Structure and Anticancer Activity of Some *bis*-pyridazine Derivatives

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The synthesis, anticancer activity and X-ray crystal structure of diethyl 2,2'-(3,3'-oxybis(4,1-phenylene))bis(6-oxopyridazine-3,1(6H)-diyl)) diacetate are reported. The in vitro anticancer activity against HeLa cell line was tested, compound showing a moderate cytotoxicity. The structure of compound was assigned by elemental and spectral analysis, the X-ray data proving unambiguously the structure. The compound crystallizes in the space group P1 with a = 16.753(1) Aº, b = 12.6931(7) Aº, c = 12.8775(8) Aº, α =90°, β= 107.328(6)°, γ = 90°, V= 2614.1(3) and Z = 4. Accurate molecular parameters for the heterocyclic system were obtained from intensity data collected at 295 K. The molecule assumes a bowl stereochemistry.

Keywords: bis-pyridazine, anticancer activity, X-ray

Nitrogen heterocycles are currently experiencing a renewed interest as highly valuable materials with a large variety of applications: agriculture (herbicidal activity and grow up factor for plants) [1-7], opto-electronics (fluorescent derivatives, sensors and biosensors, lasers, semiconductors and liquid crystal properties) [8-21], and medicinal chemistry (anticancer, antimicrobials, antihypertensive, diuretics, anti-thrombics, anticoagulants, antidepressants, anxiolytics, anticonvulsants, analgesic) [22-35].

Despite the progress achieved by modern medicinal science in cancer therapy, neoplasm is presently responsible for the deaths on about seven million people every year. Neoplasm chemotherapy is complex and complicated (because of toxicity, drug resistance and multidrug resistance mostly), developing of new chemotherapeutics being of major interest in medicine [36].

As a continuation of our work in the field of nitrogen heterocycles with anticancer activity [1, 24, 26, 29] and their structure elucidation using X-ray measurements [37-39] and, taking into consideration that diethyl 2,2'-(3,3'-oxy*bis*(4,1-phenylene))*bis*(6-oxopyridazine-3,1(6*H*)-diyl))diacetate have an excellent antituberculosis activity [28]**,** we decided to synthesize, to analyze through X-ray the structure of *bis*-pyridazine diacetate compound and to test its anticancer activity.

Experimental part

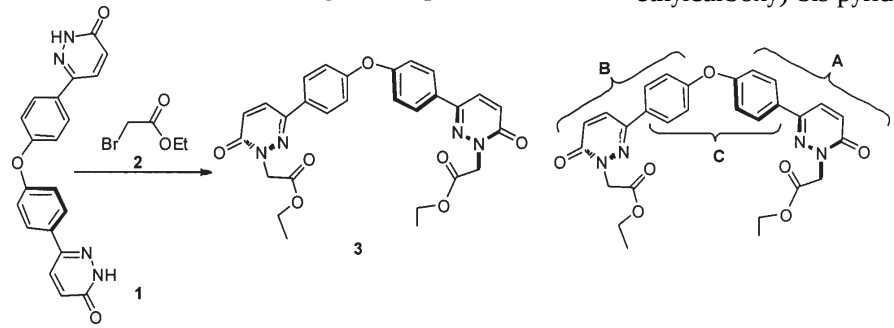
All the reagents and solvents employed were of the best grade available and were used without further purification. Melting points were determined using an electrothermal apparatus (MELTEMP II) and are uncorrected. X-Ray analysis was recorded with an Agilent SuperNova Dual diffractometer equipped with a Cu (Kα radiation) fine-focus sealed X-ray tube and a graphite monochromator. A suitable crystal was selected and mounted on the SuperNova, Eos diffractometer. Intensity data were collected using Cu-Kα radiation (λ= 1.5418 Aº), the crystal was kept at 295.00 K during data collection. All H atoms were located in difference electron density maps and were included in idealized positions in a riding model with isotropic thermal parameters equal to 1.2 times those of their parent atoms. In the final cycles of refinement, least-squares weights of the form $w = 1/[\sigma^2(F_o)^2 + (0.0387P)^2 + 0.0691P]$, $P = (F_o^2 + 2F_c^2)/3$ were employed. Crystallographic data for diethyl 2,2'-(3,3'-(4,4'-oxybis(4,1-phenylene))*bis*(6-oxopyridazine-3,1(6H)-diyl))diacetate 3 are listed in table 1. CCDC 949992, contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html.

