ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2014, *59*(9), 739-747

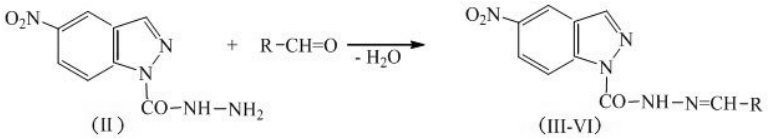
OPTIMIZATION REACTION FOR OBTAINING NEW HYDRAZIDONES WITH BIOLOGICAL ACTION

Toni ANDOR CIGU,[a] Cristina-Delia NECHIFOR,[b] Valeriu SUNEL,[a]
Dana-Ortansa DOROHOI[c,*] and Corina CHEPTEA[d]

[a] "Al. I. Cuza" University, Faculty of Chemistry, 11 Carol I Bvd., 700506, Iaşi, Roumania, vsunel@uaic.ro
[b] "Gheorghe Asachi" Technical University, Machine Manufacturing and Industrial Management Faculty, 67 D. Mangeron Bvd. RO- 700050, Iaşi, Roumania, cristina.nechifor@tuiasi.ro
[c] "Al. I. Cuza" University, Faculty of Physics, 11 Carol I Bvd. RO-700506, Iaşi, Roumania, ddorohoi@uaic.ro
[d] "Gr.T. Popa" University of Medicine and Pharmacy, Faculty of Medical Bioengineering, 9-13 Kogălniceanu Street, 700454, Iaşi, Roumania, coricheptea@yahoo.com

Received April 30, 2014

New hydrazidone derived from 5-nitroindazole were synthesized in order to obtain new substances with drug action involved. In this point of view, the ethyl ester of the 5-nitroindazol-1-yl formyl acid was synthesized and subjected to the reaction with hydrazine hydrate in order to obtain 5-nitroindazol-1-yl-formylhydrazide. A new series of hydrazidones was obtained by condensation with various aromatic aldehydes. The purpose of this paper is to establish the conditions in which the reactions for obtaining the new compounds can be realized with the highest yield. The optimization reaction for obtaining new hydrazidones of 5-nitroindazole was realized in a 3^2 factorial experiment. The chemical structure of the new synthesized compounds was confirmed through elemental and spectra (FT-IR, lH-RMN) analysis. The structural characterization of the new hydrazidones was confirmed by spectral analyses. Toxicological tests show that hydrazidones are compounds with antitumor activity.



INTRODUCTION

Data from the literature stresses the importance of hydrazidones with heterocyclic structure as a result of the presence of the group which can confer many biological activities (ex. antibacterial,[1-4] anticonvulsant,[5] antifungal,[6,7] anti-inflammatory,[8,9] antimalarial,[10] anti-tumor[11-14] and anti-HIV[15] activities). Acyl hydrazidones were cited for their action and anti-tuberculosis activity. Thus K. Bedia *et al.*[16] have recently succeeded in obtaining a series of izonicotinoyl-hydrazidones which indicates a low toxicity and high antituberculous activity of isoniazid.

Our paper reports the synthesis of four new compounds derived from hydrazidones and the optimization of their obtaining reactions. The toxicity and lethal dose LD50 were establish in order to search their pharmacological action.

When reaction yield depends by two significant variables, a 3^2 factorial experiment[17-24] allows obtaining information about the experimental conditions for which the compounds are obtained with the highest yield. The most efficient conditions for synthesis of chemical compounds are obtained by reaction optimization, in order to avoid unwanted consumption of starting compounds.

* Corresponding author: ddorohoi@uaic.ro

EXPERIMENTAL

1. General procedure for synthesis of hydrazidones (III-VI)

The literature indicates that the ≡C–NH–N=CH– group in hydrazidone molecules improves their biological potential. These experiments were leaded to the condensation of hydrazide of the 5-nitroindazolyl-formyl acid II, with various aldehydes, for synthesis a new series of N-acyl-hydrazidones containing in their structure the mentioned group. The hydrazide of 5-nitroindazolyl-formyl acid II was obtained by treating the ethyl ester of the 5-nitroindazolyl-1-formyl acid I with hydrazine hydrate. For a comparative study on the influence of the substituents from the aromatic rings of the aldehydes involved in the condensation reaction, the aromatic aldehydes, substituted with nitro, hydroxyl or dimethylamine groups in various positions, have been employed.

In the first step of the synthesis the ethyl ester of the 5-nitroindazol-1-yl-formyl acid (I) was obtained through 5-nitroindazole condensation with ethyl chloroformate, in the presence of sodium ethoxide, on refluxing in absolute ethyl alcohol.

In the next step, the esther (I) was treated with hydrazine hydrate 98% in anhydrous ethanol with obtaining the hydrazide of the 5-nitroindazolyl-formyl acid (II).

In a flask equipped with ascending refrigerator 5-nitroindazole-1-formyl-hydrazide (0,005 moles) and absolute ethyl alcohol (100 mL) were introduced and the mixture was refluxed on a water bath until a clear solution was obtained. In the next step, aldehyde derivatives (0,005 moles) dissolved in anhydrous ethyl alcohol (10-15 mL) is added, refluxing continuing for other 120 minutes. On cooling, the corresponding hydrazidones are crystallized, filtered under vacuum and washed on a filter with ethyl ether for removing the excess of aldehyde. The synthesized compounds were purified from boiling ethyl alcohol.

