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REACTION OPTIMIZATION AND QUANTUM-MECHANICAL CHARACTERIZATION OF SOME SEMISYNTHETIC PENICILLINS

Corina Cheptea[a*], Dan G. Dimitriu[b], Dana O. Dorohoi[b], Razvan N. Malancus[c] and Valeriu Sunel[d]

[a]*Medical Bioingineering Faculty, "Grigore T. Popa" University, 9-13 Kogalniceanu Street, Iasi 700454, Romania*

[b]*Faculty of Physics, "Alexandru Ioan Cuza" University, 11 Carol I Bd, Iasi 700506, Romania*

[c]*Department of Physiology and Pathophysiology, Veterinary Medicine Faculty, "Ion Ionescu de la Brad" University of Life Sciences, 3 Mihail Sadoveanu Alley, Iasi 700490, Romania*

[d]*Department of Organic Chemistry, Faculty of Chemistry, "Alexandru Ioan Cuza" University Iasi, 11 Carol I Bd, Iasi 700506, Romania*

Abstract: In this paper, we report on the synthesis of new semisynthetic penicillins by acylating 6-aminopenicillanic acid with the sulphur derivatives transformed into pivalic acid anhydrides, containing nucleus composed of 3-[(5-nitroindazol-1'-yl-methyl)]-4-aryl-5-mercapto-1,2,4-triazole. The antibacterial properties determined by the presence of β-lactam ring and 5-mercapto-1,2,4-triazoles nucleus in penicillin molecules has been previously evidenced. Factorial experiments were organized in order to establish the conditions in which the reaction yield becomes maximum. The obtained structures were analyzed from quantum-mechanical point of view and their physical-chemical properties were established by computation. The structure of the synthesized compounds was confirmed by the results of elemental and spectral analyses (FT-IR, ^{1}H- and ^{13}C-NMR). The semisynthetic penicillins were previously verified for toxicological action and antibacterial activity and the obtained results were close to those for amoxicillin, used as reference drug.

* Corina Cheptea, *e-mail:* corina.cheptea@umfiasi.ro

***Keywords*:** semisynthetic penicillins; optimization reaction; quantum-mechanical characterization

Introduction

The challenge of developing new semisynthetic penicillins is currently the focus of many research teams.[1,2] This endeavor aims to fulfill the long-standing desire for improved antimicrobial and pharmacological properties following years of penicillin therapy. Key objectives include stability against penicillinase, a wider antibacterial spectrum, resistance to acidic conditions and minimal side effects.

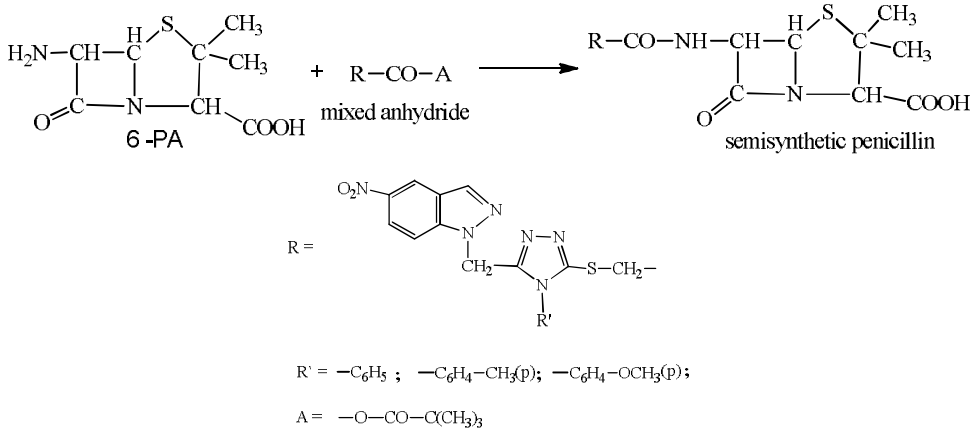
The importance of heterocyclic compounds previously recognized in the field of synthetic organic chemistry has been extensively studied due to their important properties and applications.[3-6] It is well known that several heterocyclic compounds containing nitrogen exhibit a wide variety of biological activities.[7-11]

Throughout our research, we noticed that 5-nitroindazole has important biological activities such as tuberculostatic activity,[12,13] antimicrobial activity[14] that is why we tried to obtain penicillins based on 5-nitroindazole.[15]

The synthesis of these penicillins is intriguing due to the inclusion of the triazole nucleus in certain molecules, rendering them applicability in various medical fields.[16-19] New pharmacological properties: anti-inflammatory,[20-24] antibacterial,[24-28] antitumor,[29-31] antifungal,[32-34] analgesic-antipyretic,[14,35-37] are induced by the nature and position of the substituents in the penicillin molecules.

Results and Discussion

The synthesis of penicillins goes through several stages. In general, the 6-aminopenicilanic acid (6-PA) is treated with acid chloride[16,17] or mixed anhydride[18,19] under specific conditions yields, when a semisynthetic penicillin from 6-acyl-aminopenicillanic acid is obtained (see Scheme 1).