Computing details

Data Collection: CrysAlisPro, Agilent Technologies, Version 1.171.36.28 (release 01-02-2013 CrysAlis171 .NET). Using Olex2 [40], the structure was solved with the Superflip [41] structure solution program using Charge Flipping and refined with the ShelXL [42] refinement package using Least Squares minimization.

Results and discussions

The title compound was synthesized using a straightforward and efficient setup procedure, described elsewhere [28]. Practically, the *bis*-pyridazine compound **1** was treated with ethyl 2-bromoacetate, *via* an *N*-alkylation reaction, leading to the corresponding *N*-(1-ethylcarboxy)-*bis*-pyridazinone **3**, (scheme 1).



Scheme 1. Reaction pathway to obtain *bis*-pyridazine derivatives

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Table 1

CRYSTAL AND EXPERIMENTAL DATA

Chemical formula	$C_{28}H_{26}N_4O_7$
Formula weight	530.53
Crystal Color	Yellow
Crystal size/mm^3	0.4 × 0.12 × 0.1
Crystal System	Monoclinic
Lattice Type	primitive
Space Group	C1c1
Lattice Parameters:	
Cell Lengts (Å):	
a	16.753(1)
b	12.6931(7)
c	12.8775(8)
Cell angles (°):	
α	90
β	107.328(6)
γ	90
Volume	2614.1(3)
Z value	4
ρ_{calc} (mg/mm^3)	1.348
F(000)	1112.0
μ(Cu Kα)	0.819
Reflections collected	8171
Data/restraints/parameters	3886/2/365
Final R indexes [I > 2σ(I)]	R_1 = 0.0496, wR_2 = 0.1495
Goodness of Fit on F^2	1.060

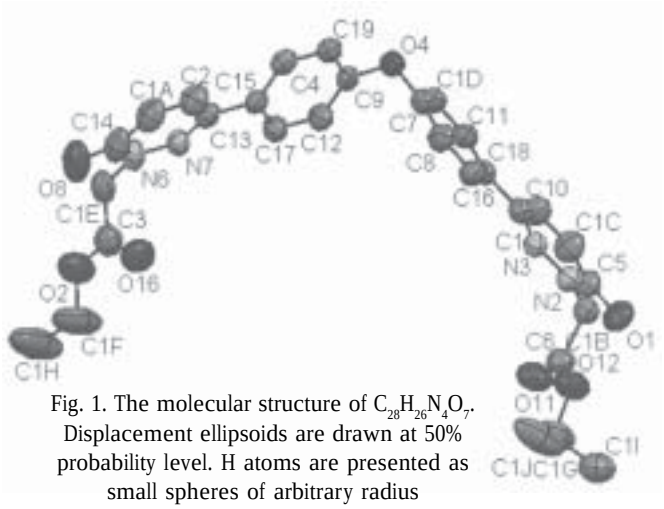

Fig. 1. The molecular structure of $C_{28}H_{26}N_4O_7$. Displacement ellipsoids are drawn at 50% probability level. H atoms are presented as small spheres of arbitrary radius

The structure of the compound was proved by elemental (C, H, N) and spectral analysis (IR, ^{1}H NMR, ^{13}C NMR, 2D-COSY, 2D-HMQC and 2D-HMBC) and are in accordance with the proposed structures. In order to establish unequivocally the structure of compound **3**, the X-ray data analysis was performed. Yellow needles crystals of compound **3** were obtained by crystallization from methanol. The crystal and structure refinement data are summarized in table 1.

The X-ray structure of the title compound with the atom numbering scheme is shown in figure 1.

There are some interesting features that have to be underlined in the X-ray structure from figure 1. The backbone of this molecule could be splitted in three main graphs: two phenyl-pyridazine graphs (**A** and **B**) and a diphenyl ether graph (**C**). In the phenyl-pyridazine graph **A**, the phenyl and pyridazine cycles are roughly coplanar, the torsion angles related to the two cycles are closely to 0° (e.g., N_3-C_{10}-C_{18}-C_{16} is 3.37° and C_1-C_{10}-C_{18}-C_{11} is 0.85°). In the other phenyl-pyridazine graph **B**, the phenyl and pyridazine cycles are under a certain angle, the torsion angles related to the two cycles being around 17° (e.g., C_{17}-C_{15}-C_{13}-N_7 is -17.11° and C_{17}-C_{15}-C_{13}-C_2 is 17.21°). In the diphenyl ether graph (**C**) the two phenyl cycles linked by oxygen are roughly perpendicular, the torsion angles related to the two cycles are: C_{19}-C_9-O_4-C_7 is 149.39°, C_{12}-C_9-O_4-C_7 is -33.09°, C_9-O_4-C_7-C_{1D} is 117.24°, C_9-O_4-C_7-C_8 is -33.63°).