The 5-nitroindazole-1-yl-formylhydrazide (II) was condensed with benzaldehyde, o- and p-nitrobenzaldehyde and o-hydroxybenzaldehyde; the corresponding series of 4 hydrazidones (III-VI) was obtained.

The substituents R of hydrazidones (III-VI) are listed below.

$III\ R = -C_6H_5$; $IV\ R = -C_6H_4 - NO_2(o)$;

$V\ R = -C_6H_4 - NO_2(p)$; $V\ IR = -C_6H_4 - OH(o)$

The reaction was performed in boiling alcohol. N-acyl hydrazidones III-VI, prepared by synthesis, are crystalline substances, yellow, melting point fixed. Purification was done by recrystallization from boiling ethanol, and the finished products were obtained with yields ranging from 63-86%. The structure of compounds III-VI inferred by the synthesis, was confirmed by elemental and spectral (FT-IR, 1H-NMR) analysis. The melting points were measured.

2. Materials and methods

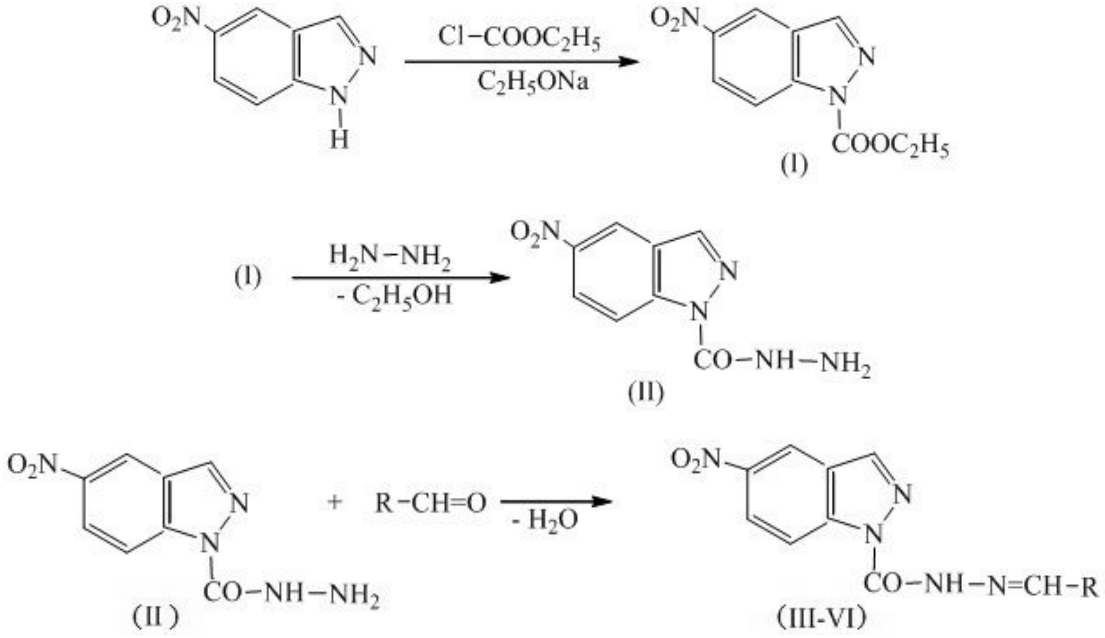
All reagents were used as purchased (Sigma-Aldrich, Fluka, Merck, S.C. Chemical Company S.A.). FT-IR spectra were registered using a FTIR spectrophotometer (ATR) Brucker Tensor-27; 1H-NMR analysis was performed on a Brucker ARX 400 spectrometer (5mm QNP probe; 1H/13C/31P/19F). The elemental analysis was made using Exeter Analytical CE 440 elemental analyzer. The melting points were determined with a Mel-Temp melting point module, provided with digital thermometer.

RESULTS

1. Spectral data

The ^{1}H NMR, ^{13}C NMR and FTIR spectra of compound III are exemplified in Figs. 1-3 respectively.

The color, purity, melting point, computed and experimental elemental composition are given below, for each compound (III-VI).



