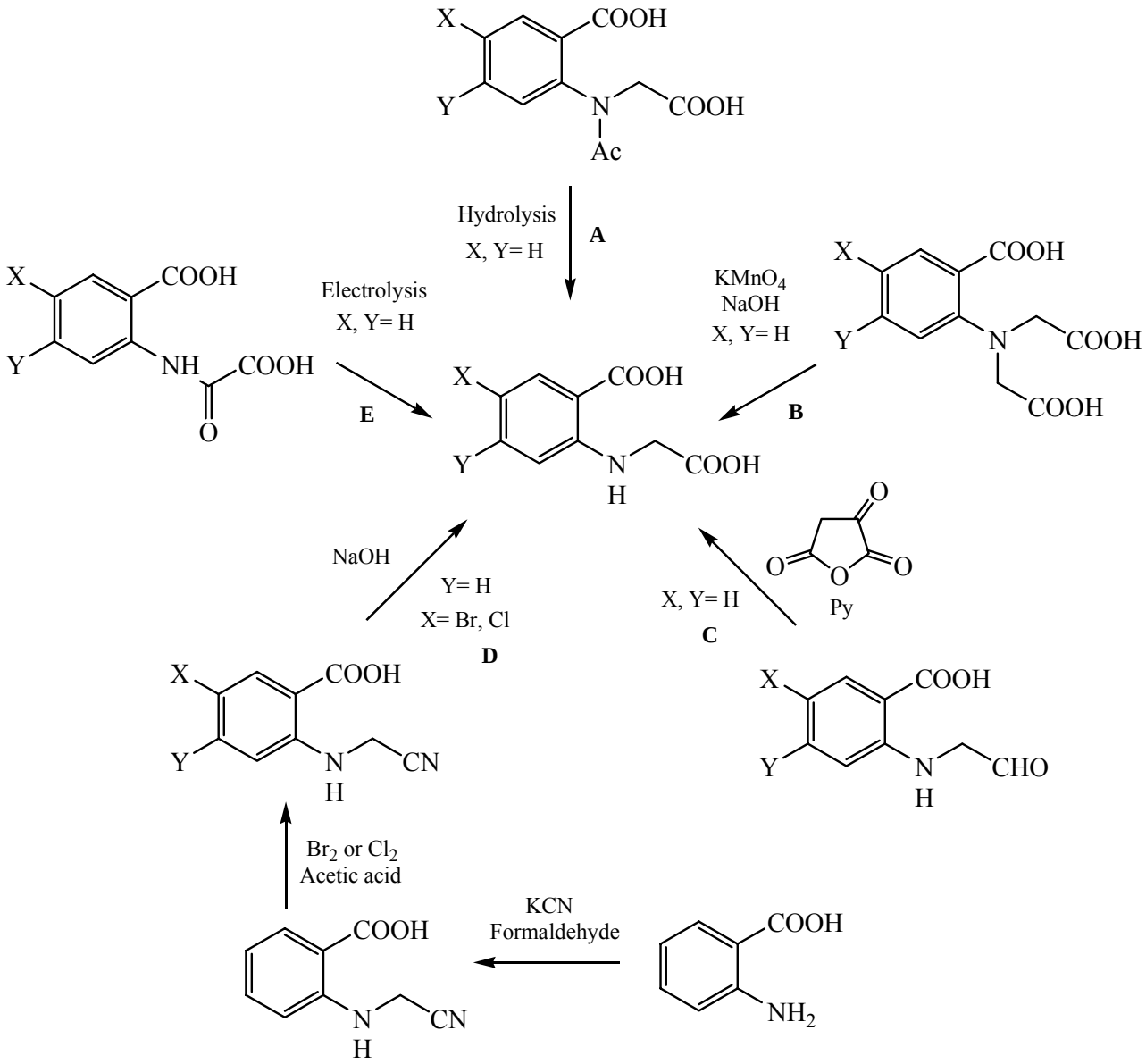


Scheme 25



Scheme 26

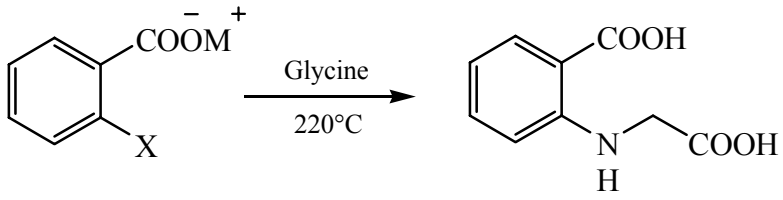
As we can observe, the key step of the process is directly related with preparing the phenyl substituted 2-[(carboxymethyl) amino] benzoic acids. Different ways have arisen in order to solve the drawbacks related with this step (Scheme 27).



Scheme 27

The strategies showed in literature, A[176], B[177], C[178], D[179], E[180] (Scheme 27), deal in most of the cases with the preparation of non-halogenated 2-[(carboxymethyl) amino] benzoic acids; the only procedure which carries out the preparation of the phenyl halogenated derivatives, involves the nitrile conversion into the carboxylic acid without showing the obtained yields (D). This procedure implies the preparation of the starting nitrile through the use of very nocive reagents either as potassium cyanide[181] or hydrogen cyanide[182].

The Ullman procedure has been an useful tool for synthesising diphenyl ethers[183,184] alkyl aryl ethers[185,] and nitrogen-carbon bonds[186,187,188]. It has been used too in the preparation of the 2-[(carboxymethyl) amino] benzoic acid through the reaction either between 2-bromobenzoic acid or 2-chlorobenzoic acid and glycine[189,190], but very high temperatures have to be used and yields are not reported (Scheme 28).



M= Na or K

X= Br or Cl

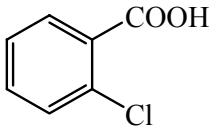
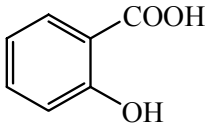
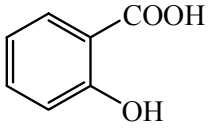
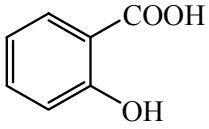
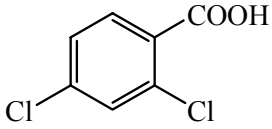
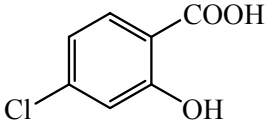
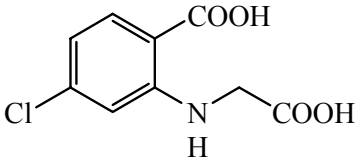
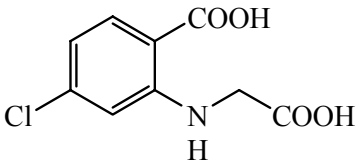
Scheme 28

3.2 RESULTS AND DISCUSSION

In this work we developed a two steps strategy taking the Ullmann's method as tool for obtaining nitrogen-carbon bonds. Its previous utilization for obtaining the non-substituted 2-[(carboxymethyl) amino] benzoic acid[189,190] allows to think in the possibility of used in the synthesis either of some halogenated or nitro 2-[(carboxymethyl) amino] benzoic acids. The Ullman's classical procedure should be followed by subsequent Rössing cyclodecarboxylation[191] to yield the halogenated or nitro -1-acetyl-1-*H*-indol-3-yl acetates.

The proposed reaction was carried out using different conditions in the concentration of glycine and type of solvent. In a first attempt water was used as reported[192]. However, the salicilic acid was obtained in each experience. These unsuccessful results involved the change from water to suitable polar aprotic solvent as DMF (Table 7).

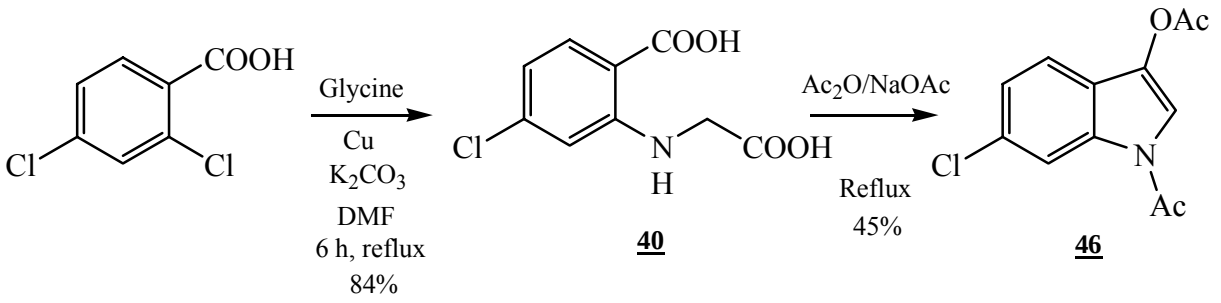
Table 7. Obtained results in order of setting the reaction conditions by the Ullmann's method.