Table 2

FRACTIONAL ATOMIC COORDINATES (×10^4) AND EQUIVALENT ISOTROPIC DISPLACEMENT PARAMETERS (Å^2×10^3) FOR COMPOUND **3**. U_{eq} IS DEFINED AS 1/3 OF THE TRACE OF THE ORTHOGONALISED U_{IJ} TENSOR.

Atom	x	y	z	U(eq)
O1	12348(2)	5111(3)	10249(3)	79.8(11)
N2	11680(2)	4854(3)	8453(3)	54.3(9)
N3	11101(2)	4409(3)	7614(3)	52.5(8)
O4	8405(2)	1742(3)	4106(3)	72.8(11)
C5	11792(3)	4655(4)	9548(4)	62.3(12)
N6	4903(2)	5272(3)	620(3)	60(1)
N7	5486(2)	4701(3)	1361(3)	53.0(8)
O8	4278(3)	5945(4)	-1050(3)	95.1(14)
C9	7820(3)	2348(3)	3373(4)	55.3(11)
C10	10604(3)	3691(3)	7820(3)	50.6(10)
O11	11509(3)	7033(3)	8602(4)	86.9(12)
O12	12441(3)	7282(3)	7688(4)	90.4(13)
C13	5970(3)	4090(4)	1005(3)	51.9(10)
C14	4810(3)	5339(5)	-470(4)	70.9(14)
C15	6612(3)	3473(3)	1817(3)	49.5(9)
O16	5368(3)	7327(4)	1063(4)	94.7(13)
C17	6832(3)	3758(4)	2910(4)	57.1(11)
C18	10001(3)	3195(3)	6846(3)	50.9(10)
C1	10650(3)	3416(4)	8909(4)	65.6(12)
O2	4206(3)	7699(3)	1471(5)	107.9(17)
C3	4720(4)	7058(5)	1196(5)	72.4(14)
C4	6997(3)	2607(4)	1529(4)	54(1)
C2	5914(3)	4052(4)	-121(4)	66.6(13)
C6	12006(4)	6703(4)	8189(4)	65.2(12)
C7	8923(3)	2253(3)	5021(4)	60.5(12)
C8	9490(3)	2997(4)	4898(4)	67.9(13)
C11	9434(3)	2443(3)	6943(4)	59.8(12)
C12	7437(3)	3197(4)	3682(4)	61.4(12)
C16	10022(3)	3463(4)	5804(4)	61.7(12)
C19	7600(3)	2045(4)	2294(4)	56.4(11)
C1A	5354(3)	4666(5)	-823(4)	74.1(15)
C1B	12237(3)	5565(3)	8130(4)	58.9(11)
C1C	11229(4)	3885(5)	9730(4)	72.8(14)
C1D	8891(3)	1970(4)	6032(4)	64.2(13)
C1E	4395(3)	5953(4)	1073(4)	66.5(13)
C1G	12364(6)	8417(5)	7717(8)	112(3)
C1I	13051(9)	8737(10)	8784(13)	120(6)
C1J	12350(18)	8731(15)	6610(20)	144(12)
C1F	4453(6)	8807(6)	1618(10)	147(4)
C1H	3778(7)	9407(7)	1751(12)	199(7)

All together the three graphs in compound **3** have a bowl shape.

Atomic coordinates and equivalent isotropic displacement parameters are given in table 2. The H atoms were positioned geometrically and refined using a riding model with C—H distances of 0.95 – 0.99 A°, and with $U_{iso}(H) = 1.2\ U_{eq}(C)$.