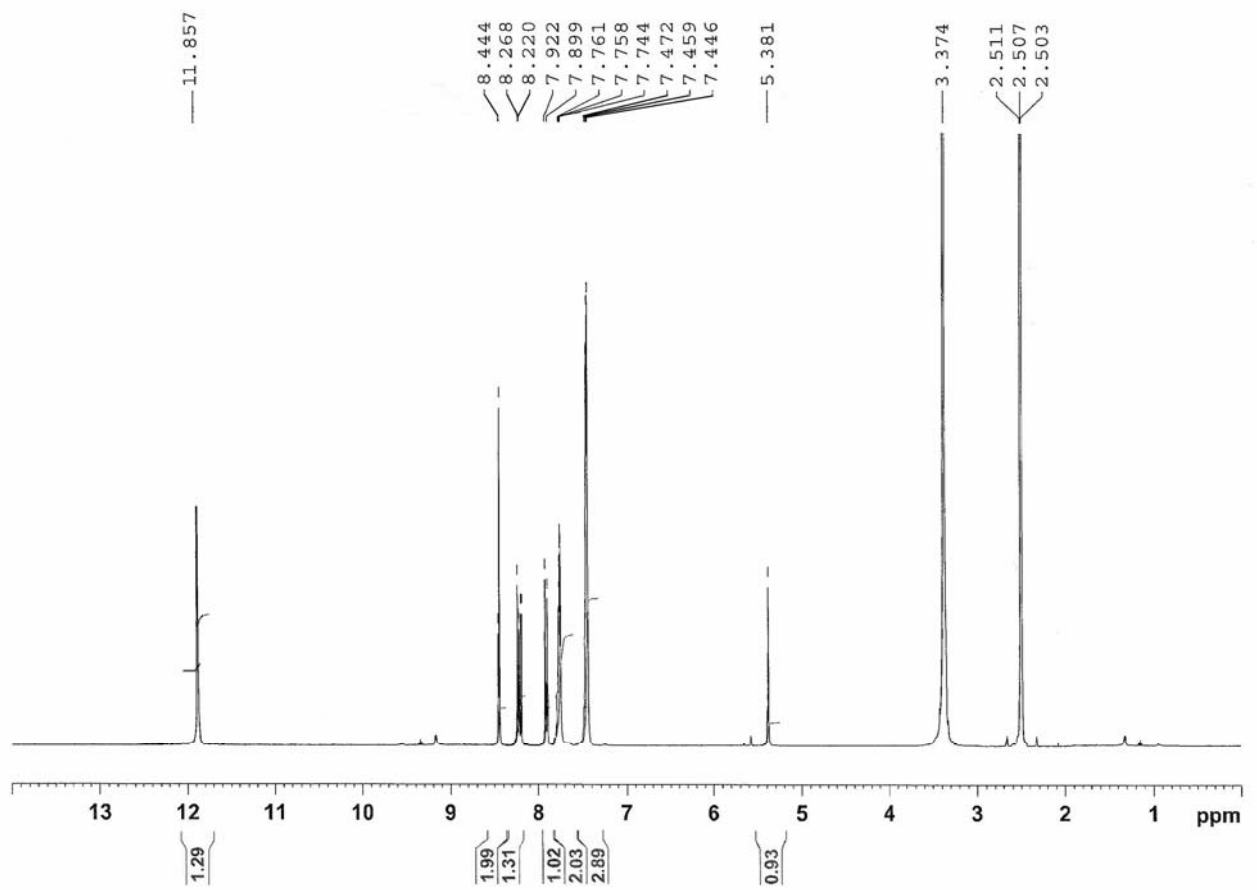


Fig. 1 – The ^{1}H-NMR spectrum of N^{1}-(5-nitroindazole-1-yl-formyl)-N^{2}-benzaldehyde-hydrazidone (III).

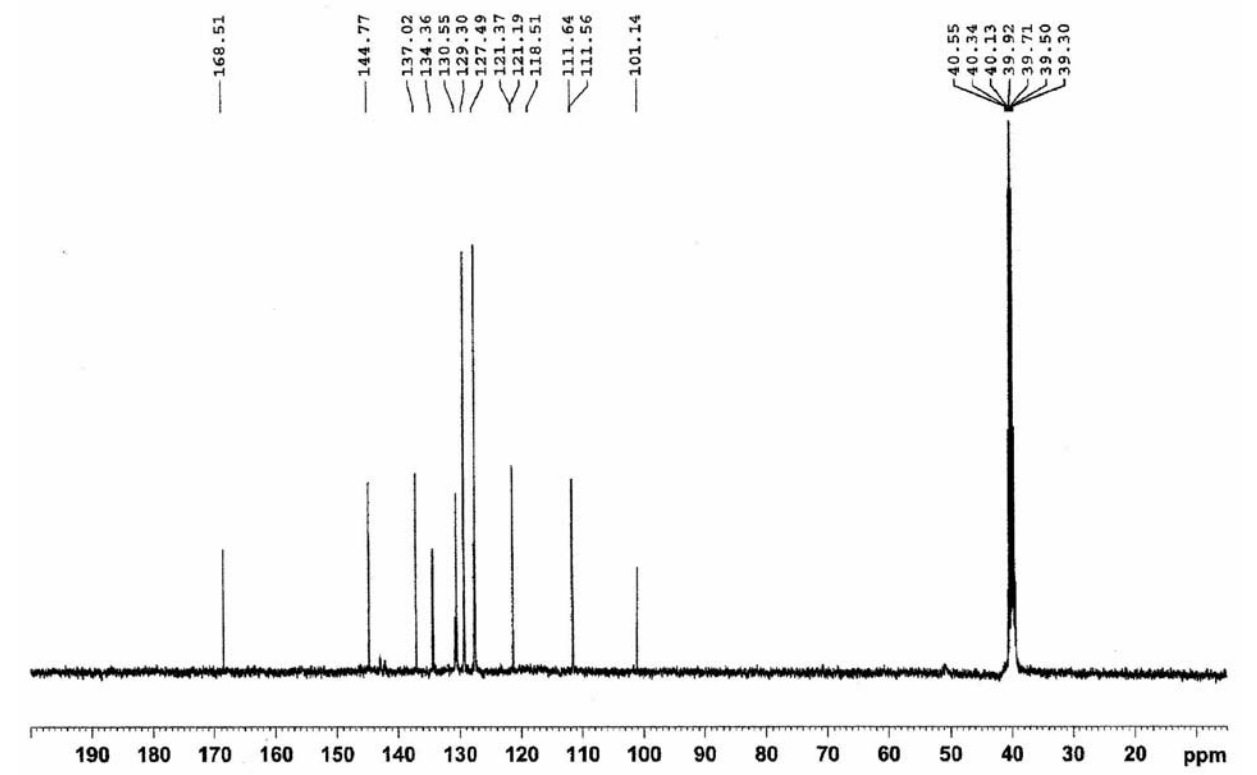


Fig. 2 – The ^{13}C-NMR spectrum of N^1-(5-nitroindazole-1-yl-formyl)-N^2-benzaldehyde-hydrazidone (III).