Starting Material	Entry	Glycine(*)	Solvent	Yield (%)	t (h)	Obtained product
	**a**	2	H_2O	92	5	
	b	3	H_2O	93	5	
	c	4	H_2O	92	5	
 2,4-dichloro benzoic acid	**d**	2.5	H_2O	95	5	
	e	1	DMF	-	6	Mixture of the 2,4-dichlorobenzoic acid and the awaited 2-[(carboxymethyl) amino] benzoic acid(**)
	f	2.55	DMF	84	6	
	g	4	DMF	84	6	

(*) mole glycine /mole benzoic acid.

(**) Showed by TLC

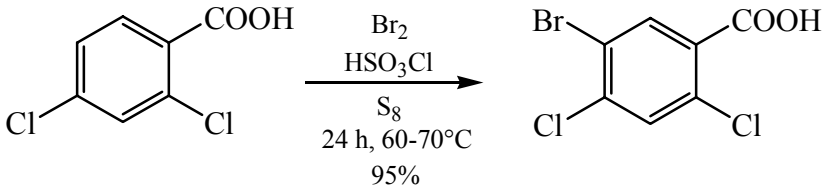
The best conditions and yields were obtained when it is used an excess of 2.55 times of glycine respecting to the 2,4-dichlorobenzoic acid, keeping constant the quantities of copper as catalyst and DMF as solvent (Entry **f**). Under these conditions yields of 84% of **40** were obtained and cyclodecarboxylation reaction was effected as next step, giving 45% of the 6-chloro-1-acetyl-1*H*-indol-3-yl acetate (**46**) (Scheme 29)[193].



Scheme 29

Encouraged by this result, we decided to carry out the preparation of other halogenated or nitro 1-acetyl-1*H*-indol-3-yl acetates starting from the commercially available 2-chloro benzoic acids. 5-Bromo-2,4-dichlorobenzoic acid, a very interesting starting material for the synthesis of chromogenic compounds, is not commercially available and had to be prepared.

The only procedure described in literature for its synthesis does not give full experimental details, uses a large quantity of chlorosulfonic acid and the final product was only characterized by its melting point[194] (Scheme 30).

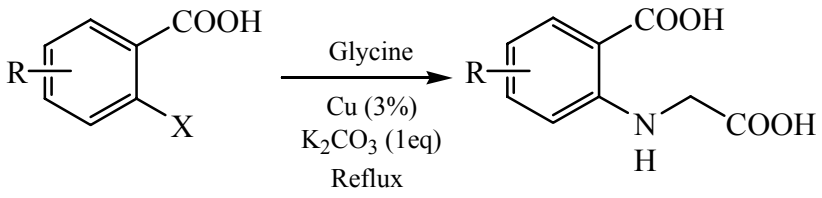


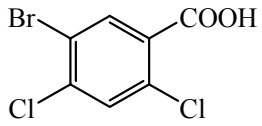
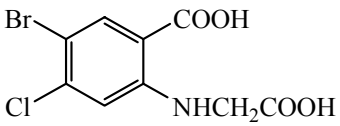
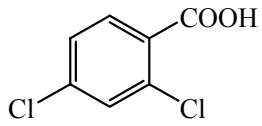
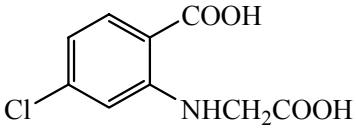
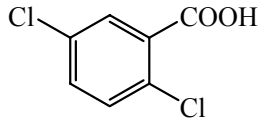
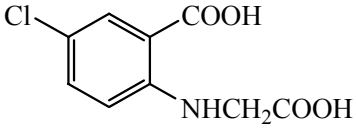
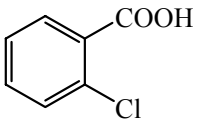
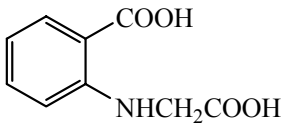
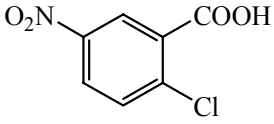
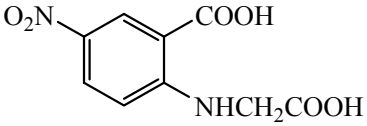
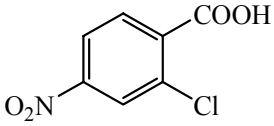
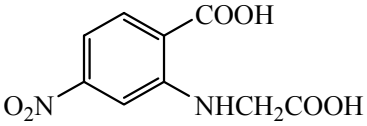
Scheme 30

In order to carry out the synthesis of 5-bromo-2,4-dichlorobenzoic acid, we followed the described procedure using less than the half of chlorosulfonic acid than in the literature method[194]. The reaction took place with within 24 hours with total consumption of the bromine and almost all the starting 2-chlorobenzoic acid, giving the awaited product in 95 % yield.

Having all the 2-chlorobenzoic acids needed, Ullmann's reaction was the next step. In a general manner, reaction took place in a range of two to six hours with good yields and purity for the obtained 2-[(carboxymethyl) amino] benzoic acids (Table 8).

Table 8. Results of obtained 2-[(carboxymethyl) amino] benzoic acids by the Ullmann's procedure.

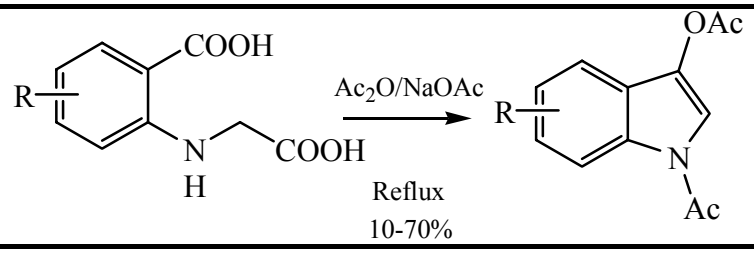


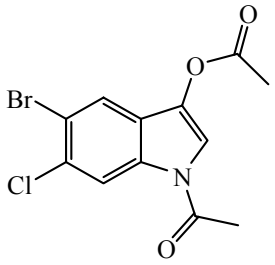
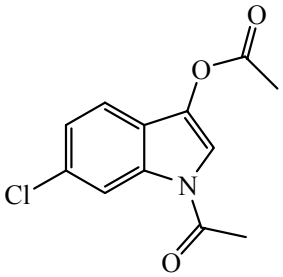
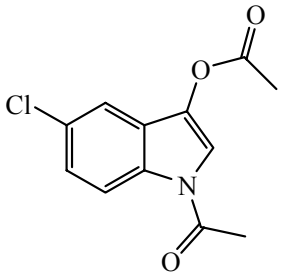
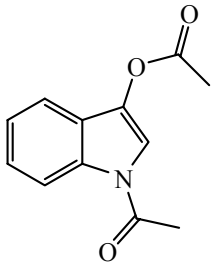
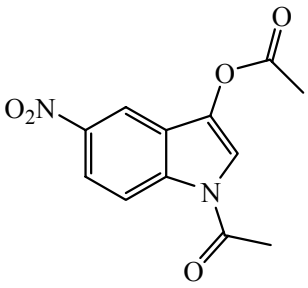
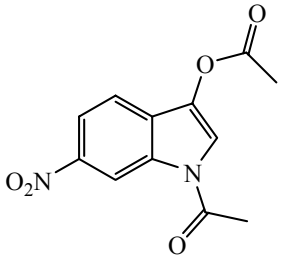
Entry	Starting material	t (h)	Yield (%)	Final product
a		4	71	
b		6	84	
c		3	87	
d		2	53	
e		2	97	
f		2	75	

2.55 eq of glycine was used

Subsequent Rössing cyclodecarboxylation[191] with sodium acetate in acetic anhydride at reflux gave the corresponding 1-acetyl-1*H*-indol-3-yl acetates in moderated to good yields (Table 9). Only in case of preparing the non-susbstituted indole lower yields were obtained (10%), but in general, no further purification was necessary.