The data from table 3 reveals that the bonds from the phenyl and pyridazine cyclic systems are as length in between the single C–C (C–N respectively) and double C=C (C=N respectively) bonds. As we already show above, the torsion angles prove the relative position of the phenyl and pyridazine cycles, one to each other. It is also interesting

Table 3
SELECTED BOND DISTANCES (Å), ANGLES (°) AND TORSION ANGLES (°)

O1	C5	1.231(6)		C14	C1A	1.418(8)	
N2	N3	1.342(5)		C15	C17	1.392(6)	
N2	C5	1.388(6)		C15	C4	1.380(6)	
N2	C1B	1.446(6)		N3	C10	1.315(6)	
C17	C12	1.387(60)		C8	C16	1.373(7)	
O4	C9	1.376(6)		C18	C11	1.378(7)	
O4	C7	1.397(5)		C18	C16	1.395(7)	
C5	C1C	1.426(8)		C1	C1C	1.342(7)	
N6	N7	1.354(5)		N6	C14	1.369(6)	
N6	C1E	1.451(7)		C3	C1E	1.495(8)	
N7	C13	1.300(6)		C4	C19	1.381(6)	
O8	C14	1.244(7)		C2	C1A	1.342(7)	
C9	C12	1.372(7)		C6	C1B	1.504(7)	
C9	C19	1.382(7)		C7	C8	1.381(7)	
C10	C18	1.495(6)		C7	C1D	1.367(7)	
N3	N2	C5	126.0(4)		C11	C18	C10	121.9(4)
N3	N2	C1B	113.8(4)		C11	C18	C16	118.1(4)
C5	N2	C1B	120.1(4)		C16	C18	C10	120.0(4)
C10	N3	N2	118.6(4)		C1C	C1	C10	118.8(5)
C9	O4	C7	117.0(3)		C3	O2	C1F	116.8(5)
O1	C5	N2	120.3(5)		O16	C3	O2	123.9(6)
O1	C5	C1C	126.4(4)		O16	C3	C1E	124.7(6)
N2	C5	C1C	113.3(4)		O2	C3	C1E	111.3(5)
N7	N6	C14	126.4(4)		C15	C4	C19	121.4(4)
N7	N6	C1E	115.0(4)		C1A	C2	C13	119.2(5)
C14	N6	C1E	118.3(4)		O11	C6	O12	125.5(5)
C13	N7	N6	117.8(4)		O11	C6	C1B	125.9(5)
O4	C9	C19	117.2(4)		O12	C6	C1B	108.6(5)
C12	C9	O4	122.6(4)		C8	C7	O4	119.8(5)
C12	C9	C19	120.2(4)		C1D	C7	O4	119.3(5)
N3	C10	C18	115.7(4)		C1D	C7	C8	120.8(4)
N3	C10	C1	121.1(4)		C16	C8	C7	119.4(5)
C1	C10	C18	123.2(4)		C18	C11	C1D	121.2(5)
C6	O12	C1G	118.1(5)		C9	C12	C17	120.0(4)
N7	C13	C15	117.7(4)		C8	C16	C18	121.1(5)
N7	C13	C2	121.3(4)		C4	C19	C9	119.6(4)
C2	C13	C15	121.0(4)		C2	C1A	C14	121.4(4)
N6	C14	C1A	113.8(4)		N2	C1B	C6	112.7(4)
O8	C14	N6	119.7(6)		C1	C1C	C5	122.2(5)
O8	C14	C1A	126.4(5)		C7	C1D	C11	119.4(5)
C17	C15	C13	119.6(4)		N6	C1E	C3	111.2(4)
C4	C15	C13	122.1(4)		O12	C1G	C1I	103.5(7)
C4	C15	C17	118.3(4)		O12	C1G	C1J	102.7(10)
C12	C17	C15	120.6(4)		C1H	C1F	O2	109.2(7)
N3-C10-C18-C16		3.37			C1-C10-C18-C11		0.85	
C17-C15-C13-N7		-17.11			C17-C15-C13-C2		17.21	
C19-C9-O4-C7		149.39			C12-C9-O4-C7		-33.09	
C9-O4-C7-C1D		117.24			C9-O4-C7-C8		-33.63	

the spatial position of the ethyl carboxylate moiety: in the phenyl-pyridazine graph **A** (where the phenyl and pyridazine cycles are coplanar) the ethyl carboxylate moiety is orientated above the plane of phenyl-pyridazine graph (N_3-N_2-C_{1B}-C_6 is -100.45° and C_5-N_2-C_{1B}-C_6 is -81.92°), the same orientation being valid in the other phenyl-pyridazine graph **B** (N_7-N_6-C_{1E}-C_3 is 97.55° and C_{14}-N_6-C_{1E}-C_3 is 29.58°). This might be related to the crystal packing structure, the most probable in order to favor the packing. Full information concerning X-ray structures could be found in the Cambridge Crystallographic Data Centre, the CCDC 949992.