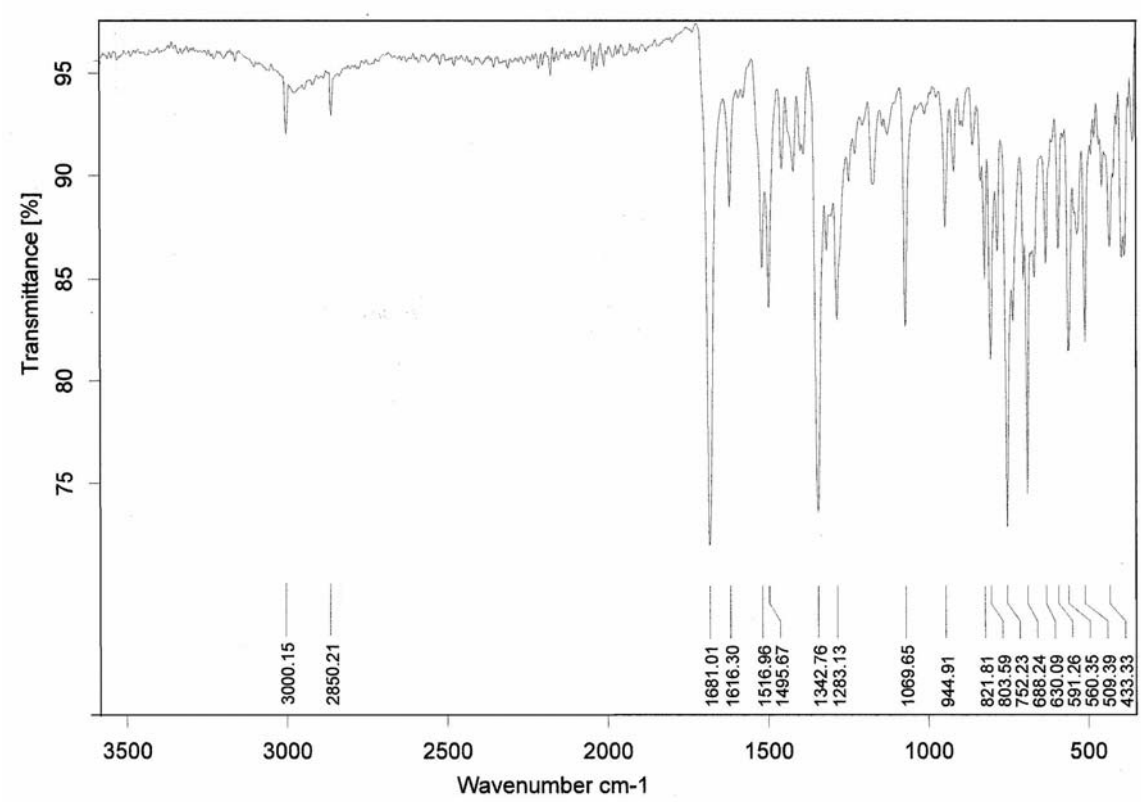


Fig. 3 – The FTIR spectrum of N^1-(5-nitroindazole-1-yl-formyl)-N^2-benzaldehyde-hydrazidone (III).

N1-(5-nitroindazole-1-yl-formyl)-N2-benzaldehyde-hydrazidone (**III**)

Yellow solid, (1.13g 73.3%); m.p.= 169-171°C; Anal. Calcd. for: C15H11N5O3; M = 309; C% 58.25; H% 3.55; N% 22.65. Found: C% 58.45; H % 3.86; N % 22.89.

FT-IR: υmax: 3000 cm^{-1} (NH);1681 cm^{-1} (C=O); 2850 cm^{-1} (CHAr); 1342 cm^{-1} (NO2 sym); 1516 cm^{-1} (NO2 assym); 1495 cm^{-1} (C=N); 803 cm^{-1} (C6H5).

1H-NMR (DMSO-d6, 400MHz), δ(ppm): 5.38 (s, 1H, CH); 7.44-7.47 (m, 3H, CHAr); 7.74-7.76 (m, 2H, CHAr); 7.89-7.92 (d, 1H, CHAr); 8.22-8.26 (d, 1H, CHAr); 8.44 (s, 2H, CHAr); 11.85 (s, 1H, NH).

13C-RMN (DMSO-d6, 400MHz), δ(ppm): 55.03 (CH2); 101.14; 111.64; 118.51; 121.37; 127.49; 129.40 (Ar); 130.55; 134.36; 137.02; 144.77 (C-N); 168.51 (C=O).

N1-(5-nitroindazole-1-yl-formyl)-N2-o-nitro-benzaldehyde-hiydrazidone (**IV**)

White- grey solid (1.11 g, 63%), m. p. = 200-202ºC. Anal. Calcd. for: C15H10N6O5; M = 354. C% 50.84; H% 2.82; N% 23.73. Found: C% 51.44; H% 3.53; N% 24.39.

FT-IR: υmax: 2900 cm^{-1} (NH); 1678 cm^{-1} (C=O); 3000 cm^{-1} (CHAr);1338 cm^{-1} (NO2 sym); 1519 cm^{-1} (NO2 assym); 1496 cm-1 (C=N); 799 cm^{-1} ortho-disubstituted benzene ring.