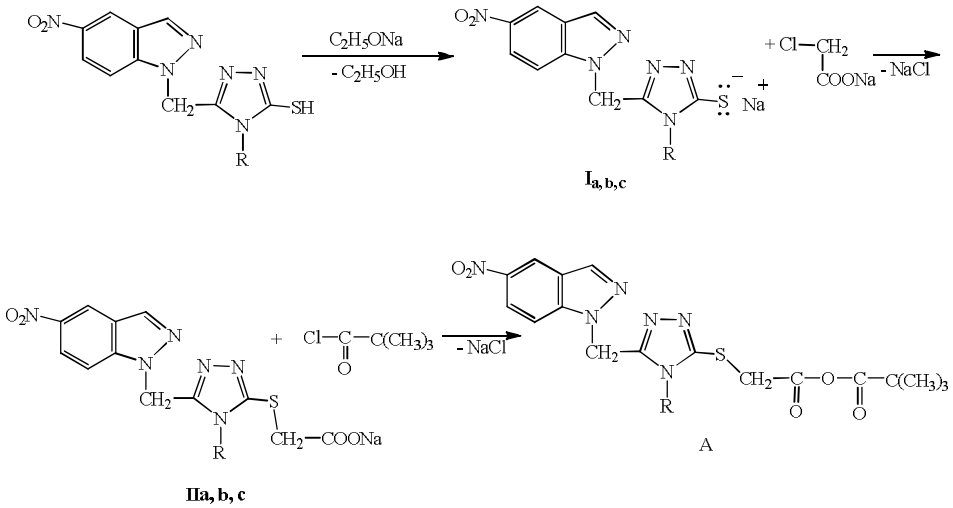
Scheme 1. Semisynthetic penicillins' synthesis reaction.

The acylation of this 6-PA with active forms of 5-mercapto-1,2,4-triazole-disubstituted derivatives follows the general scheme permitting to obtain the final product (Scheme 1).

The triazole derivatives were obtained by basic cyclization of 1-(5′-nitroindazol-1′-yl-acetyl)-4-aryl-thiosemicarbazides prepared in turn by condensation of aromatic isothiocyanates with 5-nitroindazol-1-yl-acetic acid hydrazide.[14]

In the basic environment 5-mercaptotriazoles show reaction center at the sulphur atom in position five of the triazole heterocycle. The sodium salts **Ia–Ic** are obtained by refluxing in anhydrous ethanol containing sodium ethoxide (see Scheme 2).

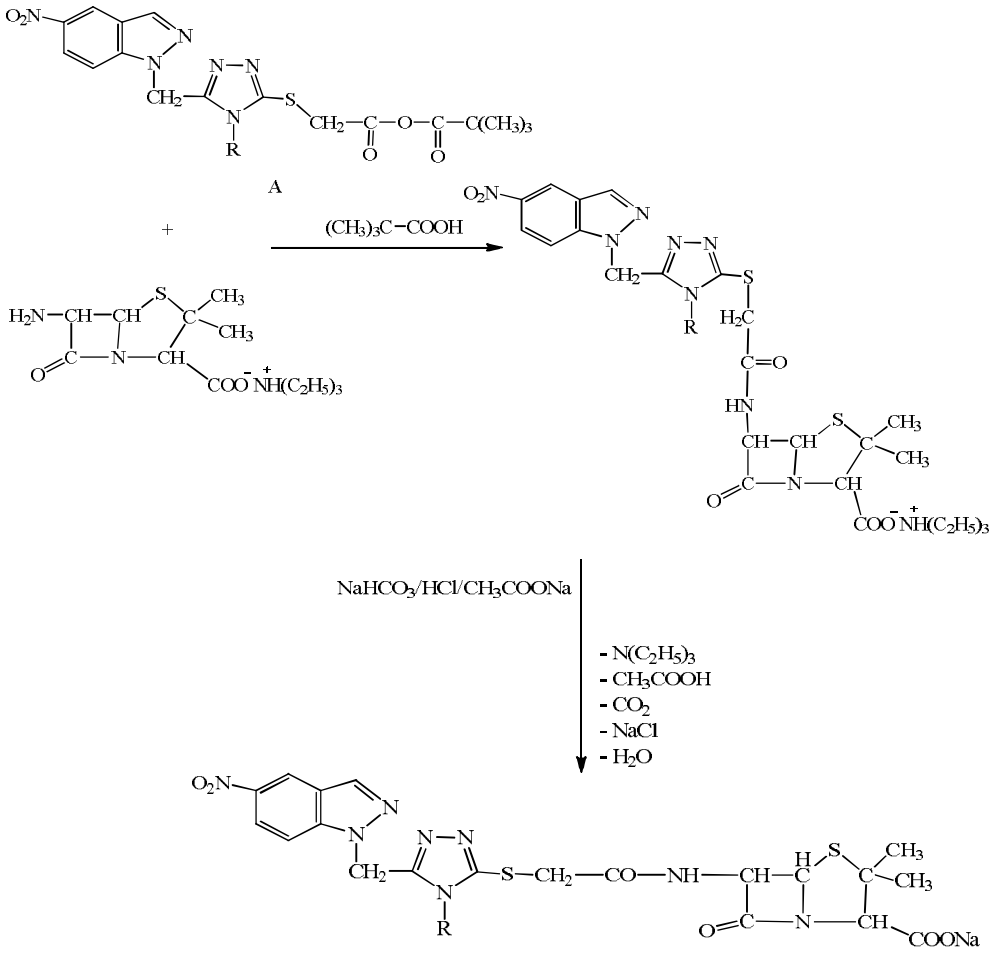
The mercapto-triazoles sodium salts (**Ia-Ic**) react with sodium monochloroacetate forming the derivatives **IIa-IIc**. For the grafting of the triazole ring on the nucleus of 6-aminopenicillanic acid, it was necessary to activate the carboxylic function of sodium thioacetates (**IIa–IIc**), transforming them into mixed type A anhydrides, able to react with the amine group of β-lactamic acid. An ideal partner for this transformation is the pivaloyl chloride which, for electronic and steric reasons due to the three methyl groups at the same carbon atom, facilitates the cleavage of the mixed anhydride at one of the two carbonyls in the desired way[14,38,39] (Scheme 2).