The *in vitro* cytotoxicity against a human cervical endothelial carcinoma (HeLa) cell line of the synthesized compound has been performed, according to Mosmann's method [43]. Compound **3** was tested at 200 μM concentration, 5-fluoro-uracil being used as standard. Compound **3** exhibited a moderate cytotoxic effect against HeLa cell line (41%), but superior to cytotoxicity of 5-fluoro-uracil (20%) used as standard.

Conclusions

We report herein the synthesis, anticancer activity and X-ray crystal structure of diethyl 2,2'-(3,3'-oxy*bis*(4,1-phenylene))*bis*(6-oxopyridazine-3,1(6*H*)-diyl))diacetate. The *in vitro* anticancer activity against HeLa cell line was tested, compound showing a moderate cytotoxicity. The structure of compound was proved unambiguously by elemental and spectral analysis, including the X-ray structure. The compound crystallizes in the space group P1 with a = 16.753(1) Aº, b = 12.6931(7) Aº, c = 12.8775(8) Aº, α =90°, β = 107.328(6)°, γ = 90°, V= 2614.1(3) and Z = 4. Accurate molecular parameters for the heterocyclic system were obtained from intensity data collected at 295 K. The molecule assumes a bowl stereochemistry.

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References

1.MANGALAGIU, I.I., Curr. Org. Chem., **15**, 2011, p. 730.
2.MOLDOVEANU, C., VASILACHE, V., RISCA, I., Rev. Chim. (Bucharest), **66**, no. 1, 2015, p. 104.
3.VASILACHE, V., MOLDOVEANU, C., FARTAIS, L., RISCA, I., Rev. Chim. (Bucharest), **65**, no. 2, 2014, p. 177.
4.RISCA, I., MOLDOVEANU, C., ASTEFANEI, D., MANGALAGIU, I.I., Rev. Chim. (Bucharest), **61**, no. 3, 2010, p. 303.
5.ZBANCIOC, GHE., BEJAN, V., RISCA, I., MOLDOVEANU, C., MANGALAGIU, I.I., Molecules, **14**, 2009, p. 403.
6.BUTNARIU, R., RISCA, I.M., CAPROSU, M., DROCHIOIU, G., MANGALAGIU, I.I., Rom. Biotech. Lett., **13**, 2008, p. 3837.
7.RISCA, I.M., ZBANCIOC, GHE., MOLDOVEANU, C., DROCHIOIU, G., MANGALAGIU, I.I., Rom. Biotech. Lett., **11**, 2006, p. 2563.
8.MANGALAGIU, I.I., BABAN, C., MARDARE, D., RUSU, G. I., RUSU, M., Appl. Surf. Sci., **108**, 1997, p. 205.
9.ARDELEANU, R., MANGALAGIU, I.I., SACARESCU, G., SIMIONESCU, M., SACARESCU, L., Macromol. Rapid. Comm., **25**, 2004, p. 1231.
10.ROTARU, A., DANAC, R., DRUTA, I., DROCHIOIU, G., CRETESCU, I., Rev. Chim. (Bucharest), **56**, no. 2, 2005, p. 179.
11.ZBANCIOC, GHE., MANGALAGIU, I.I., Synlett, **5**, 2006, p. 804.
12.SACARESCU, L., ARDELEANU, R.; SACARESCU, G.; SIMIONESCU, M.; MANGALAGIU, I.I, High Perform. Polym., **19**, 2007, p. 501.
13. LEONTIE, L., DANAC, R., DRUTA, I., CARLESCU, A., Synthetic Met., **160**, 2010, p. 2526.
14.ZBANCIOC, GHE., MANGALAGIU, I.I., Tetrahedron, **66**, 2010, p. 278.
15. ZBANCIOC, GHE., HUHN, T., GROTH, U., DELEANU, C., MANGALAGIU, I.I., Tetrahedron, **66**, 2010, p. 4298.
16. LEONTIE, L., DANAC, R., APETROAIEI, N., RUSU, G.I., Mater. Chem. Phys., **127**, 2011, p. 1471.
17. DANAC, R., LEONTIE, L., CARLESCU, A., RUSU, G.I., Mater. Chem. Phys., **134**, 2012, p. 1042.
18. MAFTEI, D., ZBANCIOC, GHE., HUMELNICU, I., MANGALAGIU, I.I., J. Phys. Chem. A, **117**, 2013, p. 3165.
19. PÂNZARIU, A., MĂLUÞAN, T., MANGALAGIU, I.I., BioResources, **9**, 2014, p. 282.
20. AL MATARNEH, C.; DANAC, R., LEONTIE, L., TUDORACHE, F.; PETRILA, I.; IACOMI, F.; CARLESCU, A., NEDELCU, G.; MANGALAGIU, I.I., Environ. Eng. Manag. J., **14**, 2015, p. 415.
21. ZBANCIOC, GHE., MANGALAGIU, I.I., MOLDOVEANU, C., Ultrason. Sonochem., **23**, 2015, p. 376.
22. DANAC, R., DANILOAIA, T., ANTOCI, V., VASILACHE, V., MANGALAGIU I. I., Lett. Drug Des. Discovery, **12**, 2015, p. 14.
23. DANAC, R., MANGALAGIU I.I., Eur. J. Med. Chem, **74**, 2014, p. 664.
24. BEJAN, V.; MANTU, D., COZNA, D.G., URSU, C., MANGALAGIU, I.I., Med. Hypotheses, **82**, 2014, p. 11.
25. KUCHKOVA, K., ARICU, A., BARBA, A., VLAD, P., SHOVA, S., SECARA, E.,UNGUR, N., TUCHILUS, C., ZBANCIOC, GHE., MANGALAGIU, I.I., Med. Chem. Res., **23**, 2014, p.1559.
26. BALAN, A.M., MIRON, A., TUCHILUS, C., ROTINBERG, P. MIHAI, C.T., MANGALAGIU, I.I., ZBANCIOC, GHE., Med. Chem, **10**, 2014, p. 476.
27. TUCALIUC, R.A., COTEA, V.V, NICULAUA, M., TUCHILUS, C., MANTU, D., MANGALAGIU, I.I., Eur. J. Med. Chem., **67**, 2013, p. 367.