1H-NMR (DMSO-d6, 400MHz), δ(ppm): 5.44 (s, 1H, CH); 7.73 (s, 1H, CHAr); 7.87-7.91 (d, 1H, CHAr); 7.96-7.99 (d, 1H, CHAr); 8.11-8.14 (m, 2H, CHAr); 8.32 (s, 1H, CHAr); 8.49 (s, 1H, CHAr); 8.91 (s, 1H, CHAr); 12.39 (s, 1H, NH).

13C-NMR (DMSO-d6, 400MHz), δ(ppm): 105.48; 112.13; 119.02; 123.25; 127.65; 129.05; 142.44 (Ar); 137.19; 139.95; 144.16; 148.16; 154.32 (C-N); 167.65 (C=O).

N1-(5-nitroindazole-1-yl-formyl)-N2-p-nitro-benzaldehide-hydrazidone (**V**)

White-yellow solid, (1.28 g, 72%). m. p.= 192-195°C. Anal. Calcd. for: C15H10N6O5; M = 354; C% 50.84; H% 2.82; N% 23.73.. Found: C% 51.18; H% 3.13 ; N% 24.16.

FT-IR: υmax: 3149 cm^{-1}; 3483 cm^{-1} (NH); 1682 cm^{-1} (C=O); 2840 cm^{-1} (CHAr); 1360 cm^{-1} (NO2 sym); 1545 cm^{-1} (NO2 assym);1490 cm^{-1} (C=N); 728 cm^{-1} para-disubstituted benzene ring.

1H-NMR (DMSO-d6, 400MHz), δ(ppm): 5.40 (s, 1H, CH); 7.64 (s, 1H, CHAr); 7.71-7.73 (d, 1H, CHAr); 7.88-7.92 (d, 1H, CHAr); 8.01-8.05 (m, 2H, CHAr); 8.22 (s, 1H, CHAr); 8.43 (s, 1H, CHAr); 8.84 (s, 1H, CHAr); 12.06 (s, 1H, NH).

13C-NMR (DMSO-d6, 400MHz), δ(ppm): 103.27; 112.44; 117.78; 121.99; 126.25; 133.09; 135.35; 138.09; 139.65 (Ar); 138.87; 140.83; 143,23; 147.13; 149.89 (C-N); 168.51 (C=O).

N1-(5-nitroindazole-1-yl-formyl)-N2-o-hydroxi-benzaldehide-hydrazidone (**VI**)
Yellow solid, (1, 14 g, 68%). m. p. = 187-189°C. Anal. Calcd. for: C15H11N5O4; M = 325. C% 56,63; H% 3,83; N% 20,64. Found: C% 56.95; H% 4.12; N% 21.05.
FT-IR: υmax: 3066 cm^{-1} (NH); 1673 cm^{-1} (C=O); 2978 cm^{-1} (CHAr); 1344 cm^{-1} (NO2 sim); 1514 cm^{-1} (NO2 asim); 1493 cm^{-1} (C=N); 1287 cm^{-1} (OH); 811 cm^{-1} ortho-disubstituted benzene ring.
1H-NMR (DMSO-d6, 400MHz), δ(ppm): 5.36 (s, 1H, CH); 6.84-6.89 (d, 1H, CHAr); 7.75 (s, 1H, CHAr); 7.86-7.89 (d, 1H, CHAr); 8.18-8.21 (d, 1H, CHAr); 8.33 (s, 1H, CHAr); 8.40-8.43 (m, 2H, CHAr); 8.80-8.83 (d, 1H, CHAr); 11.67 (s, 1H, OH); 12.06 (s, 1H, NH).
13C-NMR (DMSO-d6, 400MHz), δ(ppm): 101.92; 109.63; 117.81; 118.23; 119.32; 121.26; 122.89; 132.33; 139.24 (Ar); 134.34; 137.20; 143.61; 149.77 (C=N); 164.79 (OH); 169.12 (C=O).

The listed elemental analysis and spectral data fit the chemical structure of the new synthesized compounds III-VI.

2. Optimization data

The significant variables and their limits for which the maximum of the new compounds III-VI reaction yield is obtained were established in some previous experiments. Similar results regarding the optimization of the reaction yield were obtained for a series of amino acids.[17-18] The simplest procedure of optimization is described in some books [19-20] and scientific articles.[21-24]

The extreme values of the significant variables for the obtaining reactions of the studied compounds (III- VI) are listed in Table 1, in which the a-dimensional variables are also listed.

The values of the significant a-dimensional variables and the corresponding reaction yield experimentally determined are given in Table 2.

DISCUSSION

In the field of science, the accuracy of the measurements or of the results from numerical analysis indicates the degree of closeness of the measurement or of the calculi results to that quantity's true value.[25] Experimenters are interested in obtaining the highest yield of their experiments or in establishing the best conditions in which it happens for different economical reasons.

In numerical analysis the accuracy is the nearness of a calculation to be the true value, while the precision is the resolution of the representation, typically defined by the number of decimal or binary digits.