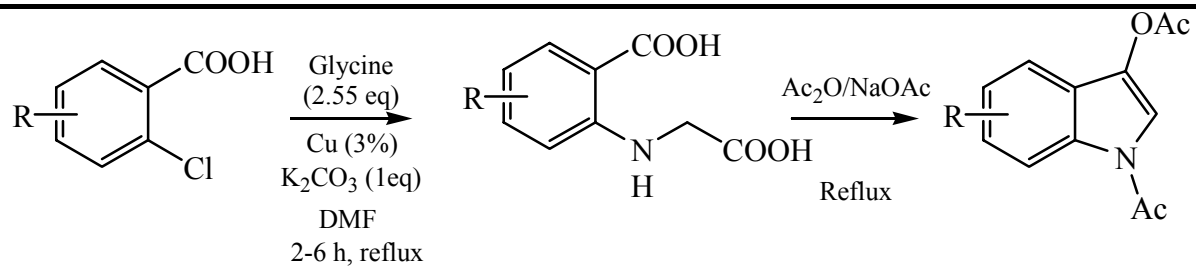
Table 9. Obtained results of Rössing cyclodecarboxylation from 2-[(carboxymethyl) amino] benzoic acids.



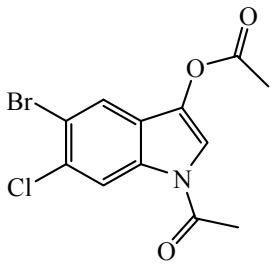
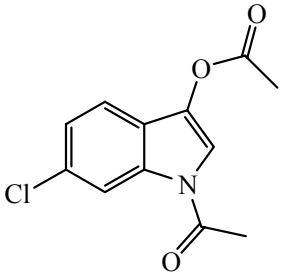
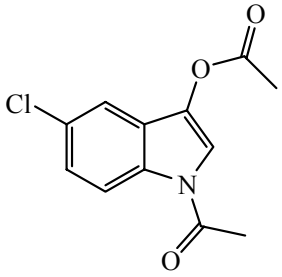
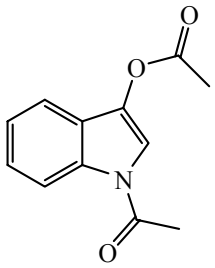
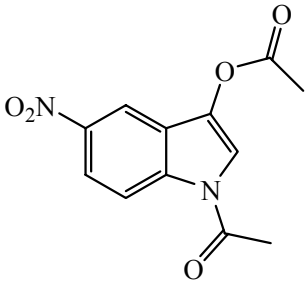
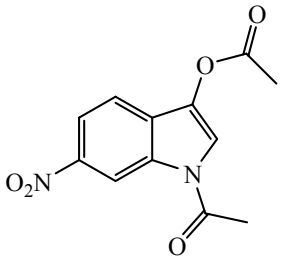
Product	Yield (%)	Product	Yield (%)	Product	Yield (%)
 **45**	64	 **46**	45	 **47**	45
 **48**	10	 **49**	70	 **50**	57

In a general manner 1-acetyl-1*H*-indol-3-yl acetates were synthesized in a two steps procedure with good yields. Overall yields of the process are showed in Table 10. Only in case of the non substituted indol the yield is lower than those previously reported[169,170], but in general yields are quite higher ranging from 38% to 68%[195].

Table 10. Obtained overall yields of synthesized 1-acetyl-1*H*-indol-3-yl acetates from 2-chlorobenzoic acids.



Overall yields: 5-68%

Product	Yield (%)	Product	Yield (%)	Product	Yield (%)
 **45**	45	 **46**	38	 **47**	39
 **48**	5	 **49**	68	 **50**	43

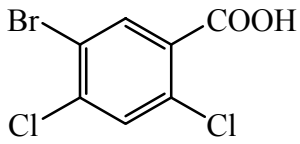
3.3 EXPERIMENTAL PART

General methods

TLC were performed on Silicagel plates ALUGRAM Sil G/UV 254 and Chloroform-ethyl acetate-AcOH (8:6:1, v:v) as the solvent system. The TLC plates were visualized by means of a Bioblock lamp with a wavelenght of 254 nm. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 250F spectrometer. The coupling constants are in Hz. Melting points were determined on a Stuart Scientific SMP 3 capillary melting point apparatus and were uncorrected.

3.3.1 *Preparation of the not commercially available 5-bromo-2,4-dichlorobenzoic acid*

***5-Bromo-2,4-dichlorobenzoic acid* (<u>38</u>)**



5 g (26.2 mmol) of 2,4-dichlorobenzoic acid were added to 20 mL of chlorosulfonic acid and when all was disolved, 0.05 g (0.2 mmol) of sulfur and 0.67 mL (13.1 mmol) of bromine were added. The mixture was heated at 60-70°C during 24 h. After total consumption of bromine the mixture was carefully poured onto ice. The mixture was stirred and the precipitate obtained was collected, washed with cold water and dried at 50°C to constant weight. The white powder was recrystallized from cyclohexane yielding 6.7 g (95 %) of **<u>37</u>** as colorless crystals.

m.p: 177-178°C mp_{lit}:187-189°C[1944] (from water).

^{1}H-NMR (DMSO-d_6): δ 8.15 (s, 1H, *H*-Ph), 7.96 (s, 1H, *H*-Ph).

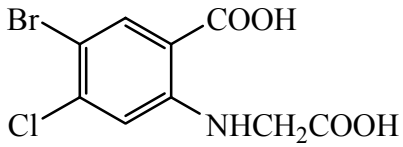
^{13}C-NMR (DMSO-d_6): δ 164.87, 137.00, 135.32, 132.17, 132.11, 131.70, 120.51.

3.3.2 *Preparation of 2-[(carboxymethyl) amino] benzoic acids from 2-chlorobenzoic acids through the Ullmann's method*.

General procedure for obtaining 2-[(carboxymethyl) amino] benzoic acids.

To a suspension of 11.11 mmol of the corresponding 2-chlorobenzoic acid, 28.11 mmol of glycine and 1.27 mmol of copper powder in 10.6 mL of dimethylformamide was slowly added 33.3 mmol of potassium carbonate. The mixture was heated at reflux with a good stirring between 2 and 6 hours. When all the corresponding 2-chlorobenzoic acid had been consumed, the mixture was poured onto 30 mL of cold 6 M hydrochloric acid and stirred for 30 min. The precipitate obtained was collected and washed with cold water until neutral pH. The solid was dried at 50°C to constant weight affording the corresponding 2-[(carboxymethyl) amino] benzoic acid.

***5-Bromo-2-[(carboxymethyl) amino]-4-chloro-benzoic acid* (39)**



Time: 4 h.

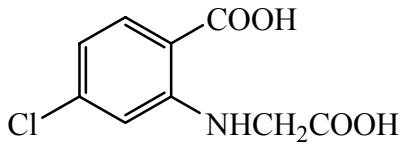
Yield: 2.43 g (71%) of **39** from 3 g of **38**.

m.p: 238°C mp_{lit}: 230°C[196]

^{1}H-NMR (DMSO-d_6): δ 13.06 (s, 2H, 2COOH), 8.22 (s, 1H, N-H), 8.00 (s, 1H, H-6), 6.90 (s, 1H, H-3), 4.05 (s, 2H, CH_2).

^{13}C-NMR (DMSO-d_6): δ 171.6, 169.6, 150.3, 140.3, 133.3, 115.3, 110.5, 109.2.

***2-[(Carboxymethyl) amino)-4-chloro-benzoic acid* (40)**



Time: 6 h.

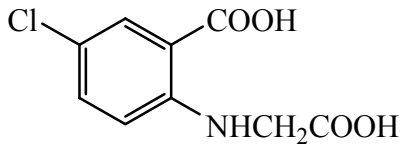
Yield: 10.1 g (84%) of **40** from 10 g of 2,4-dichloro-benzoic acid.

m.p: 225°C mp_{lit}: 228 [197]

^{1}H-NMR-(DMSO-d_6): δ 7.88 (d, $J_{6,4}$ = 8.5, 1H, H-6), 6.62 (dd, $J_{4,6}$ = 8.5, $J_{4,3}$ = 1.9, 1H, H-4), 6.53 (d, $J_{1,3}$ = 1.9, 1H, H-3), 3.97 (s, 2H, CH_2).

^{13}C-NMR (DMSO-d_6): δ 171.6, 169.6, 150.3, 140.3, 133.3, 115.3, 110.5, 109.2.

***2-[(Carboxymethyl) amino]-5-chloro-benzoic acid* (41)**



Time: 3 h.

