Utilization of Cleavage of the [1]Benzofuro[2,3-*e*][1,2,4]triazine Ring for the Synthesis of Oxygen, Nitrogen and Sulfur Derivatives of [1,2,4]Triazine [1]

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A series of 6-azacytosines **4a-4k** and **5a-5c** were prepared by nucleophilic cleavage of furan ring of [1]benzofuro[2,3-*e*][1,2,4]triazine derivative **1**. Some of them were used for the preparation of derivatives of [1,2,4]triazolo[4,3-*d*][1,2,4]triazine (**6a-6d**) and tetrazolo[1,5-*d*][1,2,4]triazine (**7**). The reaction of **1** with hydrogen sulfide afforded the corresponding 6-(2-hydroxyphenyl)-2-phenyl-5-thioxo-4,5-dihydro-1,2,4-triazin-3(2*H*)-one (**8**), while with hydrogen selenide 6-(2-hydroxyphenyl)-2-phenyl-4,5-dihydro-1,2,4-triazin-3(2*H*)-one (**9**) was formed. The prepared compounds were tested for biological activity.

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A number of cleavage reactions of heterocyclic compounds are well-known and advantageous methods for the preparation of compounds, which are otherwise available with difficulties. For example, the synthesis of 1,4-disubstituted butanes based on the opening of furan [2] or tetrahydrofuran [3,4] ring, or the synthesis of glutaconaldehyde and related compounds realized by ring cleavage of pyridinium salts [5-8].

Previously, we found that the [1]benzofuro[2,3-*e*]-[1,2,4]triazine system was very susceptible to the hydrolytic cleavage of the furan ring [9-11]. This reaction starts by nucleophilic attack of water to position 4a, thus showing a high ability of this position to react with nucleophilic reagents. It was interesting for us to know how easily these cleavage reactions could proceed with other nucleophilic agents and whether it would be possible to use this reaction for the introduction of required substituents at position 5 of the 1,2,4-triazine ring. The results of this research, using some oxygen, nitrogen and sulfur nucleophilic agents, are reported in this paper.

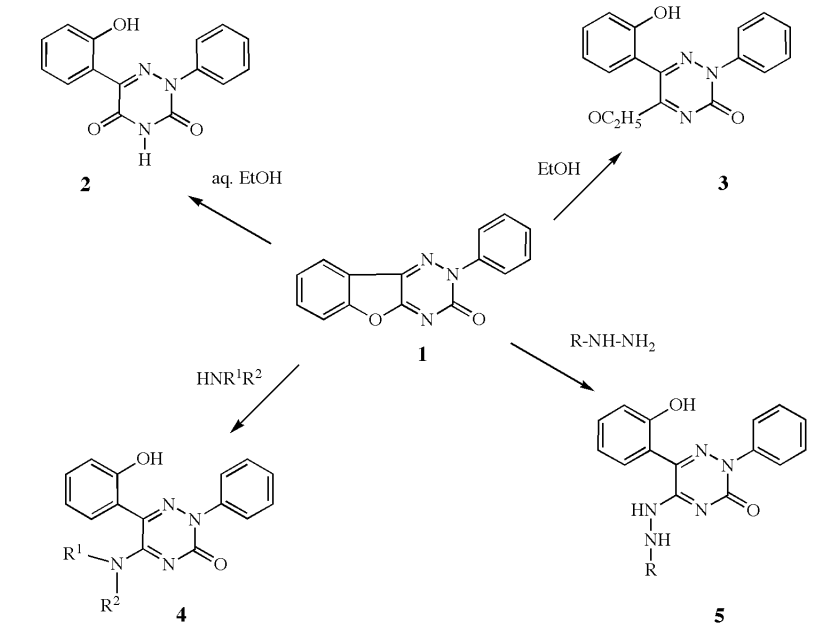
In all these cases, we found that opening of the furan ring took place and the corresponding 5-substituted 1,2,4-triazines were formed in good yields. So the reaction of 2-phenyl-2,3-dihydro[1]benzofuro[2,3-*e*][1,2,4]triazin-3-one (**1**) with anhydrous ethanol led to 5-ethoxy-6-(2-hydroxyphenyl)-2-phenyl-1,2,4-triazin-3(2*H*)-one (**3**). If the ethanol was not strictly anhydrous or if aqueous ethanol was used, the hydrolytic splitting of the furan ring prevailed thus affording 6-(2-hydroxyphenyl)-2-phenyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione (**2**) which has been prepared earlier [9,10] (Scheme 1).