Scheme 2. The obtaining of mixed type A anhydride.

It is important to state that the mixed anhydrides are unstable, so in the next step, we use them *in situ* in dichloromethane solution, in which the 6-aminopenicillanic acid is dissolved in the form of triethylammonium salt. The dichloromethane is removed *in vacuo*. The distillation residue is dissolved in saturated sodium bicarbonate solution, after completion of the coupling reaction. Penicillin passes into alkaline solution as a sodium salt. By acidifying the bicarbonate solution to pH = 1.5–2 in the presence of

butyl acetate, the acid penicillin passes into organic layer, from where it is isolated as a sodium salt (**IIIa–IIIc**) by precipitation with sodium acetate or sodium ethylhexanoate (see Scheme 3).



IIIa,b,c

R = –C_6H_5; –C_6H_4–CH_3; –C_6H_4–OCH_3

Scheme 3. The final reactions for obtaining the semisynthetic penicillins.

The penicillins were synthesized through a series of reactions, starting from sodium 1,2,4-triazole-5-α-thioacetate derivatives which reacts, in the end, with penicillanic acid.

The structure of all compounds **Ia,b,c**, **IIa,b,c**, **IIIa,b,c** was verified by elemental and spectral analysis (FT-IR, ^{1}H- and ^{13}C-NMR)[15].

Optimization reaction of penicillins

Our preliminary studies relived the marked influence of both reaction time ($X_1 = t$) and temperature ($X_2 = T$) on the yield of chemical reactions for obtaining new penicillins. Factorial experiments of 3^2 type[40-43] were organized for each reaction in order to establish the best conditions for preparing the penicillin **IIIa**, **IIIb** and **IIIc**. In the statistical analysis, the reaction yield (η) was considered as indicator for the reaction optimization and its dependence on the relevant variables X_1 and X_2 was studied.

With the extreme values of the real relevant variables [$X_{(i,min)}$,$X_{(i,max)}$] one can define the dimensionless variables, usually recommended in order to facilitate the rapidity of computations, as it follows:

$$x_i = \frac{X_i - \overline{X}_i}{\Delta X_i}, \text{ i} = 1,2 \qquad (1)$$

In equation (1), the notations:

$$\overline{X}_i = \frac{x_{i,\max} + x_{i,\min}}{2}, \text{ i} = 1,2 \qquad (2)$$

are made for the coordinate of the middle of the variation interval and

$$\Delta X_i = \frac{x_{i,\max} - x_{i,\min}}{2}, \text{ i} = 1,2 \qquad (3)$$

for the half-length of the variation range.

After determination of the dimensionless variables, one can return to the real ones, using relation:

$$X_i = \overline{X}_i + x_i \Delta X_i \qquad (4)$$

The real and dimensionless values of the relevant variables (temperature and time) of reactions are written for the compound **IIIa** in Table 1.

Table 1. Temperature, time and yield for reaction of obtaining penicillin **IIIa**.

No.	x_1; X_1 (°C)	x_2; X_2 (h)	x_1x_2	$x_1^2 - 2/3$	$x_2^2 - 2/3$	η (%)
1	-1; 8	-1;1	1	1/3	1/3	75
2	0; 9	-1;1	0	1/3	-2/3	77
3	1; 10	-1;1	-1	1/3	1/3	76
4	-1; 8	0; 1.25	0	-2/3	1/3	76
5	0; 9	0; 1.25	0	-2/3	-2/3	79
6	1; 10	0; 1.25	0	-2/3	1/3	77
7	-1; 8	1;1.5	-1	1/3	1/3	74
8	0; 9	1;1.5	0	1/3	-2/3	76
9	1; 10	1;1.5	1	1/3	1/3	74
Σ	0	0	0	0	0	684

The average value of the reaction yield is $\bar{\eta}$ = 76.00%. A polynomial dependence of the type (5) can be written:

$$\eta = a_0 + a_1x_1 + a_2x_2 + a_{12}x_1x_2 + a_{11}x_1^2 + a_{22}x_2^2 \qquad (5)$$

By the method described in [38], the correlation coefficients for reaction (**IIIa**, Scheme 3) are written below:

a_1 = - 0.667; a_2 = 0.333; a_{12} = - 0.25; a_{11} = -1.333; a_{22} = - 2.00; a_0 = 78.22.