Table 1

Extreme values of the significant variables – temperature and time – for the obtaining reactions of the studied compounds (III-VI)

Compound	Temperature $X_1(^0C)$		Time $X_2(\min)$	
	Minim	Maxim	Minim	Maxim
III	76 $(x_1=-1)$	78 $(x_1=1)$	160 $(x_2=-1)$	200 $(x_2=1)$
IV	76 $(x_1=-1)$	78 $(x_1=1)$	160 $(x_2=-1)$	200 $(x_2=1)$
V	76 $(x_1=-1)$	78 $(x_1=1)$	160 $(x_2=-1)$	200 $(x_2=1)$
VI	76 $(x_1=-1)$	78 $(x_1=1)$	160 $(x_2=-1)$	200 $(x_2=1)$

Table 2

Relevant a-dimensional parameters (for temperature and reaction time) and optimizing indicator (reaction yield) for the compounds (III), (IV), (V) and (VI)

Nr.		1	2	3	4	5	6	7	8	9
x_1		-1	-1	-1	0	0	0	1	1	1
x_2		-1	0	1	-1	0	1	-1	0	1
$\eta(\%)$	III	68	69	70	74	76	75	71	73	72
	IV	70	74	73	75	78	77	71	75	72
	V	61	68	62	67	72	68	63	71	72
	VI	53	59	55	60	66	63	54	60	57

We are interested to establish, by statistical means, in a 3^2 experiment, the significant variables and the optimal conditions for a high value of the reaction yield in order to make economy of precursors.

Let us suppose that the reaction yield (noted by η) and significant a-dimensional variables x_i ($i = 1,2$) are bonded by a relation of the second degree of the type (1):

$$\eta = a_0 + a_1 x_1 + a_2 x_2 + a_{12} x_1 x_2 + a_{11} x_1^2 + a_{22} x_2^2 \quad (1)$$

Relation (1) describes with enough precision the response function near the optimum. The orthogonal variables $x_1^2 - \frac{2}{3}$ and $x_2^2 - \frac{2}{3}$ (defined as normalized variables satisfying the conditions of orthogonallity) were also introduced to express the reaction yield:[18-24]

$$\eta = \overline{\eta} - \frac{2}{3}(a_{11} + a_{22}) + a_1 x_1 + a_2 x_2 + a_{12} x_1 x_2 + a_{11} x_1^2 + a_{22} x_2^2 \quad (2)$$

From (1) and (2), it results that:

$$a_0 = \overline{\eta} - \frac{2}{3}(a_{11} + a_{22}) \quad (3)$$

In relation (3), $\overline{\eta}$ is the average value of the reaction yield for the 3^2 experiments. The coefficients a_{ij} from (1) are calculated by the procedure described in[17-18] and listed in Table 3.

By using the values of reaction yield in the center of the variation domain, the square average deviation S_η and precision P were estimated[19-24] and listed in Table 4.

The significance of coefficients from (Table 3) is tested using expression t-student coefficient.[18] The t-student coefficients are listed in Table 5.

A maximum error in determining the yield was fixed (as being the unity) and the coefficients with lower test value than this one were neglected (for example a_{12} for compound V), because they have not a significant role in the value of the yield. All computed t-student coefficients show significant influence of the a-dimensional variables x_1 and x_2 and also of their conjugate variables on the reaction yield for obtaining compounds III-VI.

Table 3

Regression coefficients in relation (1) for compounds III-VI

Compound	a_0	a_1	a_2	a_{12}	a_{11}	a_{22}
III	75.67	1.50	0.67	-0.17	-4.50	-1.00
IV	73.89	0.17	1.00	1.00	-4.17	-2.67
V	73.11	0.83	0.50	0.00	-4.17	-6.17
VI	66.11	0.67	0.33	0.25	-6.67	-4.67

Table 4

Reaction yield in the center of the variation domain, average of the yield in the center, square average deviation S_η and precision P

Compound	η_1	η_2	η_3	$\overline{\eta}_c$	S_η	P
III	76.0	75	75	75.33	0.33	0.14
IV	78.0	78.0	79.0	78.33	0.33	0.14
V	73.0	73.0	74.0	73.33	0.22	0.16
VI	66	67	66	66.33	0.33	0.19

Table 5

t - Student tests for obtaining reactions of compounds I-IV

Compound	t_0	t_1	t_2	t_{12}	t_{11}	t_{22}
III	556.38	4.90	11.03	1.23	33.09	7.35
IV	576.80	1.23	7.35	3.68	30.64	19.61
V	465.67	5.31	3.18	0.00	26.54	39.28
VI	344.33	3.47	6.94	1.30	34.74	24.31

Final formulae for reaction yields in obtaining the compounds III-VI in optimal conditions are given in (4)-(7):

$$\eta_{III} = 75.67 + 1.50x_1 + 0{,}67x_1x_2 - 4.50x_1^2 - 1.00x_2^2 \quad (4)$$

$$\eta_{IV} = 73.89 + 0.17x_1 + 1.00x_2 + 1.00x_1x_2 - 4.17x_1^2 - 2.67x_2^2 \quad (5)$$

$$\eta_{V} = 73.11 + 0.83x_1 + 0.50x_2 - 4.17x_1^2 - 6.17x_2^2 \quad (6)$$

$$\eta_{VI} = 66.11 + 0.67x_1 + 0.33x_2 + 0.25x_1x_2 - 6.67x_1^2 - 4.67x_2^2 \quad (7)$$

