**TWO (SULFONE-AMIDOSULPHONYL)-AMIDES DERIVED FROM
N-(P-AMIDOBENZOYL)-L-GLUTAMINE; OPTIMIZED SYNTHESIS
AND TOXICITY STUDY**

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Abstract: Two derivatives of glutamine were synthesized and the most convenient physical conditions for obtaining reactions were established in factorial experiments with temperature and time of reactions considered as relevant variables. A quantum-mechanical characterization for the new compounds was performed by using HyperChem 8.0.6 Programs and their physical and chemical properties were theoretically estimated. The toxicity of two new compounds was experimentally determined.

Keywords: N-[p-(R)-Benzoyl]-L-Glutamine derivatives, factorial experiment for optimization, QSAR parameters, toxicity test.

Introduction

The sulphonamides occupy an important place between the substances used in bactericidal therapy [1].

The German pathologist and bacteriologist Gerhard Domagk was the first who establish the sulphonamide antibacterial effects [2]. The toxicity and the selectivity of these chemotherapeutic agents largely depend on the possibility of inhibition of folic acid synthesis, indispensable for the formation of nucleic acids, respectively for cell multiplication [3]. Corresponding to the structural analogy with p-aminobenzoic acid, the sulphonamides react, forming folic acid nonfunctional analogues [4-9].

Based on these data it was assumed that glutamine and its derivatives acylated with p- substituted benzoyl group, participating in the metabolism of animal bodies, reduce the toxicity of medicinal products and, at the same time, may become important folic acid antagonists involved at

the cellular level.

These data have justified researchers to synthesize new sulfonamides containing in their structure N-(p-aminobenzoyl)-L-glutamine.

2. Experimental part and molecular modeling

2.1. Synthesis of new sulfonamides of glutamine

The N-[p-methylsalicyl-amidosulphonyl)-benzoyl]-L-glutamine (I) was obtained in the following way: 0,004 moles N-(p-aminobenzoyl)-L- glutamine, 0.004 moles 5-chlorosulfonyl-methyl salicylate, 25 mL anhydrous dioxane and 0,2 mL pyridine were introduced in a flask equipped with ascending refrigerator and the mixture was refluxed for an hour, in a thermostatic silicone bath, at 116 – 118°C, in a reaction time of about 58-62 minutes. A light yellow solid compound was separated from the reaction mixture by cooling and

heating with anhydrous ethylic ether. The pure compound filtered in vacuum dried and by recrystallization from ethyl acetate, melts at 211 – 213°C. The reaction yield was 83% (1,63g).

The N-[p-methyl-phenoxyacetate-amidosulphonyl]-benzoyl]-L-glutamine (II) has been obtained in the temperature range 116-120°C and reaction time was of about an hour. The compound was prepared starting from 0,004 moles N-(p-aminobenzoyl)-L-glutamine, 30 mL anhydrous dioxane, 0,004 moles 2-hydroxy-4-chlorosulfonyl-methyl phenoxyacetate and 0,2 mL pyridine. The pure compound, purified by recrystallization from ethyl acetate, melts at 216 – 218°C. The reaction yield was 65% (1,24 g).

All reagents were used as purchased (Sigma-Aldrich, Fluka, Merck, S.C. Chemical Company S.A.). The melting points of the obtained compounds were determined with a Mel-Temp melting point module, provided with digital thermometer.

2.2. HyperChem calculations

HyperChem 8.0.6 [10] is a molecular modelling program that allows to build and analyze different molecular structures and to determine the physico-chemical properties. The molecules of N-[p-(methylsalicylamidosulfonyl)-benzoyl]-L-glutamine (I) and N-[p-(methyl-phenoxyacetate-amidosulfonyl)-benzoyl]-L-glutamine (II) have been studied using molecular modelling program HyperChem 8.0.6 by one of the semi-empirical method PM3 [11-13].

The molecule was geometrically optimized using the Polak – Ribiere algorithm as convergence criterion and the maximum gradient used to optimize the studied chemical structures was 0.1 kcal / (Å.mol). A calculation type "single point" and a number of physical and structural properties have been obtained, after the molecular geometry optimization.

The energy difference ΔE is proportional to the electrophilicity index of compounds and offers information about the compound biological activity.

The ionization potential and electron

affinity can be estimated from the values of HOMO and LUMO energies, by applying theorem Koopmans [14].

$$I = -E_{\text{HOMO}} \quad (1)$$

$$A = -E_{\text{LUMO}} \quad (2)$$

Quantitative Structure – Activity Relationships (QSAR) [15] modelling correlates the molecular structure or the properties derived from molecular structure with a particular kind of chemical or biochemical activity.