The dependence (5) for obtaining reaction of penicillin **IIIa** becomes:

$$\eta = 78.22 - 0.67x_1 + 0.33x_2 - 0.25x_1x_2 - 1.33x_1^2 - 2.00x_2^2 \qquad (6)$$

The supplementary experiment to determine the yield in the centre of variation range gives for penicillin **IIIa** the values:

η = (77, 78, 77) %; with average value $\bar{\eta}$ = 77.33%.

The experiment's precision is P = 0.1924.

The results of applying the Student test for penicillin **IIIa** are shown in Table 2.

Table 2. Student test for **IIIa**.

	a_0	a_1	a_2	a_{12}	a_{11}	a_{22}
t-test	406.5	3.48	1.72	1.30	6.91	10.40

As t-Student indicates, the product of the variable has the smallest influence on the reaction yield (the t-Student value of a_{12} correlation coefficient is smaller than 1.5) and it was eliminated from the polynomial equation. One obtains a new equation for the polynomial dependence of reaction yield on the relevant dimensionless variables of chemical reaction for compound **IIIa**.

$$\eta = 78.22 - 0.67x_1 + 0.33x_2 - 1.33x_1^2 - 2.00x_2^2 \qquad (7)$$

Fisher test, F = 25.98, shows that relation (7) can be used in estimation the reaction yield for compound **IIIa**.

From the derivatives of the reaction yield on the relevant dimensionless variable one obtains the dimensionless coordinates of the extreme value of the reaction yield. For the dimensionless coordinates, one obtains M (-0.25, 0.08, 78.33%), which is expressed in real coordinates as: M (8.75°C, 1.020 h, 78.33%).

The plotting of the Eq. (7) is shown in Figure 1.

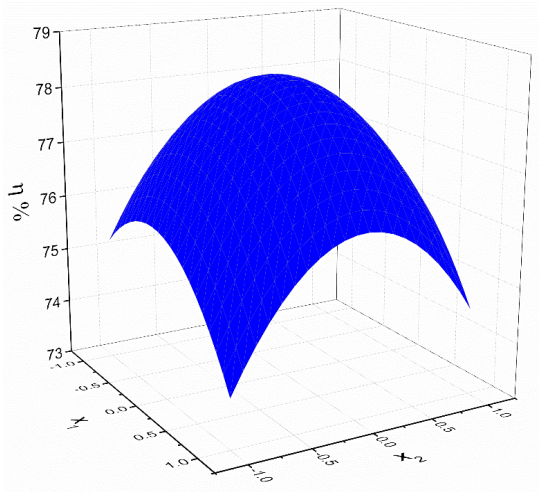


Figure 1. Dependence of the yield of reaction for obtaining the penicillin **IIIa** on the dimensionless temperature and time (according to Eq. (7)).

The real and dimensionless values of the relevant variables (temperature and time) of reactions are written for the compound **IIIb** in Table 3.

Table 3. Time, temperature and yield of reaction for obtaining penicillin **IIIb**.

No.	x_1; X_1 (°C)	x_2; X_2 (h)	x_1x_2	$x_1^2 - 2/3$	$x_2^2 - 2/3$	η (%)
1	-1; 18	-1;2	1	1/3	1/3	78
2	0; 19	-1;2	0	1/3	-2/3	80
3	1; 20	-1;2	-1	1/3	1/3	79
4	-1; 18	0; 2.25	0	-2/3	1/3	79
5	0; 19	0; 2.25	0	-2/3	-2/3	82
6	1; 20	0; 2.25	0	-2/3	1/3	80
7	-1; 18	1; 2.5	-1	1/3	1/3	79
8	0; 19	1; 2.5	0	1/3	-2/3	81
9	1; 20	1; 2.5	1	1/3	1/3	79
Σ	0	0	0	0	0	717

In the case of reaction for obtaining penicillin **IIIb**, the average value of reaction yield for the 9 experiments organized in 3^2 factorial analysis is: $\overline{\eta}$ = 79.67 % and the values of the correlation coefficients of polynomial (5) are computed as being: a_1 = 0.67; a_2 = 0.17; a_{12} = - 0.25; a_{11} = - 1.00; a_{22} = - 3.34; a_0 = 81.56. The polynomial dependence for **IIIb** is written in (8).

$$\eta = 81.56 + 0.67x_1 + 0.17x_2 - 0.25x_1x_2 - 1.00x_1^2 - 3.34x_2^2 \quad (8)$$

The supplementary experiment to determine the yield in the centre of the variation range gives: η = (79, 78, 78) %; $\overline{\eta}$ = 78.33%; P = 0.1924 and t-Student results for **IIIb** are given in Table 4.