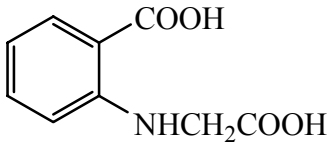
Yield: 6.27 g (87%) of **41** from 5 g of 2,5-dichloro-benzoic acid.

m.p: 210-215°C mp_{lit}: 210°C[198]

^{1}H-NMR (DMSO-d_6): δ 8.15 (s, 1H, N-H), 7.62 (d, *J*=2.53, 1H, H-6,), 7,24 (dd, *J*=2.53 and *J*=8.95, 1H, H-4), 6.82 (d, *J*=8.95, 1H, H-3,), 4.16 (s, 2H, CH_2).

^{13}C-NMR (DMSO-d_6): δ 171.62, 168.10, 149.57, 133.26, 129.71, 122.40, 120.92, 118.45, 47.46.

***2-[(Carboxymethyl) amino] benzoic acid* (42)**



Time: 2 h.

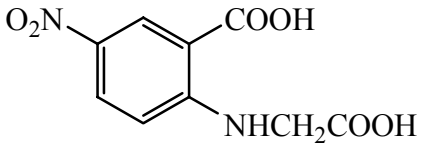
Yield: 3.52 g (53%) of **42** from 5 g of 2-chloro-benzoic acid.

m.p: 220°C mp_{lit}: 220°C[199]

^{1}H-NMR (DMSO-d_6): δ 7.91 (d, *J*= 7.95, 1H, H-Ph), 7.33 (m, 1H, H-Ph), 6.57 (m, 2H, H-Ph).

^{13}C-NMR (DMSO-d_6): δ 172.28, 150.49, 134.94, 132.15, 129.77, 129.08, 115.26, 112.03, 44.67.

***2-[(Carboxymethyl) amino]-5-nitro-benzoic acid* (43)**



Time: 2 h.

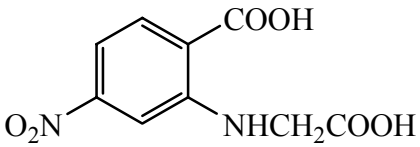
Yield: 5.8 g (97%) of **43** from 5 g of 2-chloro-5-nitro-benzoic acid.

m.p: 222-223°C mp_{lit}: 226-227°C[175]

^{1}H-NMR (DMSO-d_6): δ 8.05 (m, 2H, H-Ph), 7.37 (m, 2H, H-Ph), 4.10 (s, 2H, CH_2).

^{13}C-NMR (DMSO-d_6): δ 171.04, 168.65, 154.31, 135.47, 129.59, 128.68, 112.64, 109.92, 44.54.

***2-[(Carboxymethyl) amino]-4-nitro-benzoic acid* (<u>44</u>)**



Time: 2 h.

Yield: 4.45 g (75%) of **<u>44</u>** from 5 g of 2-chloro-4-nitro-benzoic acid.

m.p: 243-244°C (from absolute ethanol) mp_{lit}: 240-242°C[1744] (from water).

^{1}H-NMR (DMSO-d_6): δ 8.35 (s, 1H, N-H), 8.00 (d, *J*=8.5, 1H, H-Ph), 7.34-7.30 (m, 2H, H-Ph), 4.10 (s, 2H, CH_2).

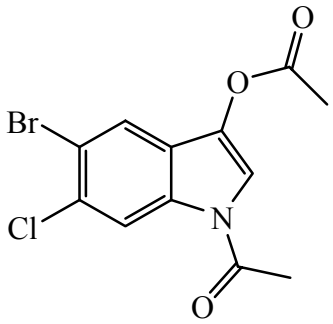
^{13}C-NMR (DMSO-d_6): δ: 171.77, 168.99, 151.67, 150.72, 133.82, 115.92, 109.02, 106.38, 44.66.

3.3.3 *Cyclodecarboxylation of 2-[(carboxymethyl) amino] benzoic acids for synthesizing (1-acetyl-indol-3-yl) acetates.*

General procedure of cyclodecarboxylation for obtaining (1-acetyl indol-3-yl) acetates from 2-[(carboxymethyl) amino] benzoic acids

A mixture 8.36 mmol of the corresponding 2-(carboxymethyl amino) benzoic acid 12.8 mL of acetic anhydride and 32.06 mmol of dry sodium acetate were heated at reflux. When the gas evolution had finished, the mixture while still hot was poured into a beaker and left to cool overnight at 0°C. The precipitate was collected and poured into 35 mL of ice water and stirred for 1 h, collected again and dried in vacuum to yield the corresponding (1-acetyl indol-3-yl) acetate.

***(1-Acetyl-5-bromo-6-chloro-indol-3-yl) acetate* (45)**



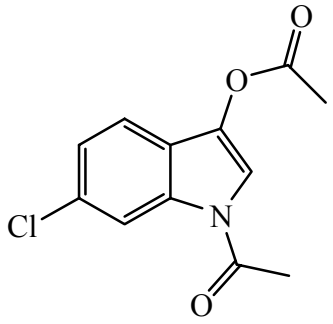
Yield: 1.77 g (64%) of **45** from 2.58 g of **39**.

m.p: 171-172°C mp_{lit}: 176°C[196]

^{1}H-NMR (DMSO-d_6): δ 8.51 (s, 1H, H-Ph), 7.99 (s, 2H, H-Ph and H-pyrrole), 2.62 (s, 3H, $NCOCH_3$), 2.39 (s, 3H, $OCOCH_3$).

^{13}C-NMR (DMSO-d_6): δ 172.24, 169.86, 132.29, 131.89, 129.92, 124.33, 122.84, 118.37, 117.52, 116.28, 23.76, 20.71.

***(1-Acetyl-6-chloro-indol-3-yl) acetate* (46)**



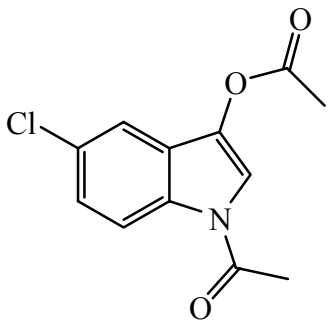
Yield: 4.8 g (45%) of **46** from 10.5 g of **40**.

m.p: 111-113 mp_{lit}: 112-113[196]

^{1}H-NMR (DMSO-d_6): δ 8.34 (d, $J_{7,5}$ = 1.9, 1H, H-7), 7.90 (s, 1H, H-2), 7.52 (d, $J_{4,5}$ = 8.5, 1H, H-4), 7.34 (dd, $J_{5,7}$ = 1.9, $J_{5,4}$ = 8.5, 1H, H-5), 2.61 (s, 3H, CH_3, $NCOCH_3$), 2.28 (s, 3H, $OCOCH_3$).

^{13}C-NMR (DMSO-d_6): δ 169.36, 167.95, 133.02, 132.57, 130.08, 123.56, 122.27, 119.06, 116.33, 115.59, 23.36, 20.25.

***(1-Acetyl-5-chloro-indol-3-yl) acetate* (47)**



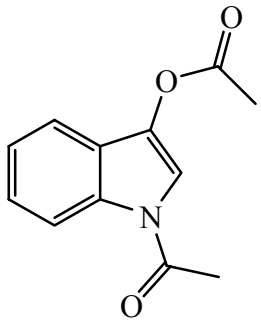
Yield: 2.44 g (45%) of **47** from 5 g of **41**.

m.p: 134-135°C mp_{lit}: 130°C[196]

^{1}H-NMR (DMSO-d_6): δ 8.3 (d, *J*= 8.95, 1H, H-Ph), 7.91 (s, 1H, H-pyrrole), 7.57 (d, *J*= 2.07, 1H, H-Ph), 7.36 (dd, *J*= 8.95 and *J*= 2.07, 1H), 2.62 (s, 3H, $NCOCH_3$), 2.39 (s, 3H, $OCOCH_3$).

^{13}C-NMR (DMSO-d_6): δ 169.96, 168.91, 133.14, 131.56, 131.57, 128.53, 126.03, 125.58, 118.05, 117.95, 24.06, 20.98.

***(1-Acetyl-indol-3-yl) acetate* (48)**



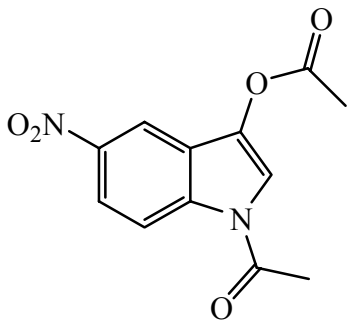
Yield: 0.32 g (10%) of **48** from 3.15 g of **42**.

m.p: 84-86°C mp_{lit}: 83[196], 88 [200]

^{1}H-NMR (DMSO-d_6): δ 8.36 (d, *J*= 7.9, 1H, H-Ph), 7.90 (s, 1H, H-Pyrrole), 7.41 (m, 3H, H-Ph), 2.62 (s, 3H, $NCOCH_3$), 2.39 (s, 3H, $OCOCH_3$).