As expected, the cleavage of the furan ring of compound **1** was easier when stronger nucleophilic agents, such as primary and secondary amines or hydrazine and its derivatives were used. We found that reaction with amines and hydrazines took place even in aqueous solutions. This means, that the aminolysis is preferred to hydrolysis. For preparative reasons, we performed most experiments without solvent or in a chloroform solution. The reaction of compound **1** with aqueous ammonia afforded smoothly 5-amino-6-(2-hydroxyphenyl)-2-phenyl-1,2,4-triazin-3(2*H*)-one (**4a**). The same reaction was carried out using primary and secondary amines, affording corresponding *N*-substituted 6-azacytosines **4b-4k**. To verify the use of compound **1** as a reagent capable of binding to the free amino groups of amino acids in proteins we also used lysine in the above mentioned reactions to obtain the corresponding N^6-[6-(2-hydroxyphenyl)-3-oxo-2-phenyl-2,3-dihydro-1,2,4-triazin-5-yl]lysine (**4d**). Hydrazinolysis of compound **1** with hydrazine, phenylhydrazine or semicarbazide proceeded smoothly and afforded the corresponding 5-hydrazino-6-(2-hydroxyphenyl)-2-phenyl-1,2,4-triazin-3(2*H*)-one (**5a**) respectively their derivatives **5b** and **5c** (Scheme 1).

To demonstrate that the reaction can also proceed with compounds containing more amino groups, we used putrescine and spermidine as reactants to get more complex derivatives **4j** and **4k**. These prepared substances are interesting for their potential biological activity (Scheme 2).

Hydrazino derivatives **5a** and **5c** served as starting compounds for the preparation of derivatives of [1,2,4]triazolo-[4,3-*d*][1,2,4]triazine **6a-6d** and tetrazolo[1,5-*d*]-[1,2,4]triazine **7**. Heating of compound **5a** in formic acid gave compound **6a** without formylation of phenolic OH group. In contrast, acetic anhydride acetylated the hydroxy group to the derivative **6b** as it was expected. The closure of the fused triazole ring was also successful using carbon disulfide affording compound **6c**. An analogous oxo derivative **6d** was obtained by thermal cyclization of semicarbazide derivative **5c**. 8-(2-Hydroxyphenyl)-6-phenyltetrazolo[1,5-*d*][1,2,4]triazin-5(6*H*)-one (**7**) was obtained smoothly by the nitrosation of the hydrazino derivative **5a** (Scheme 3).

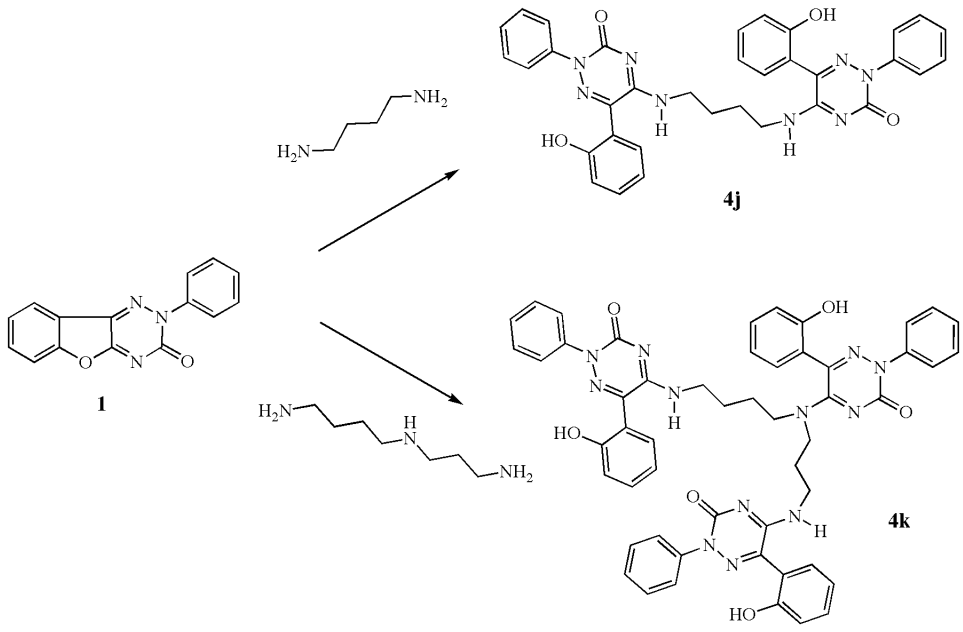
Scheme 1



4a $R^1 = R^2 = H$
4b $R^1 = H$; $R^2 = CH_3$
4c $R^1 = H$; R^2 = cyclohexyl
4d $R^1 = H$; $R^2 = (CH_2)_4CH(NH_2)COOH$
4e $R^1 = H$; R^2 = phenyl
4f $R^1 = R^2 = CH_2CH_3$
4g $R^{1+} R^2 = CH_2CH_2CH_2CH_2CH_2$
4h $R^1 = H$; $R^2 = CH_2CH_2NH_2$
4i $R^1 = H$; $R^2 = CH_2CH_2CH_2CH_2NH_2$