Table 4. Student test for penicillin **IIIb**.

	a_0	a_1	a_2	a_{12}	a_{11}	a_{22}
t-test	423.9	3.48	0.88	1.30	5.20	17.36

The dimensionless variable x_2 has no influence on the reaction yield as the small value of t-Student shows. According to Student test it results that the polynomial equation

$$\eta = 81.56 + 0.67x_1 - 0.25x_1x_2 - 1.00x_1^2 - 3.34x_2^2 \qquad (9)$$

correctly describes the chemical reaction yield dependence on the time and temperature.

The high value of Fisher test F = 81.49, shows that relation (9) correctly describes the reaction yield dependence on the dimensionless variables.

From the derivatives of the reaction yield on the relevant dimensionless variable one obtains the dimensionless coordinates of the extreme value of the reaction yield. For the dimensionless coordinates, one obtains *M* (- 0.33, - 0.01, 81.23%), which is expressed in real coordinates as *M* (18.67°C, 2.247 h, 81.23%).

The plotting of the Eq. (9) is shown in Figure 2.

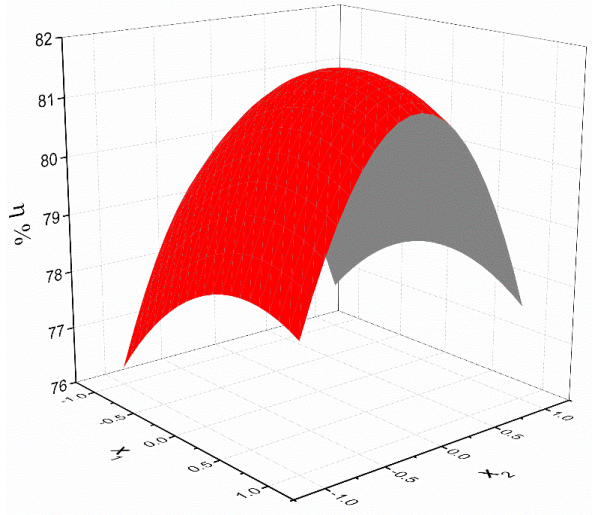


Figure 2. Dependence of the yield of reaction for obtaining the penicillin **IIIb** on the dimensionless temperature and time (according to Eq. (9)).

The real and dimensionless values of the relevant variables (temperature and time) of reactions are written for the compound **IIIc** in Table 5.

Table 5. Time, temperature and yield of reaction for obtaining penicillin **IIIc**.

No.	x_1; X_1 **(°C)**	x_2; X_2 **(h)**	x_1x_2	$x_1^2 - 2/3$	$x_2^2 - 2/3$	η **(%)**
1	-1; 28	-1;3	1	1/3	1/3	85
2	0; 29	-1;3	0	1/3	-2/3	87
3	1; 30	-1;3	-1	1/3	1/3	85
4	-1;28	0; 3.25	0	-2/3	1/3	86
5	0; 29	0; 3.25	0	-2/3	-2/3	89
6	1; 30	0; 3.25	0	-2/3	1/3	87
7	-1; 28	1;3.5	-1	1/3	1/3	87
8	0; 29	1;3.5	0	1/3	-2/3	88
9	1; 30	1;3.5	1	1/3	1/3	86
Σ	0	0	0	0	0	760

In the case of compound **IIIc**, from Table 5 it results the following values: average value of reaction yield $\overline{\eta}$ = 84.44 % and the correlation coefficients of polynomial (5) a_1 = 0.67; a_2 = 0.00; a_{12} = - 0.75; a_{11} = - 1.00; a_{22} = - 2.00; a_0 = 86.44. The polynomial dependence for reaction of penicillin **IIIc** is written in the form

$$\eta = 86.44 + 0.67x_1 - 0.75x_1x_2 - 1.00x_1^2 - 2.00x_2^2 \qquad (10)$$

The supplementary experiment to determine the yield in the centre of range η = (87, 86, 86) %; $\overline{\eta}$ = 86.33%; P = 0.1924 and t-Student results for penicillin **IIIc** are given in Table 6.

Table 6. Student test for **IIIc**.

	a_0	a_1	a_2	a_{12}	a_{11}	a_{22}
t-test	449.3	3.48	0	3.90	5.20	10.40

The dimensionless variable x_2 has no influence on the reaction yield as the small value of t-Student shows. According to the t-Student test it results that the polynomial equation (10) correctly describes the chemical reaction yield dependence on the time and temperature, in the case of penicillin **IIIc**. This conclusion is confirmed by the Fisher test value, 15.59.