^{13}C-NMR (DMSO-d_6): δ 169.29, 168.18, 133.43, 132.44, 125.53, 123.46, 123.34, 117.64, 115.90, 115.50, 23.57, 20.37.

***(1-Acetyl-5-nitro-indol-3-yl) acetate* (<u>49</u>)**



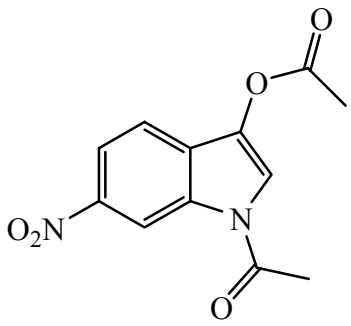
Yield: 1.88 g (70%) of **<u>49</u>** from 2.47 g of **<u>43</u>**.

m.p: 221-222°C mp_{lit}: 219-220°C[175]

^{1}H-NMR (DMSO-d_6): δ 8.55 (m, 2H, H-Ph), 8.27 (d, *J*= 9.15, 1H, H-Ph), 8.15 (s, 1H, H-Pyrrole), 2.68 (s, 3H, $NCOCH_3$), 2.44 (s, 3H, $OCOCH_3$).

^{13}C-NMR (DMSO-d_6): δ 170.14, 168.66, 143.72, 135.50, 133.64, 124.04, 120.89, 119.58, 116.94, 114.62, 24.04, 20.79.

***(1-Acetyl-6-nitro-indol-3-yl) acetate* (<u>50</u>)**



Yield: 3.85 g (57%) of **<u>50</u>** from 6.15 g of **<u>44</u>**.

m.p: 195-198°C mp_{lit}: 195°C[201]

^{1}H-NMR (DMSO-d_6): δ 9.18 (d, *J*= 1.83, 1H, H-Ph), 8.29 (s, 1H, H-Pyrrole), 8.21-8.17 (m, 1H, H-Ph), 7.77 (d, *J*= 9.15, 1H, H-Ph), 2.70 (s, 3H, $NCOCH_3$), 2.48 (s, 3H, $OCOCH_3$).

^{13}C-NMR (DMSO-d_6): δ: 169.73, 168.14, 144.92, 132.62, 130.95, 128.09, 121.60, 118.57, 118.40, 111.82, 23.46, 20.31.

3.4 CONCLUSIONS

With this simple procedure, halogenated and nitro 1-acetyl-1*H*-indol-3-yl acetate derivatives were obtained in moderated to good yields, less steps are required and yields are higher than those reported in literature.

4. GENERAL CONCLUSIONS AND PERSPECTIVES

- The use of MAEM as starting material for preparing both cefotaxime (**1**) and cefpodoxime proxetil (**3**) (chapter I), allowed to eliminate the drawbacks of the classic pathways of synthesis, to obtain better yields diminishing the production cost of the final products. It should also be noted that the mercaptobenzothiazole (**6**), byproduct in both process, can be recovered with good purity.

- To avoid using diketene, the development of two procedures for protecting the amino function by phenylacetylation in the synthesis of **11** and using DMA as solvent for obtaining **18**, allowed to increase yields in the preparation of cefdinir (**15**).

- In chapter II, bringing the catalysts concentration down to 1% is a great improvement over the large quantities of sulfuric acid employed in the classical Pechmann method and even the 10% catalyst in already improved procedures. These two zirconium catalysts bring alternative ways for obtaining hydroxy coumarins in good yields through environmentally safe procedures as they does not produce acidic and/or toxic waste.

- Applaying the Ullmann's procedure (chapter III) for the preparation of 2-[(carboxymethyl) amino] benzoic acids is a great improvement in order to obtain halogenated and nitro 1-acetyl-1*H*-indol-3-yl acetate derivatives, saving time and increasing yields of the final products.

- The procedure described in this work to obtain indole derivatives could be a useful tool for preparing polycyclic nitrogen containing compound derivatives.

REFERENCES

1 Dürckheimer, W.; Blumbach, J.; Lattrell, R.; Scheunemann, K. H.; *Angew. Chem. Int. Ed. Engl.,* 24, **1985**, 180-202.

2 Thomson, T. D.; Quay, J. F.; Webber, J. A.; *JAVMA,* 5, 10, **1984**, 1109-1113.

3 Williams, J. D.; *Drugs,* 34, (Supp. 2), **1987**, 15-22.

4 Martindale. The Extra Pharmacopeia. *29th Edition London., The Pharmaceutical Press,* **1989**, 151-156.

5 Fujimoto, K.; Ishihara, S.; Yanagisawa, H.; Ide, J.; Nakayama, E.; Nakao, H.; Sugawara, S.; Iwata, M.; *J. Antibiotics,* 40, 3, **1987**, 370-384.

6 Tood, W. M.; *Int. J. Atimicrobial Agents,* 4, **1994**, 37-62.

7 Hitzel, V.; Latrell, R.; Bormann, D.; **1979**, *DE* 2804040.

8 Heymes, R.; Lutz, A.; **1979**, *US* 4152432.

9 Ochiai, M.; Okada, T.; Aki, O.; Morimoto, A.; Kawakita, K., Matsushita, Y., **1978**, *US* 4098888.

10 Ochiai, M.; Morimoto, A.; Miyawaki, T.; Matsushita, Y.; Okada, T.; Natsugari, H.; Kida, M. V.; *J. Antibiotics,* 34, 2, **1981**, 171-185.

11 Heymes, R.; Pronine, D.; **1981**, *EP* 0034536.

12 Bucourt, R.; Heymes, R.;, Lutz, L.;, Pénasse, L.;, Perronet, J.; *Tetrahedron,* 34, **1978**, 2233-2243.

13 Blumbach, J.; Dürckheimer, W.; Reden, J.; Sliger, H.; **1979**, *DE* 2758000.

14 Kim, W. J.; Lee, C. H.; Kim, B. J.; Lee, G. S.; **1985**, *GB* 2158432.

15 Lim, S. K.; Moon, S. K.; Lee, G. S.; **1986**, *EP* 0175814.

16 Ascher, G.; **1981**, *EP* 0037380.

17 Farge, D.; Moutonnier, C.; Peyronel, J. F.; **1981**, *DE* 3105136.

18 Heymes, R.; Lutz, A.; 1979, *US* 4152432; Chem. Abstr.; **1977**, 87: P168063t.

19 Bonfanti, G.; **1988**, *EP* 0273156.

20 Lab-base R 2 13, TRIO 1, Copyrights Fisons plc. 1988-1992, Hampden Dates Services Ltd., Eclipse Computer Solutions, Switzerland, **1992**.

21 T. Mills, J.C. Roberson, Instrumental Data for Drug Analysis, second ed., vol. 1, Elsevier, Amsterdam, **1987**, 367.

22 Rodríguez, J. C.; Hernández, R.; González, M.; López, M. A.; Fini, A.; *Il Farmaco*, 55, **2000**, 393-396.

23 Xu, K.; Handbook of Organic Materials of Chemical Engineering and Intermediate, Chemical Industry Press, China, 1998.

24 Muthadi, F. J.; Hassan, M. A.; Florey, K.; (Ed.) Analytical Profiles of Drug Substances, Academy Press, New York, vol. 11, **1982**, 159.

25 United States XXII Pharmacopeia, The United States Pharmacopeia Convention Inc., 12601 Twinbrook Parkway, Roockville, MD 20852, **1990**, 249-250.

26 Mills, T. J.; Roberson, C.; Instrumental Data for Drug Analysis, second ed., vol. 1, Elsevier, Amsterdam, **1987**, 367.

27 Dean, J.A.; Handbook of Organic Chemistry, McGraw-Hill, New York, **1987**, 1-273.

28 Adam, D.; Bergogne-Berezin, E.; Jones, R. N.; *Drugs*, 42, 3, **1991**, 1-66.

29 Sankyo Company Limited, *JP* 58994886, **1983**, Chem. Abstr. 99,139644a

30 Fujimoto, K.; Nakayama, E.; Nakao, I.; *FP* 2517308, **1983**. Sankyo Company Limited.

31 Nakao, H; Ide, J; Yanagisawa, H; Iwata, M; Kamai, T; Masuda, H; Hirasawa, Sankyo Kenkyusho Nenpo, 39, **1987**, 1-44.

32 Sankyo Company Limited, *JP* 59163387, **1984**.

33 Ochiai, M.; Morimoto, A.; Matsusshida, Y.; Okada, T.; *J. Antibiotics*, 34, 2, **1981**, 160-170.

34 Hantzsch, A.; *Ann. Chem.*; 1, **1888**, 249.

35 Cephalosporins and Penicillins, Chemistry and Biology. Edited by E. H. Flynn, Academic Press, New York and London, 84, **1972**.