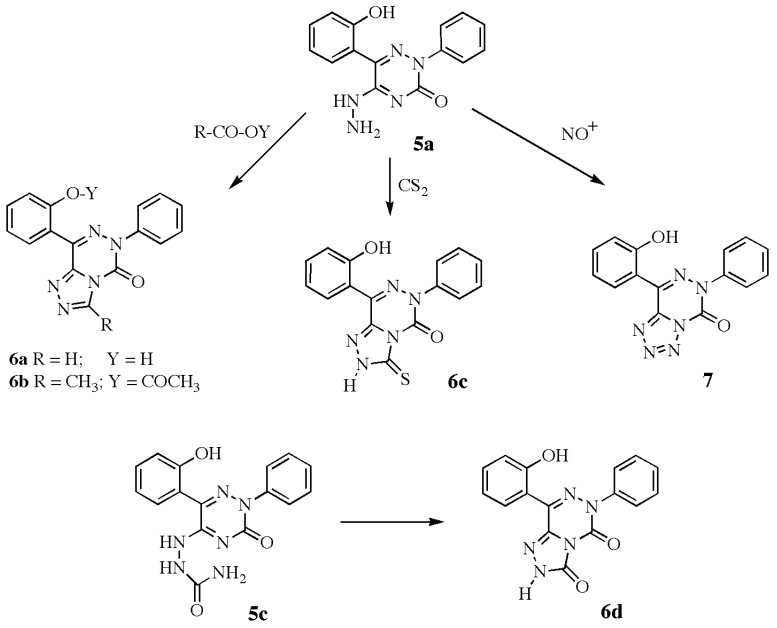
5a R = H
5b R = phenyl
5c R = $CONH_2$

Scheme 2



Our further interest was to find out whether the nucleophilic opening of the furan ring could be achieved using hydrogen sulfide. We found that the reaction took place smoothly under base catalysis and afforded 6-(2-hydroxy-

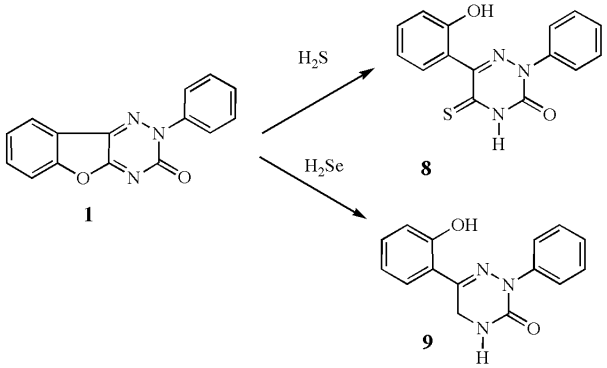
Scheme 3



phenyl)-2-phenyl-5-thioxo-4,5-dihydro-1,2,4-triazin-3(2*H*)-one (**8**). In contrast, the reaction of **1** with H_2Se proceeded with the reduction of the triazine ring affording 6-(2-hydroxyphenyl)-2-phenyl-4,5-dihydro-1,2,4-triazin-3(2*H*)-one (**9**). The cleavage of the furan ring in compound **1** with H_2F_2 was unsuccessful (Scheme 4).

Most of the prepared compounds have a similar structure with that of pyrimidine nucleobases. The prepared compounds were tested for biological activity. Human breast adenocarcinoma cell line *MCF7* was used for cytotoxicity determination of prepared compounds by calcein AM assay [12]. The tested azacytosines **4a-4k** and **5a-5c** showed poor cytostatic activity (IC_{50} = 60-80 μmol/L) with the exception of moderately active compounds **5c** (IC_{50} = 3.5 μmol/L) and **4a** (IC50 = 23 μmol/L).

Scheme 4



EXPERIMENTAL

The melting points were determined on a Boetius stage and are uncorrected. The IR spectra were recorded in KBr wafers on an ATI Unicam Genesis FTIR instrument. The NMR spectra were registered on a Bruker AMX-360 spectrometer (360 MHz) and on a Bruker Avance 300 MHz DRX spectrometer; chemical shifts are reported in ppm, coupling constants *J* in Hz. Elemental analyses were performed with an EA 1108 Elemental Analyser (Fison Instrument). Mass spectra were recorded on an ion trap mass spectrometer LCQ Finnigan Mat.

Cytotoxicity Determination of Prepared Compounds.