From the derivatives of the reaction yield on the relevant dimensionless variable one obtains the dimensionless coordinates of the extreme value of the reaction yield. For the dimensionless coordinates, one obtains *M* (0.18, - 0,08, 86.53%), which is expressed in real coordinates as *M* (28.18°C, 3.23 h, 86.52%).

The plotting of the Eq. (11) is shown in Figure 3.

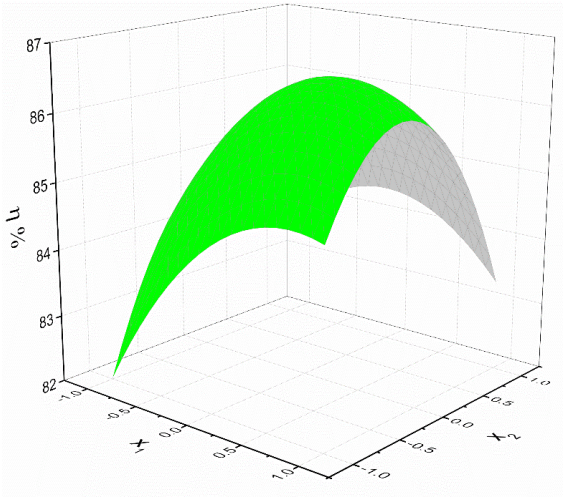


Figure 3. Dependence of the yield of reaction for obtaining the penicillin **IIIc** on the dimensionless temperature and time (according to Eq. (11)).

Quantum mechanical characterization of penicillins IIIa,b,c

As new obtained chemical compounds, the penicillins **IIIa,b,c** were subjected to a quantum mechanical characterization using the program Spartan'14[44,45] for isolated molecules in their electronic ground state. The aim of this analysis was to obtain information on the reactivity of the new compounds, as well as some molecular parameters of interest for

applications (such as the dipole moment, polarizability or polar surface area).

Some results obtained by molecular modeling[46-48] are illustrated in the following. The optimized spatial distribution of the molecular atoms in the studied penicillins is illustrated in Figure 4. The optimized distribution of the molecular atoms corresponds to the best stability (minimum of the molecule's energy) of the isolated molecule in its ground electronic state.

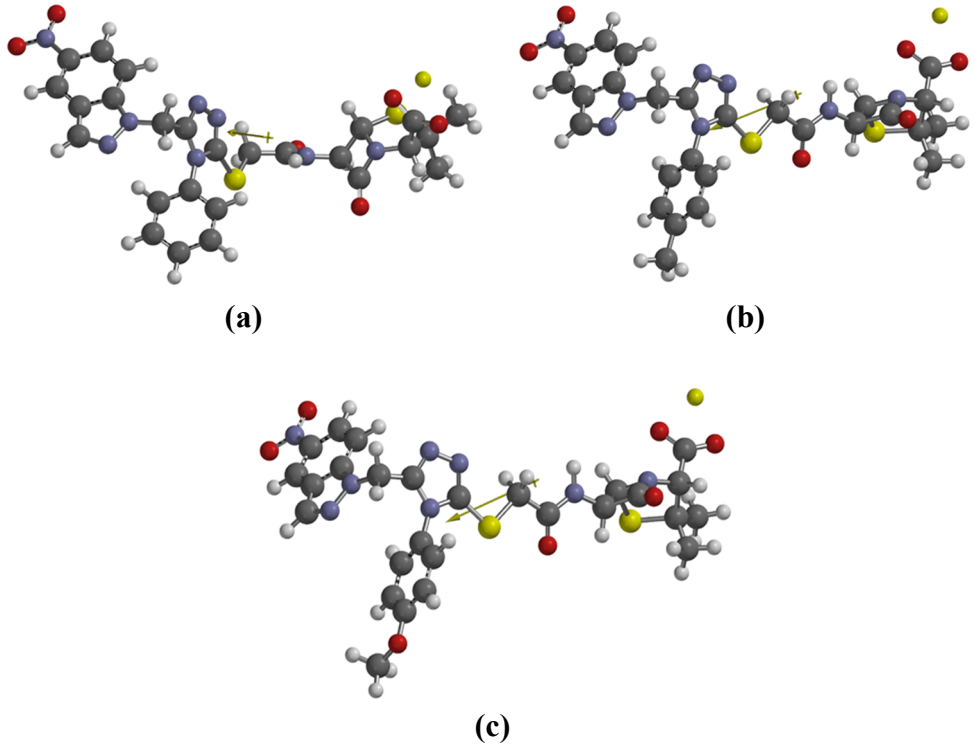


Figure 4. Optimized structures of the penicillins **IIIa** (a), **IIIb** (b) and **IIIc** (c). The colors of the atoms are: red for nitrogen, white for hydrogen, yellow for sulphur (large spheres) and sodium (small sphere), respectively. The arrow indicates the electrical dipole moment of the molecule.

Figure 4 shows that the substituents influence the change in the orientation of the electric dipole moment in the compounds under study.