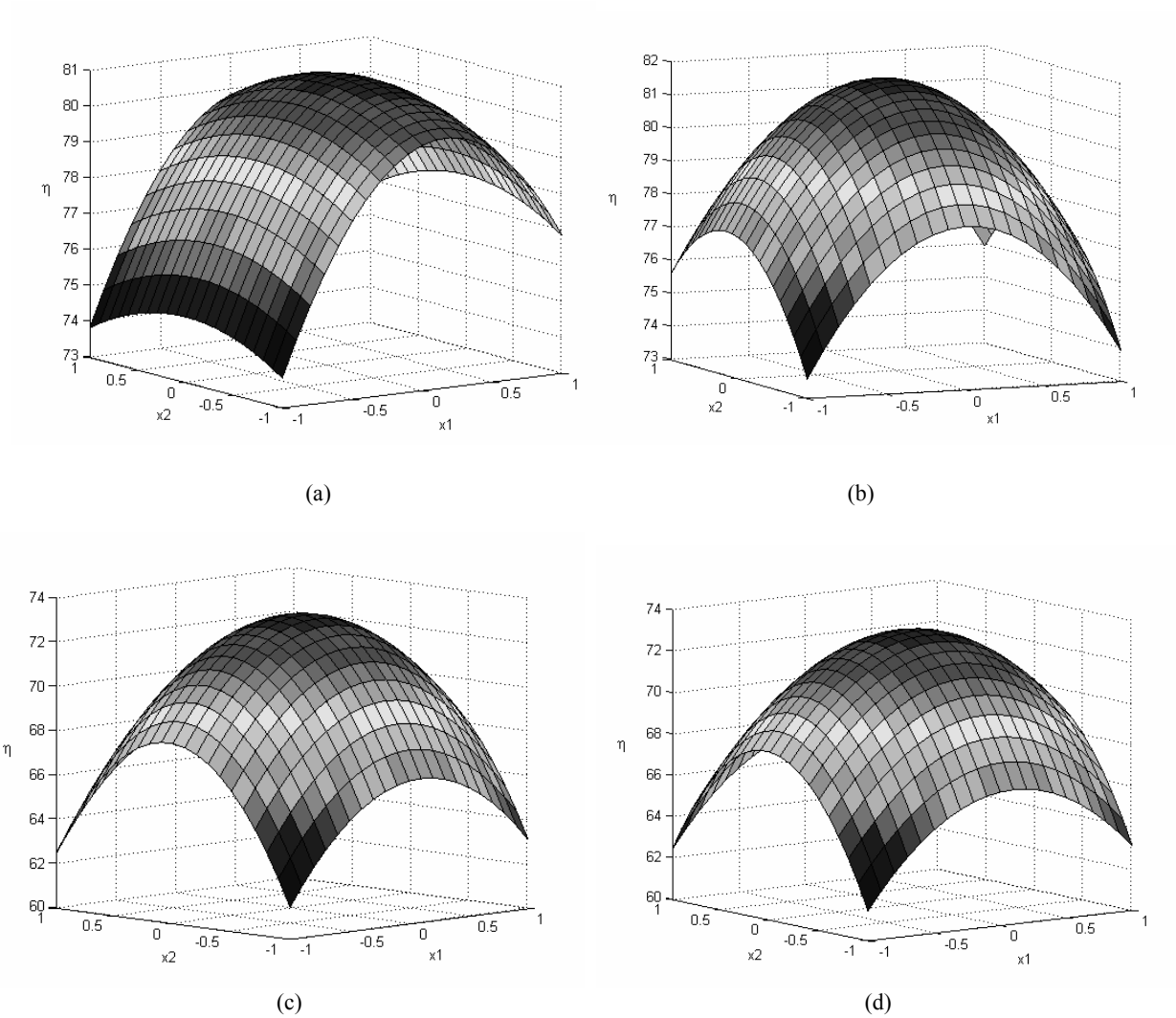


Fig. 4 – Reaction yield vs. a-dimensional coordinates for compounds: (a) – III; (b) – IV; (c) – V; (d) – VI.

Table 6

Extreme values of the reaction yield, $\eta(x_{1,e}, x_{2,e})$ and the corresponding coordinates $x_{1,e}, x_{2,e}$

Compound	$x_{1,e}(X_{ie})$	$x_{2,e}(X_i)$	$\eta(x_{1,e}; x_{2,e})$
III	0.17 (77.17 0C)	-0.16 (176 min 48sec)	75.75
IV	0.02 (77.02 0C)	0.06 (181 min 12sec)	73.94
V	0.01 (77.1 0C)	0.04 (180 min 48sec)	71.18
VI	0.05 (77.05 0C)	0.14 (182 min 48sec)	61.08

Table 7

The LD50 of the synthesized compounds

Compound	24 hours	48 hours	7 days	The average	Administration route	Test animals
III	1384	1398	1368	1383	i.p.	mice
IV	1060	1070	1050	1060	i.p.	mice
V	1125	1135	1115	1125	i.p.	mice
VI	1590	1620	1560	1590	i.p.	mice

The graphs of the equations (4)-(7) are shown in Fig. 4.

The reaction yield dependence on the a-dimensional variables x_1 and x_2 is plotted in 3D representation in Fig. 4. The zones from Fig.4a-4d characterized by appropiate values of the reaction yield have the same color.

The models with quadratic terms describe with sufficient accuracy the region where it is supposed (and experimentally proved) that the reaction yield is maxim. The disadvantage of the method is that in order to determine the t-student coefficients additional experiments should be organized in the center of the variability interval.

3. Toxicity studies

The obtained compound were tested for their toxicity degree and the lethal dose LD50 was also established. The toxicological data obtained (Table 7) confirm that N-formyl-hydrazidones synthesized have low toxicity, which recommends them for biological tests.

The toxicity was estimated through intra-peritoneal (i.p.) administration of the substances under analysis, as suspensions, in Tween 80, n groups formed of 14 mice each (20±5g), according to the Kärber method.[26] The tested animals were followed, their mortality being recorded of 7 days intervals.