The tumour cells were maintained in plastic tissue culture flasks and grown on Dulbecco's modified Eagle's cell culture medium (DMEM) at 37 °C in 5% CO_2 atmosphere and 100% humidity. The cell suspension of approximate density 1.25x105 cells ml^{-1} was redistributed into 96-well microtitre plates (Nunc, Denmark). After 12 h of preincubation, the tested compounds (in the 0.5–200 μmol/l range) were added. Incubation lasted for 72 h. At the end of this period, the cells were incubated for 1 h with calcein AM and fluorescence of the live cells was measured at 485/538 nm (ex/em) with a Fluoroskan Ascent (Labsystems, Finland). IC_{50} values, the drug concentrations lethal to 50% of the tumour cells, were estimated.

6-(2-Hydroxyphenyl)-2-phenyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione (**2**).

A solution of compound **1** (20.2 mg, 0.077 mmol) in 50% aqueous ethanol (5 ml) was refluxed for 1 h and then evaporated to dryness. The residue was mixed with water (3 ml), collected by filtration and dried at 120 °C for 1 h. Sample for analysis was prepared by crystallization from ethanol. Yield 19.5 mg (90.3 %), mp 261-263 °C. Spectral data were identical to the data of the compound prepared earlier [9].

5-Ethoxy-6-(2-hydroxyphenyl)-2-phenyl-1,2,4-triazin-3(2*H*)-one (**3**).

Compound **1** (49.1 mg, 0.186 mmol) was dissolved in anhydrous ethanol (10 ml) in a sealed vial. After 10 min, quantitative yield of the product was found (TLC). The solution was evaporated and the resulting oily product mixed with hexane (3 ml). Solids were collected by filtration, washed with hexane and crystallized from a small amount of ethanol by standing at – 20 °C. Yield: 46.7 mg (81.1 %), mp 140-144 °C (dec.). IR: 3440, 3194, 2981, 1656, 1591, 1455, 1427, 1260, 1153, 757. ^{1}H NMR (360 MHz, $CDCl_3$): δ 1.49 (t, 3H, CH_3, *J* = 7.0 Hz); 4.66 (q, 2H, CH_2, *J* = 7.0 Hz); 6.93-7.01 (m, 2H, arom.); 7.33 (t, 1H, arom., *J* = 7.6 Hz); 7.40 (d, 1H, arom., *J* = 7.1 Hz); 7.48 (t, 2H, arom., *J* = 7.6 Hz); 7.61 (d, 2H, arom., *J* = 7.2 Hz); 7.99 (d, 1H, arom., *J* = 7.2 Hz); 9.52 (s, 1H, OH). MS (ESI, *m/z* (rel %)): 332.2 (100) $[M+Na]^+$, 310.3 (20) $[M+H]^+$.

Anal. Calcd. for $C_{17}H_{15}N_3O_3$ (309.3): C, 66.01; H, 4.89; N, 13.58. Found N, 65.78; H, 4.74; N, 13.34.

6-(2-Hydroxyphenyl)-5-imino-2-phenyl-4,5-dihydro-1,2,4-triazin-3(2*H*)-one (**4a**).

A mixture of compound **1** (23.5 mg, 0.089 mmol) and 26% aqueous ammonia (3 ml) was stirred until a solution was obtained (6 h). The yellow solution was evaporated *in vacuo* to dryness. Yield 26.3 mg (99.1 %) of hydrate of **4a**. Anhydrous compound was obtained by crystallization from ethanol and drying at 140 °C for 2 h. Mp 235-237 °C. IR: 3474, 3393, 3074, 1673, 1644, 1593, 1451, 1338, 1266, 576. ^{1}H NMR (360 MHz, DMSO-d_6): δ 6.87 (bs, 1H, NH); 6.94 (t, 1H, arom., *J* = 7.4 Hz); 6.98 (d, 1H, arom., *J* = 8.2 Hz); 7.31-7.39 (m, 3H, arom.); 7.45-7.49 (m, 2H, arom.); 7.57-7.60 (m, 2H, arom.); 8.18 (bs, 1H, OH); 10.03 (bs, 1H, NH); ^{13}C NMR (300 MHz, DMSO-d_6): δ 117.0, 118.1, 119.8, 126.5, 129.1, 129.9, 131.3, 132.8, 137.2, 140.1, 141.6, 143.7, 156.4; MS (ESI, *m/z* (rel %)): 303.1 (20) $[M+Na]^+$, 281.3 (100) $[M+H]^+$.

Anal. Calcd. for $C_{15}H_{12}N_4O_2$ (280.3): C, 64.28; H, 4.32; N, 19.99. Found C, 63.98; H, 4.05; N, 19.71.

6-(2-Hydroxyphenyl)-5-(methylamino)-2-phenyl-1,2,4-triazin-3(2*H*)-one (**4b**).