36 Nakao, H.; Fujimoto, K.; Ishihara, S.; Sugawara, S.; Igarachi, I.; *US* 4486425, **1984**. Sankyo Company Limited.

37 Nakao, H; Fujimoto, K; Ishihara, S; Sugawara, S; Igarachi, I.; *US* 4716158, **1987**. Sankyo Company Limited.

38 Rodríguez, J. C.; Hernández, R.; González, M; Rodríguez, Z; Tolón, B; Vélez, H; Valdés, B; López, M. A.; Fini, A.; *Il Farmaco*, 58, **2003**, 363-369.

39 Fischer, G.; Defossa, E.; Ggerlach, U.; Hörlein, R.; Krass, N.; Latrell, R.; Stache, U.; Wollmann, T.; Isert, D.; *EP* 0531875 A2, Der. Chem. Abstr.; **1993**, C1993-086926.

40 Fujimoto, K.; Ishihara, S.; Yanagisawa, H.; Ide, J.; Nakayama, E.; Nakao, H.; Sugawara, S.; *J. Antibiot.*, 40, **1987**, 370-384.

41 Neu, H. C.; Saha, G.; Chin, N-X.; *Antimicrob. Agents Chemother.*; 33, **1989**, 17955-18000.

42 Cohen, M. A.; Joannides, E. T.; Roland, G. E.; *Diagn. Microbiol. Infect. Dis.*; 18, **1994**, 31-39.

43 Cohen, M. A.; Wold, S. A.; Merservey, M. A.; *Diagn. Microbiol. Infect. Dis*.; 18, **1994**, 41-47.

44 Sultan, T.; Baltch, A.; Smith, R. P.; Ritz, W.; *Chemotherapy* (Switzerland); 40, **1994**, 80-91.

45 *Pharmaprojects PLUS. PBJ publications LTD, Richmond, Surrey, UK,* **1998.**

46 Scriver, S. R.; Willey, B.M.; Low, D.E. *Eur. J. Clin. Microbiol. Infect. Dis.*; 11, **1992**, *646-52.*

47 Dreholb, M.; Bianchi, P.; Keyserling, C. H.; *Antimicrob*. *Agents Chemother*; 41, 7, **1997**, 1579-1583.

48 Kenneth, J. T.; James, A. H.; Rothestein, E. ; *Arch. Pediatr. Adolesc. Med*.; 151, 1, **1997**, 45-49.

49 Gwaltney, J. M.; Savolainen, S.; Rivas, P.; *Antimicrob*. *Agents Chemother*.; 41, 7, **1997**, 1517-1520.

50 Tack, K. J.; Keyserling, C. H.; Mccarty, J.; *Antimicrob. Agents Chemother*.; 41, 4, **1997**, 739-742.

51 Cohen, M. A.; Roland, G. E.; Huband, M.D.; *In Program and Abstracts of the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL. Washington, DC: American Society for Microbiology,* **1991**, 354.

52 Medrano, J. L.; *Farmacia Clínica*; 8, 3, **1991**, 270-278.

53 Forti, I.; *Medicina (Buenos Aires)*, 54, **1994**, 54, 439-458.

54 Kees, F.; Grobecker, H.; *Antibiot. Chemother*.; 47, **1995**, 7-9.

55 Inamoto, Y.; Chiba, T.; Kamimura, T. Takaya, T.; *J. Antibiot*.; 41, **1988**, 828-830.

56 Takaya, T.; Takasugi, H.; Masugi, Yamanaka, T.; Kawabata, H. K.; *US Patent*. 4559334, Chem. Abstr.; **1981**, 95: P150687e.

57 Kamachi, H.; Narita, Y.; Okita, T.; Abe, Y.; Imura, S.; Tomatsu, K.; Yamasaki, T.; Okumura, J.; Naito, T.; Oki, T.; Kawaguchi, H.; *J. Antibiot.*; 41, **1988**, 1602-1616.

58 Takaya, T.; Takasugi, H.; Murakawa, T.; Nakano, H.; *J. Antibiot*.; 34, **1981**, 1300-1318.

59 Schotten, C.; *Ber.*; 17, **1884**, 2544.

60 Baumann, E.; *Ber.*; 19, **1886**, 3218.

61 Yamanaka, H.; Chiba, T.; Kawabata, K.; Takasugi, H.; Masugi, T.; Takaya, T.; *J. Antibiot.*; 38, **1985**, 1738-1751.

62 Chauvette, R. R.; Hayes, H. B.; Huff, G. L.; Pennington, P. A.; *J. Antibiot*.; 2, **1972**, 248-250.

63 Lunn, W. H. W.; Burchfield, R. W.; Elzey, T. K.; Mason, E. V.; *Tetraedron Lett*.; 14, **1974**, 1307-1310.

64 Glaxo Group Ltd.; *FR. Patent*. 2426695, Chem. Abstr.; **1980**, 92: 198413c.

65 Latrell, R.; Blumbach, J.; Duerckheimer, W.; Fehlhaber, H. W.; Fleischmann, K.; Kirrstetter, R.; Mencke, B.; Scheunemann, K. H.; Schrinner, E.; Schwab, W.; Seeger, K.; Seibert, G.; Wieduwitt, M.; *J. Antibiot*.; 41, **1988**, 1374-1394.

66 González, M.; Rodríguez, Z.; Tolón, B.; Rodríguez, J. C.; Velez, H.; Valdés, B.; López, M. A.; Fini, A.; *Il Farmaco*, 58, **2003**, 409-418.

67 Murray, R. D. H.; Méndez, J.;Brown, S. A.; The Natural Coumarins-Occurrence, Chemistry and Biochemistry, John Wiley & Sons Ltd., Chichester, Uk, **1982**.

68 O-Kennedy, R.; Thornes, R. D.; Coumarins: Biology, Applications and Mode of Action; Wiley & Sons, Chichester, **1997**.

69 Silván, A. M.; Abad, M. J.; Bermejo, P.; Sollhuber, M.; Villar, A.; *J. Nat. Prod*.; 59, **1996**, 1183-1185.

70 Curini, M.; Epifano, F.; Maltese, F.; Marcotullio, M. C.; Prieto-González, S.; Rodríguez, J. C.; *Aust. J. Chem*.; 56, **2003**, 59-60.

71 Curini, M.; Epifano, F.; Maltese, F.; Marcotullio, M. C.; Tubaro, A.; Altinier, G.; Prieto-González, S.; Rodríguez, J. C.; *Bioorganic & Medicinal Chemistry Letters*; 14, **2004**, 2241-2243.

72 Manolov, I.; Danchev, N. D.; *Arch. Pharm. Pharm. Med. Chem.*, 2, **2003**, 83-94.

73 Singer, L. A.; Kong, N. P.; *J. Am. Chem. Soc*.; 88, **1966**, 5213.

74 Brühlmann, C.; Ooms, F.; Carrupt, P. A.; Testa, B.; Catto, M.; Leonetti, F.; Altomare, C.; Carotti, A.; *J. Med. Chem*.; 44, **2001**, 3195-3198.

75 Kleiner, H. E.; Reed, M. J.; DiGiovanni, J.; *Chem. Res. Toxicol*.; 16, **2003**, 415-422.

76 Appendino, G.; Mercalli, E.; Fuzzati, N.; Arnoldi, L.; Stavri, M.; Gibbons, S.; Ballero, M.; Maxia, A.; *J. Nat. Prod.*; 67, **2004**, 2108-2110.

77 El-Agrody, A. M.; Abd El-Latif, M. S.; El-Hady, N. A.; Fakery, A. H.; Bedair, A. H.; *Molecules*, 6, **2001**, 519-527.

78 Foti, M.; Piattelli, M.; Baratta, M.; T.; Ruberto, G.; *J. Agric. Food Chem*.; 44, **1996**, 497-450.

79 Yu, J.; Wang, L.; Walzem, R. L.; Miller, E. G.; Pike, L. M.; Patil, B.; *J. Agric. Food Chem*.; 53, **2005**, 2009-2014.

80 Miyake, Y.; Murakami, A.; Sugiyama, Y.; Isobe, M.; Koshimizu, K.; Ohigashi, H.; *J. Agric. Food Chem.*; 47, **1999**, 3151-3157.