The highest occupied molecular orbitals (HOMO) and the lowest unoccupied molecular orbitals (LUMO)[49] of each studied penicillin are shown in Figure 5, in which the arrows suggest the electronic transitions

between these orbitals. From these figures it results that the UV absorption bands of penicillins are determined by intramolecular charge transfer (ICT).

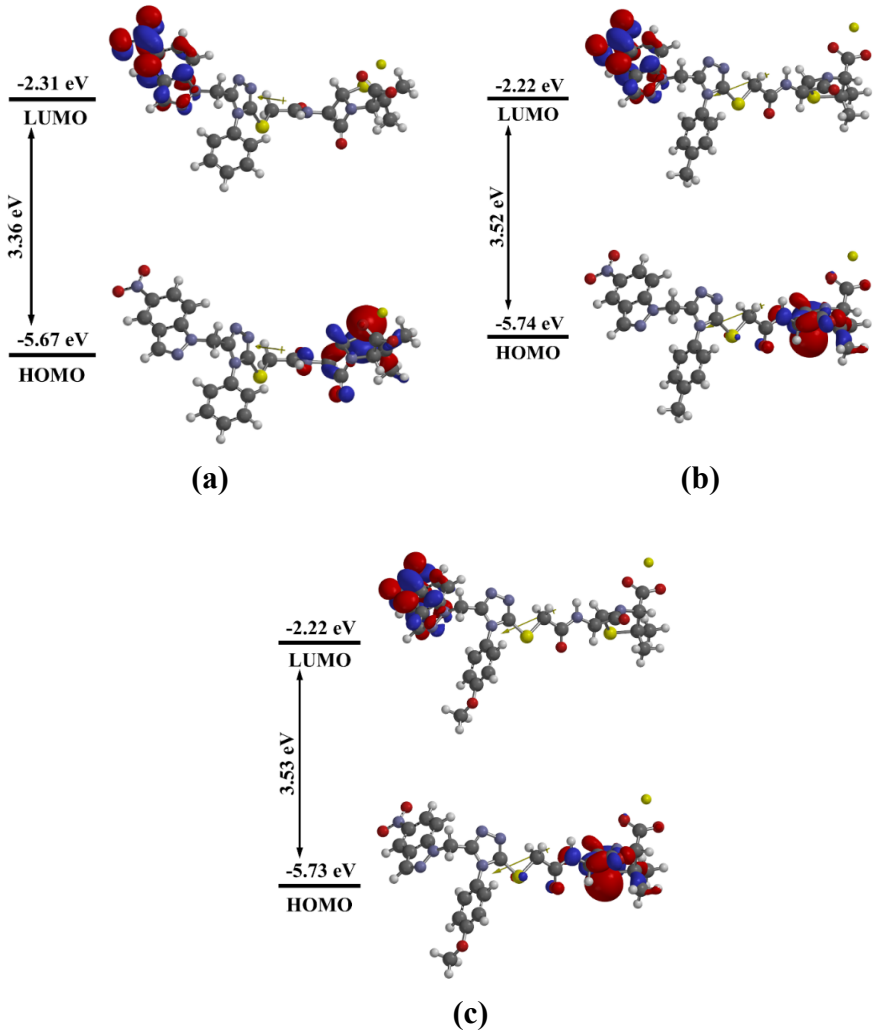


Figure 5. Charge transfer transitions between HOMO and LUMO for penicillins **IIIa** (**a**), **IIIb** (**b**) and **IIIc** (**c**).

From Fig. 5 it results that the absorption of the UV photons induces similar modifications in the three penicillins studied in this paper.

Some parameters[47,48] corresponding to the optimized atomic configuration of the studied penicillins, computed with DFT (Density Functional Theory) method EDF2, together with the basis set 6-31 G* from Spartan'14, are given in Table 7.

Table 7. Computed parameters of penicillins **IIIa – IIIc**.

Parameter	Penicillin		
	IIIa	**IIIb**	**IIIc**
Weight (a.m.u.)	630.642	644.669	660.668
Energy (a.u.)	-2851.38432	-2890.66758	-2965.84333
E_{HOMO}	-5.67	-5.74	-5.73
E_{LUMO}	-2.31	-2.22	-2.22
Dipole moment (D)	12.38	6.58	5.54
Tautomers	1	1	1
Conformers	7776	7776	15552
Area (Å^2)	578.44	603.68	612.98
Volume (Å^3)	549.30	567.64	576.32
Polar surface area (Å^2)	136.298	137.865	144.525
Polarizability (Å^3)	85.14	86.59	87.30
Hydrogen bond donor (HBD) count	1	1	1
Hydrogen bond acceptor (HBA) count	15	15	16

From Table 7 it results the increase of the molecular stability when passes from penicillin **IIIa** to **IIIc**, induced by the substituents.