A mixture of **1** (21.1 mg, 0.080 mmol) and 35% aqueous methylamine was stirred for 30 min. The obtained solution was evaporated *in vacuo* to dryness to yield 27.4 mg (98.3 %) of trihydrate of **4b**. Anhydrous compound was obtained by crystallization from ethanol and drying at 140 °C for 2 h. Mp 273-275 °C; IR: 3405, 3337, 3059, 2935, 1653, 1586, 1556, 1453, 1363, 1257, 747; ^{1}H NMR (360 MHz, DMSO-d_6): δ 2.86 (s, 3H, CH_3); 6.94 (t, 1H, arom., *J* = 7.4 Hz); 6.99 (d, 1H, arom., *J* = 8.2 Hz); 7.31-7.39 (m, 3H, arom.); 7.45-7.49 (m, 2H, arom.); 7.50-7.56 (bs, 1H, NH); 7.56-7.59 (m, 2H, arom.); 9.90 (bs, 1H, OH); MS (ESI, *m/z* (rel %)): 317.2 (70) $[M+Na]^+$, 295.3 (100) $[M+H]^+$.

Anal. Calcd. for $C_{16}H_{14}N_4O_2$ (294.3): C, 65.30; H, 4.79; H, N 19.04. Found C, 65.12; H, 4.76; N, 18.79.

5-(Cyclohexylamino)-6-(2-hydroxyphenyl)-2-phenyl-1,2,4-triazin-3(2*H*)-one (**4c**).

Compound **1** (48.7 mg, 0.185 mmol) was dissolved in cyclohexylamine (0.5 ml) at room temperature. After 30 min with occasional stirring, the solid began to precipitate from the solution. The mixture was diluted with water (4 ml) and neutralized with dilute acetic acid to pH ~ 8. The precipitate compound was collected by suction, washed with water and dried. Sample for analysis was prepared by crystallization from anhydrous ethanol. Yield 64.4 mg (96.1 %), mp 265-268 °C; IR: 3394, 3146, 2931, 2854, 1648, 1586, 1542, 1451, 1333, 756; ^{1}H NMR (360 MHz, $CDCl_3$): δ 1.06-1.96 (m, 10H, CH_2); 4.07 (bs, 1H, CH); 6.07 (bs, 1H, NH); 6.97 (t, 1H, arom., *J* = 7.6 Hz); 7.25-7.43 (m, 6H, arom.); 7.57-7.60 (m, 2H, arom.); 10.30 (bs, 1H OH); ^{13}C NMR (300 MHz, DMSO-d_6): δ 25.2, 25.6, 31.6, 49.5, 116.7, 119.3, 119.9, 125.6, 127.3, 128.8, 131.7, 131.9, 136.3, 142.3, 153.0, 155.2, 156.2; MS (ESI, *m/z* (rel %)): 385.2 (50) $[M+Na]^+$, 363.3 (100) $[M+H]^+$.

Anal. Calcd. for $C_{21}H_{22}N_4O_2$ (362.4): C, 69.59; H, 6.12; N, 15.46. Found C, 69.45; H, 5.92; N, 15.54.

N^6-[6-(2-Hydroxyphenyl)-3-oxo-2-phenyl-2,3-dihydro-1,2,4-triazin-5-yl]lysine (**4d**).

Compound **1** (44.0 mg, 0.167 mmol) was suspended, with stirring, in aqueous solution (5 ml) of DL-lysine (0.334 mmol) prepared from DL-lysine hydrochloride and an equivalent amount of sodium hydroxide. After 24 h stirring, the obtained solution was neutralized with dilute acetic acid to pH 7. After 1 h stirring, the precipitate was collected on filter, washed with water and dried; Yield 52.4 mg (76.5 %), mp 275 °C (dec.); IR: 3400, 3068, 2936, 1651, 1586, 1546, 1453, 1356, 754; ^{1}H NMR (360 MHz, DMSO-d_6): δ 1.35-1.81 (m, 6H, CH_2); 3.0-3.8 bs (COOH, CH, CH_2 and NH protons together with residue of water); 6.88 (t, 1H, arom., *J* = 7.4 Hz); 7.01 (d, 1H, arom., *J* = 7.9 Hz); 7.27-7.34 (m, 3H, arom.); 7.40-7.46 (m, 2H, arom.); 7.53-7.56 (m, 2H, arom.); OH proton is missing in spectrum; MS (ESI, *m/z* (rel %)): 410.3 (100) $[M+H]^+$; negative mode: 408.2 (100) $[M-H]^-$.

Anal. Calcd. for $C_{21}H_{23}N_5O_4$ (409.4): C, 61.60; H, 5.66; N, 17.10. Found C, 61.43; H, 5.79; N, 17.03.