81 Ito, I.; Itoigawa, M.; Mishina, Y.; Filho, V. C.; Enjo, F.; Tokuda, H.; Nishino, H.; Furukawa, H.; *J. Nat. Prod*.; 66, 3, **2003**, 368-371.

82 Fahr, E.; *Pharmazeutische Zeitung*, 127, **1982**, 163.

83 Edelson, R. L.; *J. Photochem. Photobiol., B: Biol*.; 10, **1991**, 165.

84 Guiotto, A.; Chilin, A.; Manzini, P.; Dall'Aqua, F.; Bordin, F.; Rodighiero, P.; *Il Farmaco*; 50, **1995**, 479.

85 Halliwell, B.; Gutteridge, J. M. C.; *Free Radicals in Biology and Medicine*, 3rd ed.; Oxford University Press: New York, **1999**.

86 Yu, B. P., *Free Radicals in Aging*; CRC Press: Boca Raton, FL, **1993**.

87 Kostova, I.; *Curr. Med. Chem*. Anti-Cancer Agents, 5, **2005**, 29-46.

88 Kolodziej, H.; Kayser, O.; Woerdenbag, H. J.; van Uden, W.; Pras, N. *Z. Naturforsch*, 52, **1997**, 240.

89 Lin, W. L.; Wang, C. J.; Tsai, Y. Y.; Liu, C. L.; Hwang, J. M.; Tseng, T. H.; *Arch. Toxicol*.; 74, **2000**, 467.

90 Chu, C. Y.; Tsai, Y. Y.; Wang, C. J.; Lin, W. L.; Tseng, T. H.; *Eur. J. Pharmacol*.; 25, **2001**, 416.

91 Piller, N. B.; *Br. J. Exp. Pathol.*, 56, **1975**, 554.

92 Piller, N. B.; Casley-Smith, J. R.; *Br. J. Exp. Pathol.*; 56, **1975**, 439.

93 Paya, M.; Halliwell, B.; Hoult, J. R. S.; *Biochem Pharmacol.*; 44, **1992**, 205.

94 Laughton, M. J.; Evans, P. J.; Moroney, M. A.; *Biochem.Pharmacol.*; 42, **1991**, 1673.

95 Nicolaides, D. N.; Fylaktakidou, K. C.; Litinas, K. E.; Hadjipavlou-Litina, D.; *Eur. J. Med. Chem.*; 33, **1998**, 715.

96 Nicolaides, D. N.; Fylaktakidou, K. C.; Litinas, K. E.; Papageorgiou, G. K.; Hadjipavlou-Litina, D.; *J. Heterocycl. Chem.*; 35, **1998**, 619.

97 Nicolaides, D. N.; Fylaktakidou, K. C.; Litinas, K. E.; Hadjipavlou-Litina, D.; *J. Heterocycl. Chem.*; 33, **1996**, 967.

98 Cichewicz, R. H.; Nair, M. G.; *J. Agric. Food Chem.*; 50, **2002,** 87-91.

99 Barclay, L. R. C.; Vinqvist, M. R.; Mukai, K.; Itoh, S.; Morimoto, H.; *J. Org. Chem*.; 58, **1993**, 7416-7420.

100 Wang, T. C.; Lee, K. H.; Chen, Y. L.; Liou, S. S.; Tzeng, C. C.; *Bioorg. Med. Chem. Lett*.; 8, **1998**, 2773-2776.

101 Tzeng, C. C.; Lee, K. H.; Wang, T. C.; Han, C. H.; Chen, Y. L.; *Pharm. Res*.; 17, **2000**, 715-719.

102 Tyagi, Y. K.; Kumar, A.; Raj, H. G.; Vohra, P.; Gupta, G.; Kumari, R.; Kumar, P.; Gupta, R. K.; *Europ. J. of Med. Chem*.; 40, **2005**, 413-420.

103 Bailly, F.; Maurin, C.; Teissier, E.; Vezina, H.; Cotelle, P.; *Bioorg. & Med. Chem*.; 12, **2004**, 5611-5618.

104 Obaseki, A. O.; Coker, H. B.; *J Pharm Pharmacol*.; 39, **1987**, 142-144.

105 Garazd, Y. L.; Panteleimonova, T. N.; Garazd, M. M.; Khilya, V. P.; *Chem. of Natural Compounds*, 39, 4, **2003**, 330-336.

106 Griffin, R. J.; Fontana, G.; Golding, B. T.; Guiard, S.; Hardcastle, I. R.; Leahy, J. J. J.; Martin, N.; Richardson, C.; Rigoreau, L.; Stockley, M.; Smith, G. C. M.; *J. Med. Chem*.; 48, **2005**, 569-585.

107 Knoevenagel, E.; *Chem*. *Ber*.; 29, **1896**, 172, 31, 1898, 730.

108 Jones, G.; *Org*. *React*.; 15, **1967**, 204.

109 Tietze, L. F.; Beifuss, U.; In *Comprehensive Organic Synthesis*; Trost, B. M.; Fleming, I.; Heathcock, C. H., Eds.; Pergamon Press: Oxford, Vol. 2, Chapter 1.11, **1991**, 3341-3394.

110 Johnson, J. R.; *Org. React*.; 1, **1942**, 210.

111 Shriner, R. L.; *Org. React*.; 1, **1942**, 1.

112 Narasimahan, N. S.; Mali, R. S.; Barve, M. V.; *Synthesis*, **1979**, 906.

113 Yavari, I.; Hekmat-Shoar, R.; Zonouzi, A.; *Tetrahedron Lett*.; 39, **1998**, 2391.

114 Pechmann, V. H.; Duisberg, C. *Ber. Dtsch. Chem. Ges.*; 17, **1884**, 929.

115 Koelsch, C. F.; Masley, P. T.; *J. Am. Chem. Soc*.; 75, **1953**, 3596.

116 Dey, B. B.; Sankaranarayanan, Y.; *J. Indian Chem. Soc*.; 11, **1934**, 687.

117 Peipei, S.; Zhixin, H.; *Synth. Commun*.; 35, **2005**, 1875-1880.

118 Sethna, S.; Phadke, R.; *Org. React*.; 7, **1953**, 1.

119 Simmonis, H.; Remmert, P.; *Chem. Ber*.; 47, **1914**, 2229.

120 Robertson, A.; Sandrock, W. F.; Henry, C. B.; *J. Chem. Soc*.; **1931**, 2426.

121 Sethna, S. M.; Shah, N. M.; Shah, R. C.; *J. Chem. Soc*.; **1938**, 228.

122 Appel, H.; *J. Chem. Sot*., **1935**, 1031.

123 Woods, L. L.; Sapp, J.; *J. Org. Chem*. 27, **1962**, 3703.

124 Gunnewegh, E. A.; Hoefnagel, A. J.; van Bekkum, H.; *J. of Molecular Catalysis A: Chemical*, 100, **1995**, 87-92.

125 Potdar, M. K.; Mohile, S; S.; Salunkhe, M. M.; *Tetrahedron Lett*.; 42, **2001**, 9285-9287.

126 Bose, D. S.; Rudradas, A. P.; Babu, M. H.; *Tetrahedron Lett*.; 43, **2002**, 9195-9197.

127 Singh, P. R.; Singh, D. U.; Samant, S. D.; *Synlett*, **2004**, 1909.

128 Valizadeh, H.; Shockravi; A.; *Tetrahedron Lett*.; 46, **2005**, 3501-3503.

129 Sharma, G. V. M.; Reddy, J. J.; Lakshmi, P. S.; Krishna, P. R.; *Tetrahedron Lett*.; 46, **2005**, 6119-6121.

130 Russell, A.; Frye, J.R.; *Org. Synth. Coll*. Vol.3, **1955**, 281, Vol. 21, **1941**, 22.

131 Tong-Shuang, L.; Zhan-Hui; Z.; Feng, Y.; Cheng-Guang; F.; *J. Chem. Res. Synop*.; 1, **1998**, 38-39.

132 Smitha, G.; Sanjeeva-Reddy, C.; *Synth. Commun*.; 34, **2004**, 3997-4004.

133 Ahmed, B.; Khan, S. A.; Alam, T.; *Pharmazie*; 58; **2003**; 173-176.

134 Desai, R.D.; Mavani, G.K.; *Pr. Indian Acad*.; 11, **1942**.

135 Hale, D. K.; Hawdon, A. R.; Jones, J. I.; Packham, D. I.; *J. Chem. Soc*.; **1952**; 3503-3506.

136 Pechmann, V.; Cohen ; *Chem. Ber*.; 17, **1884**, 2190.

137 Pechmann, V. Chem. Ber.; 34, **1901**, 354-362.

138 Campos-Toimil, M.; Orallo, F.; Santana, L.; Uriarte, E.; *Bioorg. Med. Chem. Lett*.; 12, **2002**, 783-786.

139 Dey; T.; *J. Chem. Soc*.; 107, **1915**, 1637.

140 Yadav, G. D.; Nair, J. J.; *Catal. Lett*.; 62, **1999**, 49-52.

141 Wilson, N. G.; McCreedy, T.; *Chem. Commun*.; **2000**, 733-734.

142 Reddy, B. M.; Sreekanth, P. M.; *Tetrahedron Lett*.; 44, **2003**, 4447-4449.

143 Adeeva, V.; Liu, H. Y.; Xu, B. Q.; Sachtler, W. M. H.; *Topics Catal*.; 6, **1998**, 61-76.

144 Deutsch, J.; Quaschning, V.; Kemnitz, E.; Auroux, A.; Ehwald, H.; Lieske, H.; *Topics Catal*.; 13, **2000**, 281-285.