LogP [16] is related to the hydrophobic character of the molecule, which plays an important role in biochemical interactions. The lipophilicity [17] is a determining factor in the adsorption and distribution of the compounds in the human body.

2.3. Factorial experiment for reaction optimization

The 3^2 factorial program (in which 3 is the level of the variation of the relevant variables and 2 is the number of significant variables) was used in order to establish the most convenient conditions for chemical reaction in which the studied compounds [18,19] were obtained.

In our experiment the variables are: the reaction yield (y), temperature (x_1) and the time interval of reaction (x_2). The adimensional relevant variables x_i , ($i=1, 2$) are computed [20-22] as function of:

$$\overline{X_i} = \frac{X_{i\min} + X_{i\max}}{2} \quad (3)$$

The correlation between the adimensional variable x_i ($i=1,2$) and the real variable X_i ($i=1, 2$) is given by :

$$X_i = \overline{X_i} + x_i \Delta X_i \quad (4)$$

Where $\overline{X_i}$ is the average value of the real variation domain and ΔX_i is the half of the real variation domain [19].

The estimations are made in adimensional variables which allow rapid calculations and then the real values are obtained by using relation (4).

The polynomial model used for optimization reaction takes into consideration

the both singular influence of the relevant variables and its sinergetic influence:

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

This statistic technique of the 3^2 factorial program permits to avoid a large

series of experiments for determining the model coefficients.

3. Results and discussions

3.1. Optimization of the obtaining reactions for compounds I and II

For the beginning, $3^2 = 9$ experiments were organised and the results are given in Table 1 for the studied substances (I) and (II).

Table 1 Adimensional (real) variables $x_i(X_i)$, $i=1,2$ and reaction yield y for compounds I and II

$x_1 (X_1^\circ\text{C})$	$x_2 (X_2\text{min})$	x_1x_2	$x_1^2 - \frac{2}{3}$	$x_2^2 - \frac{2}{3}$	y_I	y_{II}
-1 (116)	-1 (58)	1	1/3	1/3	76	60
-1 (116)	0 (60)	0	1/3	-2/3	80	63
-1 (116)	1 (62)	-1	1/3	1/3	77	60
0 (118)	-1 (58)	0	-2/3	1/3	79	60
0 (118)	0 (60)	0	-2/3	-2/3	83	65
0 (118)	1 (62)	0	-2/3	1/3	80	63
1 (120)	-1 (58)	-1	1/3	1/3	77	61
1 (120)	0 (60)	0	1/3	-2/3	81	64
1 (120)	1 (62)	1	1/3	1/3	79	62

The coefficients $a_0 - a_{22}$ of the model (5) were computed by statistical means.

The results for the studied compounds are listed in Table 2.

Table 2 Polynomial coefficients of relation (5)

Compound	a_0	a_1	a_2	a_{12}	a_{11}	a_{22}
I	82.888	0.667	0.667	0.25	-2.333	-3.333
II	64.666	0.667	0.667	0	-1	-3

The t-student test must be made in order to decide if these coefficients are the best. Three experiments in the center of the

variation domain were organized and the obtained data in these experiments are listed in Table 3.

Table 3 Yield values in the middle of the variation domain η_{ic} ($i=1,2,3$), the average yield in center of variation range $\bar{\eta}_c$, square average deviation S_η , precision P

Compound	η_{1c}	η_{2c}	η_{3c}	$\bar{\eta}_c$	$\Delta\eta_{ic}$	S_η	P
I	82	83	82	82.333	0.333	0.333	0.192
II	65	64	63	64	1	1	0.333

The t-student coefficients can be computed using the same precision P for the

three experiments. The values of the t-student coefficients are listed in Table 4.

Table 4 T-student coefficients of obtaining reaction for compounds I and II

Compound	t_0	t_1	t_2	t_{12}	t_{11}	t_{22}
I	431.718	3.489	3.489	1.302	12.151	17.359
II	194.19	2.003	2.003	0	3.003	9.009

The t-student coefficient smaller than unity indicates a small influence of the

corresponding variable on the reaction yield and the term with this coefficient must be

eliminated. The real values for the maximum yield of reactions are listed in Table 5.