5-Anilino-6-(2-hydroxyphenyl)-2-phenyl-1,2,4-triazin-3(2*H*)-one (**4e**).

Compound **1** (22.3 mg, 0.085 mmol) was dissolved in aniline (0.5 ml) with stirring at room temperature. After 20 min, water (7 ml) was added to the solution and compound **4e** was slowly precipitated under stirring with dilute acetic acid. Sample for analysis was prepared by crystallization from ethanol; Yield 22.7 mg (75.2 %), mp 245-248 °C; IR: 3365, 3145, 1650, 1549, 1518, 1449, 1363, 1250, 751, 693; ^{1}H NMR (360 MHz, DMSO-d_6): δ 6.93 (d, 1H, arom., *J* = 7.5 Hz); 7.00 (d, 1 H, arom., *J* = 8.4 Hz); 7.18 (t, 1H, arom., *J* = 7.4 Hz); 7.33-7.47 (m, 7H, arom.); 7.57 (m, 2H, arom.); 6.67 (d, 2H, arom., *J* = 7.7 Hz); 9.01 (s, 1H, OH); 10.13 (bs, 1H, NH); MS (ESI, *m/z* (rel %)): 379.2 (100) $[M+Na]^+$, 357.4 (40) $[M+H]^+$.

Anal. Calcd. for $C_{21}H_{16}N_4O_2$ (356.4): C, 70.77; H, 4.53; N, 15.72. Found C, 71.00; H, 4.42; N, 15.54.

5-(Diethylamino)-6-(2-hydroxyphenyl)-2-phenyl-1,2,4-triazin-3(2*H*)-one (**4f**).

This compound was prepared in a similar way as compound **4e** using compound **1** (47.1 mg, 0.179 mmol) and diethylamine (0.5 ml); Yield 56.2 mg (93.3 %), mp 216-219 °C (dec.); IR: 3445, 3087, 2986, 2939, 1632, 1568, 1519, 1431, 1352, 1262, 1140, 755; ^{1}H NMR (360 MHz, DMSO-d_6): δ 0.98 (bs, 6H, CH_3); 3.32 (bs, 4H, CH_2); 6.88-6.93 (m, 2H, arom.); 7.27-7.44 (m, 5H, arom.); 7.52-7.55 (m, 2H, arom.); 10.03 (bs, 1H, OH); ^{13}C NMR (300 MHz, DMSO-d_6): δ 13.1, 43.5, 115.9, 119.9, 124.7, 125.1, 127.3, 128.9, 130.4, 131.1, 134.5, 142.0, 152.2, 155.3, 156.2; MS (ESI, *m/z* (rel %)): 359.2 (100) $[M+Na]^+$, 337.4 (35) $[M+H]^+$.

Anal. Calcd. for $C_{19}H_{20}N_4O_2$ (336.4): C, 67.84; H, 5.99; N, 16.66. Found C, 67.59; H, 5.88; N, 16.49.

6-(2-Hydroxyphenyl)-2-phenyl-5-piperidino-1,2,4-triazin-3(2*H*)-one (**4g**).

This compound was prepared in a similar way as compound **4e** using compound **1** (50.9 mg, 0.193 mmol) and piperidine (0.5 ml); Yield 64.1 mg (95.1 %); mp 222-225 °C (dec.); IR: 3325, 3063, 2946, 1635, 1558, 1507, 1448, 1340, 753; ^{1}H NMR (360 MHz, DMSO-d_6): δ 1.44 (bs, 4H, CH_2); 1.53 (q, 2H, CH_2, J = 5.3 Hz); 3.47 (bs, 4H, CH_2); 6.90-6.94 (m, 2H, arom.); 7.28-7.36 (m, 3H, arom.); 7.41-7.45 (m, 2H, arom.); 7.54-7.56 (m, 2H, arom.); 10.10 (bs, 1H, OH); MS (ESI, *m/z* (rel %)): 371.3 (100) $[M+Na]^+$, 349.4 (25) $[M+H]^+$.

Anal. Calcd. for $C_{20}H_{20}N_4O_2$ (348.4): C, 68.95; H, 5.79; N, 16.08. Found C, 68.86; H, 5.64; N, 15.79.

5-[(2-Aminoethyl)amino]-6-(2-hydroxyphenyl)-2-phenyl-1,2,4-triazin-3(2*H*)-one (**4h**).