145 Sun, Y.; Ma, S.; Du, Y.; Yuan, L.; Wang, S.; Yang, J.; Deng, F.; Xiao, F. S.; *J. Phys. Chem. B* 109, **2005**, 2567-2572.

146 Liu, Z. Q.; Yu, W.; Liu, Z. L.; *Chem. Phys. Lipids*, 103, 1-2, **1999**, 125-135.

147 Torres, R.; Faini, F.; Modak, B.; Urbina, F.; Labbe, C.; Guerrero, J.; *Phytochemistry*, 67, 10, **2006**, 984-987.

148 Yang, H.; Protiva, P.; Gil, R. R.; Jiang, B.; Baggett, S.; Basile, M. J.; Reynertson, K. A.; Weinstein, I. B.; Kenelly, E. J.; *Planta Medica*, 71, **2005**, 352-360.

149 Benzie, I. F. F.; Strain, J. J.; *Anal. Biochem.*; 239, **1996**, 70-76.

150 Sharma, V. M; Reddy, J. J; Lakshmi, P. S.; Krishna, P. R.; *Tetrahedron Lett.*; 46, **2005**, 6119.

151 John, E.V.O; Israelstam, S.S.; *J. Org. Chem.*; **1961**, 240.

152 Canter, J.; *J. Chem. Soc.*; **1931**, 1255.

153 Wheelock, C.E.; *J. Am. Soc.*; **1959**, 1348.

154 Chakravarti D.; *J.Indian Chem. Soc.*; **1931**, 407-409.

155 Khaikin, M. S.; Petrova, N. L.; Kukhtin, V. A.; *Zh. Obshch. Khim.*; 33, **1963**, 3941-3943.

156 Barris, E.; Israelstam, S.S.; *J. S. African Chem. Inst.*; **1960**, 125.

157 Reddy, B. M.; Reddy, V. R.; Giridhar, D. *Synth. Commun.*; 31, **2001**, 3603-3608.

158 Grover, J. K.; *J. Sci. Ind. Res.*; 11B, **1952**, 50-55.

159 Singh, R. P.; Singh, R. V.; Malik, O. P.; *Indian J. Chem. Sect. B*; 27; **1988**; 1031.

160 Rodríguez-Domínguez, J. C.; Kirsch, G.; *Synthesis*, 11, **2006**, 1895-1897.

161 Rodríguez-Domínguez, J. C.; Kirsch, G.; *Tetrahedron Lett.*; 47, **2006**, 3279-3281.

162 Burstone, M. S.; Enzyme Histochemistry and its application in the study of neoplasms, Academic Press, New York, N. Y., **1962**, 304.

163 Ferguson, W. J.; *US Patent* 5358854, **1994**.

164 Manafi, M.; *J. of Food Microb.*; 60, **2000**, 205.

165 Szameit, C.; Miech, C.; Balleininger, M.; Schmidt, B.; Von Figura, K.; Dierks, T.; *The J. Biol. Chem.*; 274, **1999**, 15375.

166 Ono, K.; Tsuji, H.; Rai, S. K.; Yamamoto, A.; Masuda, K.; Endo, T.; Hotta, H.; Kawamura, T.; Uga, S.; *Appl Environ Microbiol.*, 67, **2001**, 3832.

167 Holt, S. J.; Sadler, P. W.; *Proc. Roy. Soc. London*, B, 148, **1958**, 481.

168 Sadler; P. W.; Warren; R. L.; *J. Am. Chem. Soc.*, 78, **1956**, 1251.

169 Mauthner, J.; Suida, W.; *Monatsh. Chem*.; 9, **1888**, 732.

170 Mauthner, J.; Suida, W.; *Monatsh. Chem*., 9, **1890**, 374.

171 Roth, J. N.; Ferguson, W. J.; *US Patent 5,210,022*, **1993**; *Chem. Abstr*.; 119, 90788, **1993**.

172 Borsche, K.; Weussmann, T.; Fritzsche, F.; *Chem. Ber*.; 57; **1924**; 1773.

173 Bad. Anilin-Sodaf.; *DE Patent* 148615, **1904.**

174 Schwarz, J.; *Monatsh. Chem*., 26, **1905**, 1262

175 Holt, S. J.; Petrow, V.; *J. Chem. Soc*.; **1947**, 607.

176 Bayer & Co.; *DE Patent* 102893.

177 B.A.S.F.; *DE Patent*149346, **1903**.

178 Farbwerk, M.; Leonhardt, A. & Co.; *DE Patent*120105, **1901**.

179 Bad. Anilin-Sodaf.; *DE Patent* 148615, **1904**.

180 Kinzlberger & Co.; *DE Patent* 163842, **1903**.

181 Heller, G.; Fiesselmann, G.; *Justus Liebigs Ann. Chem*.; 324; **1902**; 127.

182 B.A.S.F; *DE Patent* 158346; **1903**.

183 Brewster, R. Q.; Groening, T.; *Organic Syntheses, Coll.* Vol. 2, **1943**, 445*;* Vol. 14, **1934**, 66.

184 Rao, A. V. R.; *Pure & Appl. Chem.*; Vol. 70, No. 2, **1998**, 391-396.

185 Xiao, X. Y.; Li, R.; Hurst, D.; Zhuang, H.; Shi, S.; Czarnik, A. W.; *J. Comb. Chem.*; 4, **2002**, 536-539.

186 Hassan, J.; Sévignon, M.; Gozzi, C.; Schulz, E.; Lemaire, M.; *Chem. Rev.*; 102, **2002**, 1359.

187 Sourdon, V.; Mazoyer, S.; Pique, V.; Galy, J. P.; *Molecules*; 6, **2001**, 673-682.

188 Sugahara, M.; Ukita, T.; *Chem. Pharm. Bull.*; 45, **1997**, 719-721.

189 Hoechster-Farbwerke; *DE Patent* 125456, **1900**.

190 Hoechster-Farbwerke; *DE Patent* 142507, **1903**.

191 Rössing, A.; *Ber*.; 17, **1884**, 2988.

192 Pellón, R. F.; Estévez-Braun, A.; Docampo, M. L.; Martín, A.; Ravelo, A. G.; *Synlett*; 10, **2005**, 1606-1608.

193 Balbuzano-Deus, A.; Rodríguez-Domínguez, J. C.; Fernández-Villalobo, López-López, M. A.; Kirsch, G.; *Org. Prep. and Proced. Int*.; 37, **2005**, 87.

194 Farbenindustrie, I. G.; *FR Patent* 835727, 1, **1938**. *Chem. Abst*. 33: 50047

195 Rodríguez-Domínguez, J. C.; Balbuzano-Deus, A.; López-López, M. A.; Kirsch, G.; *J. Het. Chem*.; for publishing.

196 Holt, S. J.; Sadler, P. W.; *Proc. Roy. Soc. London*, B, 148, **1958**, 481.

197 Heller, M.; Hessel, A.; *J. prakt. Chem*.; 2, 120, **1929**, 73.

198 Sadler; P.; Warren, W.; *J. Am. Chem. Soc*.; 78, **1956**, 1251.

199 Raileanu, D.; Constantinescu-Simon, O.; Mosanu, E; Nenitzescu, C. D.; *Rev. Roum. Chim*.; 12, **1967**, 105.

200 Mardenborough, G. L.; Fan, C. P.; Ablordeppey, Y. S.; Nimrod, A. C.; Alice, M.; *Med. Chem. Res*., 9, **1999**, 118.

201 Holt, S. J.; Petrow, V.; *J. Chem. Soc*.; **1958**, 1217.