The energy gap $\Delta E = E_{HOMO} - E_{LUMO}$ is in the UV range of the spectrum with the values: 27102 cm^{-1} for penicillin **IIIa**, 28392 cm^{-1} for penicillin **IIIb** and 28312 cm^{-1} for penicillin **IIIc**. These values[47,48] show that the highest chemical reactivity corresponds to penicillin **IIIa**, while for the last two penicillins the values are very close. At the same time, by passing from penicillins **IIIa** to **IIIc**, the molecular dipole moment diminishes from 12.38 D to 5.54 D. The hydrogen substitution with –CH_3

and with –O–CH_3 determines the decrease of the dipole moment from 12.38D to 6.58 D, respectively to 5.54 D.

The molecular polar surface area (PSA)[50] of the studied penicillins increases from **IIIa** to **IIIc** (see Table 7), showing a worst penetrability of the molecules in which the hydrogen atom is substituted. These values are probably diminished in polar liquids (as water or alcohols) in which the penicillins are dissociated, and their spatial configuration is modified.

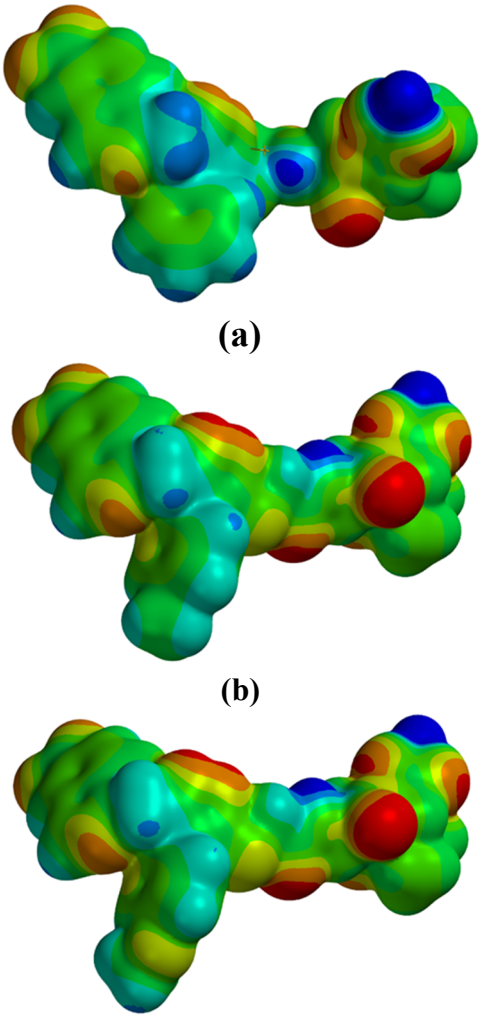


Figure 6. Electrostatic potential maps of the penicillins **IIIa (a)**, **IIIb (b)** and **IIIc (c)**.

Some information about the penicillins reactivity and about the places at which it can add protons, or it can donate protons can be obtained from the electrostatic potential maps[51] shown in Fig. 6. Electrostatic potential map (an overlay of the electrostatic potential on the electron density) describes the overall molecular charges distribution and helps to anticipate the sites of the electrostatic addition[47,48].

From Fig. 6 it results some small modifications in the aspect of electrostatic potential in the molecular region of the hydrogen substitution by $–CH_3$, or by $–O–CH_3$.

The biological activity of the obtained **IIIa**, **IIIb** and **IIIc** semisynthetic penicillins was analyzed, and the results are published in a previous article[15], were their non-toxic character and antibacterial activity are established.

In this paper 3^2 factorial experiments were made in order to establish the optimal conditions for synthesis. Additionally, quantum mechanical characterization with Spartan'14 evidenced the physical-chemical parameters of the semisynthetic penicillins.

Conclusions

The penicillins were synthesized through a series of reactions, starting from sodium 1,2,4-triazole-5-α-thioacetate derivatives which reacts, in the end, with penicillanic acid.

The favorable conditions for the obtaining reactions of semisynthetic penicillins **IIIa – IIIc**, established by 3^2 factorial experiments, showed that the reaction temperature was near the ambiental value. The reaction time was between one to three hours, increasing from **IIIa** to **IIIc**. The maximum yield was near 90% for compound **IIIc**.

According to the quantum mechanical calculations one can find the penicillins stability, their dipolar and polarizable character. The small value of the energy gap, ΔE, demonstrates a good chemical reactivity of studied

penicillins. The dipole moments of the semisynthetic penicillins decrease from **IIIa** to **IIIc**, while the molecular polarizability increases in the same sense. These characteristics come in addition to the non-toxic and antibacterial properties previously established. The polar surface area (PSA) of the studied penicillins is high (higher than 90 $Å^2$) and do not facilitate the cell penetration. The hydrogen bond acceptance (HBA) count (equal with 15) shows that the synthetized compounds can add protons by hydrogen bonds.

We honor the memory of *Emeritus University Professor Valeriu Șunel*, we will keep alive his dedication to science and to the undergraduate, master and doctoral students with whom he worked tirelessly and with great pleasure in the chemistry laboratory.
With gratitude and respect, let's pay a final tribute to the one who guided our steps on the path of science,
Professor Emeritus Chemist Dr. Valeriu Șunel

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