This compound was prepared in a similar way as compound **4e** using compound **1** (70.0 mg, 0.266 mmol) and ethylenediamine (0.5 ml); Yield 60.1 mg (69.9 %), mp 260-270 °C (dec.); IR: 3309, 2960, 1665, 1548, 1453, 1340, 756; ^{1}H NMR (360 MHz, DMSO-d_6): δ 2.81 (t, 2H, CH_2, J = 6.2 Hz); 3.44 (t, 2H, CH_2, J = 6.2 Hz); 3.6-4.5 (bs, NH together with residue of water); 6.88 (t, 1H, arom. J = 7.4 Hz); 6.95 (d, 1H, arom., J = 8.5 Hz); 7.32-7.38 (m, 3H, arom.); 7.45-7.50 (m, 2H, arom.); 7.56-7.60 (m, 2H, arom.); OH proton is missing in spectrum; MS (ESI, *m/z* (rel %)): 324.3 (80) $[M+H]^+$.

Anal. Calcd. for $C_{17}H_{17}N_5O_2$ (323.4): C, 63.15; H, 5.30; N, 21.66. Found C, 62.99; H, 5.66; N, 21.25.

5-[(4-Aminobutyl)amino]-6-(2-hydroxyphenyl)-2-phenyl-1,2,4-triazin-3(2*H*)-one (**4i**).

Compound **1** (57.4 mg, 0.218 mmol) was dissolved in a solution of butane-1,4-diamine (111.8 mg, 1.268 mmol) in chloroform (9 ml). After 18 h stirring, the precipitated solid was collected by filtration, washed with chloroform (2 x 2 ml) and dried. This product was suspended in chloroform (5 ml) (to remove traces of butane-1,4-diamine) and after 30 min stirring it was again collected by filtration and dried. Sample for analysis was prepared the crystallization from DMSO; Yield 60.3 mg (78.8 %), mp 220 °C (dec.); IR: 3353, 3064, 2946, 1652, 1554, 1452, 1154, 756; ^{1}H NMR (360 MHz, DMSO-d_6): δ 1.46-1.69 (m, 4H, CH_2); 2.66 (t, 2H, CH_2, J = 7.0 Hz); 3.34 (t, 2H, CH_2, J = 6.8 Hz); 3.4-4.2 (bs, NH together with residue of water) 6.76 (t, 1H, arom., J = 7.4 Hz); 6.84 (d, 1H, arom., J = 8.5 Hz); 7.26-7.33 (m, 3H, arom.); 7.40-7.45 (m, 2H, arom.); 7.53-7.58 (m, 2H, arom.); OH proton is missing in spectrum; MS (ESI, *m/z* (rel %)): 352.3 (70) $[M+H]^+$.

Anal. Calcd. for $C_{19}H_{21}N_5O_2$ (351.4): C, 64.94; H, 6.02; N, 19.93. Found C, 64.68; H, 6.32; N, 19.79.

5,5′-[(Butane-1,4-diyl)diamino]bis[6-(2-hydroxyphenyl)-2-phenyl-1,2,4-triazin-3(2*H*)-one] (**4j**).

Compound **1** (40.3 mg, 0.153 mmol) was dissolved in a solution of butane-1,4-diamine (6.21 mg, 0.070 mmol) in anhydrous chloroform (2.5 ml). After 18 h stirring in a sealed 5-ml glass vial, the precipitate was collected by filtration and washed with choroform (2 x 0.5 ml). Sample for analysis was prepared by crystallization from DMSO; Yield 41.6 mg (96.1 %), mp 300-305 °C (dec.); IR: 3404, 3065, 2941, 1649, 1586, 1551, 1452, 1353, 755; ^{1}H NMR (360 MHz, DMSO-d_6): δ 1.64 (bs, 4H, CH_2); 3.3-4.1 (bs, 2x CH_2 and NH together with residue of water); 6.94 (t, 2H, arom., J = 7.4 Hz); 7.00 (d, 2H, arom., J = 8.5 Hz); 7.31-7.39 (m, 6H, arom.); 7.45-7.49 (m, 4H, arom); 7.57-7.60 (m, 4H, arom.); OH protons are missing in spectrum; MS (ESI, *m/z* (rel %)): 637.2 (65) $[M+Na]^+$, 615.3 (100) $[M+H]^+$.

Anal. Calcd. for $C_{34}H_{30}N_8O_4$ (614.7): C, 66.44; H, 4.92; N, 18.23. Found C, 66.81; H, 4.88; N, 18.07.

1,5,10-Tris[6-(2-hydroxyphenyl)-3-oxo-2-phenyl-1,2,4-triazin-5-yl]-1,5,10-triazadecane (**4k**).