Table 5 Real values for the maximum of the reaction yield

Compound	$x_{1e}(X_1\text{ }^{\circ}\text{C})$	$x_{2e}(X_2\text{ min})$	$y_e(\%)$
I	0.15 (117.15)	0.10 (60.20)	82.38
II	0.35 (118.7)	0.13 (60.25)	64.82

The relations in which the reaction yield is expressed by relevant variables and by their products are the following:

$$y_I = 82.89 + 0.67x_1 + 0.67x_2 +$$

$$0.25x_1x_2 - 2.333x_1^2 - 3.333x_2^2 \quad (6)$$

$$y_{II} = 64.67 + 0.67x_1 + 0.67x_2 + 0.25x_1x_2 - x_1^2 - 3x_2^2 \quad (7)$$

3.2. Calculation results using HyperChem 8.0.6

By using the molecular modelling software, the main physical-chemical characteristics of sulfonamides were

calculated.

The optimized geometry of compounds (I) and (II) are represented in figure 1 (a and b).

a) Compound I

b) Compound II

Figure 1 The optimized structures of compounds (colors for atoms are as follows: carbon-green, nitrogen-blue, oxygen-red, hydrogen-white and sulfur-yellow)

The QSAR parameters calculated from HyperChem program for compounds I and II are listed in table 6 and the electro-

optical properties resulting from HyperChem program are listed in table 7.

Table 6 QSAR parameters calculated by HyperChem using semi-empirical method PM3

Parameters QSAR	Compound I	Compound II
Log P	-3.55	-4.38
Hydration energy (kcal/mol)	-25.69	-28.54
Refractivity ($\text{\AA}^3$)	120.73	127.25
Polarizability ($\text{\AA}^3$)	42.22	44.69
Mass (u.a.m)	479.46	509.48
Surface Area ($\text{\AA}^2$)	667.60	742.62
Volume ($\text{\AA}^3$)	1242.18	1333.83

Table 7 The electro-optical properties resulting from HyperChem program by method PM3 for compound I and II

Properties	Compound I	Compound II
Total energy (kcal/mol)	-138588.44	-148793.3931
Binding energy (kcal/mol)	-5768.4577	-6132.8584
Heat of formation (kcal/mol)	-315.0847	-344.8324
Dipole moment (Debye)	4.878	5.456
E _{LUMO} (eV)	-1.21276	-1.1913
E _{HOMO} (eV)	-9.29476	-9.2786
ΔE = E _{HOMO} - E _{LUMO} (eV)	8.082	8.0873

A negative value of logP indicates that the compound is hydrophilic while the positive values of logP shows that the studied compound is lipophilic. The studied compounds are hydrophilic.

The stability of the studied molecular structures is given by their high values of negative total energy.

4. Toxicity evaluation

The Spearman-Kärber arithmetic method [23] was used in determining the lethal dose 50% LD₅₀. The final formula in this method is (8):

$$DL_{50} = DL_{100} \frac{\sum(a \times b)}{n} \quad (8)$$

where: a – the difference between two successive doses of the substance administered; b – the average number of dead animals of two consecutive batches; n – the number of mortalities in a batch; DL₁₀₀ – the

lethal dose 100%.

The results of the toxicity study (Table 8), based on Spearman-Kärber method, confirm that the using N-(p-aminobenzoyl)-L-glutamine as transport agent for sulphonamide residues significantly influence the toxicity diminishing and requires further research to establish their bacteriostatic activity.

Lots of 6 white male mice were used in order to determine the acute toxicity of the two new sulphonamides.

The substances tested (I, II) were administered intraperitoneally as a suspension in Tween 80 and the mortality was recorded 24 hours, 48 hours and 7 days.

The values of DL₅₀ for the synthesized compounds were compared with the acute toxicity of sulfamethoxazole finding the values below than these.

Table 6 The results of the toxicity study for compound I and II

Compounds	DL ₅₀ (mg/kg body)			
	24 hours	48 hours	7 days	Average
I	8500	8500	8350	8460
II	8720	8730	8640	8693
Sulfamethoxazole	2600	2600	1750	2316

The new synthesized compounds are non-toxic and the DL₅₀ values recommend them for the laboratory screening.

5. Conclusions

The 3² experiments organised in order to optimize the reaction yield for obtaining compounds I and II avoid the big number of experiments made for establish the favorable conditions of the chemical reaction.

The highest values of the reaction yield for obtaining the compounds I and II are of about 85% and 63%, respectively.

Having in view the values of the relevant variables corresponding to the maximum of the reaction yield, the reaction can be made at real values of the relevant variables with the precursors quantities conducting to a given quantity of the chemical product.

The electro-optical properties and QSAR parameters of the synthesized compounds obtained by using HyperChem 8.0.6 program recommend compounds I and II as biologically active substances.

The toxicity evaluation of synthesized sulphonamides was established by DL_{50} ,

which recommend them for antibacterial and antifungal activity testing.

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