28. MANTU, D., ANTOCI, V., MANGALAGIU, I.I., Infect. Dis. Drug Target, **13**, 2013, p. 344.
29. LUCA, M.C., TURA, V., MANGALAGIU, I.I., Med. Hypotheses, **75**, 2010, p. 627.
30. BUTNARIU, R., MANGALAGIU, I.I., Bioorg. Med. Chem., **17**, 2009, p. 2823.
31. BALAN, A.M., FLOREA, O., MOLDOVEANU, C., ZBANCIOC, GHE., IUREA, D., MANGALAGIU, I.I., Eur. J. Med. Chem., **44**, 2009, p. 2275.
32. BUTNARIU, R., CAPROSU, M., BEJAN, V., UNGUREANU, M., POIATA, A., TUCHILUS, C., MANGALAGIU, I.I., J. Heterocyclic Chem., **44**, 2007, p. 1149.
33. UNGUREANU, M., MOLDOVEANU, C., POEATA, A., DROCHIOIU, G., PETROVANU, M., MANGALAGIU, I.I., Ann. Pharm. Fr., **64**, 2006, p. 287.
34. CAPROSU, M., BUTNARIU, R., MANGALAGIU, I.I., Heterocycles, **65**, 2005, p. 1871.
35. MANGALAGIU, I.I., UNGUREANU, M., MANGALAGIU, G., GROSU, G., PETROVANU, M., Ann. Pharm. Fr., **56**, 1998, p. 181.
36. AVENDAO, C., MENENDEZ, J.C. Medicinal chemistry of anticancer drugs, Elsevier: Amsterdam, 2008.
37. ANTOCI, V., APOSTU, M., CIOBANU, C., MANTU, D., Acta Chemica Iasi, **22**, 2014, p. 25.
38. MAFTEI, D., MANTU, D., BEJAN (ANTOCI), V., Rev. Chim. (Bucharest), **64**, no. 3, 2013, p. 301.
39. MANTU, D., MAFTEI, D., IUREA, D., BEJAN (ANTOCI), V., Rev. Chim. (Bucharest), **63**, no. 12, 2012, p. 1239.
40. DOLOMANOV, O.V., no. 12, , L.J., GILDEA, R.J., HOWARD, J.A.K., PUSCHMANN, H., J. Appl. Cryst., **42**, 2009, p. 339.
41. DOLOMANOV, O.V., BOURHIS, L.J., GILDEA, R.J., HOWARD, J.A.K., PUSCHMANN, H., J. Appl. Cryst., **40**, 2007, p.786.
42. SHELXL, SHELDRICH, G.M., Acta Cryst., **A64**, 2008, p. 112.
43. MOSMANN, T., J. Immunol. Methods., **65**, 1983, p. 55.

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