CONCLUSIONS

The new five hydrazidones derived from 5-nitroindazole were synthesized by reaction of corresponding hydrazides condensation with different aromatic aldehydes. The structure of prepared compounds was confirmed by elemental and spectral (FT-IR, 1H-NMR, 13C-NMR and MS) analysis.

The synthesis reactions were optimized for establishing the conditions to obtain the highest yield of the synthesized compounds. By using a mathematic 3^2 factorial model, the conditions for the most efficient synthesis of compounds III-VI were obtained in this paper. Optimization is necessary to avoid unwanted consumption of starting compound. The toxicity of hydrazidones was established.

Acknowledgements: This work was supported by the Grant ERA NET – ERA IB 6-003 authorized by The National Council for Scientific Research – Executive Unit for Financing Higher Education, Research, Development and Innovation (CNCS-UEFISCDI).

REFERENCES

1. C. Lonelle, J. M. Brunel, N. Vidal, M. Dherbomez and Y. Letourneux, *Eur. J. Med. Chem*., **2004**, *39*, 1067-1071.
2. A. M. Pieczonka, A. Strzelczyk, B. Sadowska, G. Mlosto and P. Staczek, *Eur. J. Med. Chem.*, **2013**, *64*, 389-395.
3. A. Upadhyay, S. K. Srivastava and S. D. Srivastava, *Eur. J. Med. Chem.*, **2010**, *45*, 3541-3547.
4. D. Sriram, P. Yogeeswari and R. V. Devakaram, *Bioorg. Med. Chem.*, **2006**, *14*, 3113-3118.
5. S. G. Kucukguzel, A. Mazi, F. Sahin, S. Ozturk and J. P. Stables, *Eur. J. Med. Chem*., **2013**, *38*, 1005-1013.
6. S. Papakonstantinou, N. Pouli, P. Marakos and A. Chytyroglou, *Farmaco*., **2002**, *57*, 973-977.
7. P. Vicini, F. Zani, P. Cozzini and I. Doytchinova, *Eur. J. Med. Chem.*, **2002**, *37*, 553-564.
8. A. R. Todeschini, A. L. Miranda, C. M. Silva, S. C. Parrini and E. J. Barreiro, *Eur. J. Med. Chem.*, **1998**, *33*, 189-199.
9. M. A. Gaston, L. R. Dias, A. C. Freitas, A. L. Miranda and E. J. Barreiro, *Acta Helvet*. **1996**, *213*, 71-90.
10. P. Melnyk, V. Leroux, C. Sergheraert and P. Grellier, *Bioorg. Med. Chem. Lett*., **2006**, *16*, 31-35.
11. Y. Xia, C. D. Fan, B. X. Zhao, J. Zhao, D. S. Shin and J. Y. Miao, *Eur. J. Med. Chem.*, **2008**, *43*, 2347-2353.
12. O. I. Sabbagh, and H. M. Rady, *Eur. J. Med. Chem*., **2009**, *44*, 3680-3686.
13. K. Effenberger, S. Breyer and R. Shobert, *Eur. J. Med. Chem*., **2010**, *45*, 1947-1954.
14. A. Kamal, N. Shankaraiah, V. Devaiah, K. L. Reddy, A. Juvekar, S. Sen, N. Kurian and S. Zingde, *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 1468-1473.
15. P. Vicini, M. Incerti, P. L. Colla and R. Loddo, *Eur. J. Med. Chem*., **2009**, *44*, 1801-1807.

16. K. Bedia, O. Elcin, U. Seda, K. Fatma, S. Nathaly, R. Sevim and A. Dimoglo, *Eur. J. Med. Chem.*, **2006**, *41*, 1253-1261.
17. C. Cheptea, L. M. Ivan, D. O. Dorohoi, V. Sunel, Ion Hurjui, C. I. Saveanu and A. I. Galaction, *Ukr. Journal of Phys.*, **2014**, *59*, 313-318.
18. M. Moise, V. Sunel, M. M. Dulcescu and D.O. Dorohoi, *Rev. Chim. (Bucharest)*, **2010**, *61*, 799-804.
19. L. Fisher, "Design for Environment, Creating Eco-Efficient Products and Processes", Ed. McGraw Hill, New York, 1996, 123-157.
20. A. Azzouz, M. Leontie and D.O. Dorohoi, C. Gheorghies, "Elemente de strategie in design industrial", Ed. Plumb, Bacau, 1998, 43-87.
21. C. Cheptea, M. M. Dulcescu, D. O. Dorohoi, V. Sunel and L. Profire, *D.J.N.B.*, **2012**, *7*, 287-297.
22. C. Cheptea, V. Sunel, C. Stan and D. O. Dorohoi, *Rev. Roum. Chim.*, **2012**, *57*, 229-234.
23. I. Hurjui, C. Cheptea, C. F. Dascalu, L. Hurjui, C. Peptu, V. Sunel, D.O. Dorohoi, *D. J. N. B.*, **2012**, 7, 1747-1756.
24. D. Grebinisan, M. Burlea, C, Cheptea, C. Lionte, D. O. Dorohoi, V. Sunel, M. Popa and I. Hurjui, *D.J.N.B.*, **2013**, *8*, 777-785.
25. J.R. Taylor, "An Introduction to error analysis; The study of Uncertainties in Physical measurements", University Science Books, **1999**, 128-129.
26. S. Kärber, *Sci. Technol.*, **1978**, *12*, 417-419.