Compound **1** (50.2 mg, 0.191 mmol) was dissolved in a solution of spermidine (9.23 mg, 0.063 mmol) in anhydrous chloroform (3.0 ml). The solution was heated at 50 °C in a sealed 5-ml glass vial for 18 hrs and then evaporated to dryness. Sample for analysis was prepared by crystallization from DMSO; Yield 57.3 mg (96.3 %), mp 196-201 °C (dec.); IR: 3402, 3069, 2945, 1648, 1586, 1559, 1451, 1353, 756; ^{1}H NMR (360 MHz, DMSO-d_6): δ 1.60-1.73 (m, 6H, CH_2); 3.1-4.0 (bs, 4x CH_2 and NH together with residue of water); 6.87-7.00 (m, 4H, arom.); 7.03-7.12 (m, 5H, arom.); 7.15-7.20 (m, 6H, arom.); 7.31-7.42 (m, 8H, arom.); 7.44-7.52 (m, 4H, arom.); OH protons are missing in spectrum; MS (ESI, *m/z*): 935.3 (100) $[M+H]^+$.

Anal. Calcd. for $C_{52}H_{46}N_{12}O_6$ (935.0): C, 66.80; H, 4.96; N, 17.98. Found C, 66.53; H, 4.78; N, 17.71.

5-Hydrazino-6-(2-hydroxyphenyl)-2-phenyl-1,2,4-triazin-3(2*H*)-one (**5a**).

Compound **1** (318.2 mg, 1.209 mmol) was dissolved in 80% hydrazine hydrate (5.0 ml). After 1 h stirring at room temperature, the solution was diluted with water (10 ml) and neutralized with dilute acetic acid. The precipitated solid was collected on a filter and washed with water. Sample for analysis was prepared by crystallization from anhydrous ethanol; Yield 345.9 mg (96.9 %), mp 235-240 °C (dec.); IR: 3346, 3241, 3162, 3073, 1693, 1661, 1584, 1455, 1337, 755; ^{1}H NMR (360 MHz, DMSO-d_6): δ 5.50-6.50 (bs, 3H, NH); 6.77-6.83 (m, 2H, arom.); 7.18-7.21 (m, 2H, arom.); 7.27 (t, 1H, arom., J = 7.4 Hz); 7.40 (m, 2H, arom.); 7.50 (m, 2H, arom.); 9.48 (bs, 1H, OH); MS (ESI, *m/z* (rel %)): 296.4 (70) $[M+H]^+$.

Anal. Calcd. for $C_{15}H_{13}N_5O_2$ (295.3): C, 61.01; H, 4.44; N, 23.72. Found C, 60.74; H, 4.32; N, 23.56.

6-(2-Hydroxyphenyl)-2-phenyl-5-(phenylhydrazino)-1,2,4-triazin-3(2*H*)-one (**5b**).

Compound **1** (45.0 mg, 0.171 mmol) was suspended in phenylhydrazine (0.5 ml) at room temperature. After 4 h stirring, water (4 ml) was added to the bright yellow suspension, which was then neutralized with dilute acetic acid, then collected by filtration, washed with water and crystallized for analysis from acetic acid – DMSO (3:1 v/v) mixture; Yield 60.1 mg (94.5 %), mp 277-280 °C; IR: 3345, 3342, 3062, 1696, 1601, 1501, 1362, 1244, 470; ^{1}H NMR (360 MHz, DMSO-d_6): δ 6.71 (t, 1H, arom., J = 7.3 Hz); 6.79 (d, 2H, arom., J = 7.7 Hz); 6.87 (t, 1H, arom., J = 7.4 Hz); 6.92 (d, 1H, arom., J = 8.0 Hz); 7.14 (t, 2H, arom., J = 8.0 Hz); 7.25-7.35 (m, 3H, arom.); 7.40-7.44 (m, 2H, arom.); 7.54-7.57 (m, 2H, arom.); 9.15 (s, 1H, NH); 9.49 (s, 1H, NH); 10.56 (bs, 1H, OH); MS (ESI, *m/z* (rel %)): 394.2 (60) $[M+Na]^+$, 372.3 (40) $[M+H]^+$.

Anal. Calcd. for $C_{21}H_{17}N_5O_2$ (371.4): C, 67.91; H, 4.61; N, 18.86. Found C, 68.05; H, 4.51; N, 18.54.

2-[6-(2-Hydroxyphenyl)-3-oxo-2-phenyl-2,3-dihydro-1,2,4-triazin-5-yl]hydrazinecarboxamide (**5c**).

A mixture of **1** (108.8 mg, 0.411 mmol), semicarbazide hydrochloride (1276.0 mg, 11.44 mmol) and solution of sodium hydroxide (458.0 mg, 11.44 mmol) in water (6 ml) was stirred for 24 h. A pale yellow solid was collected by filtration and washed with water; Yield 146.0 mg (99.8 %) of hydrate of **5c**, mp 200-210 °C (with formation of **6d**). Sample for analysis was prepared by crystallization from ethanol and dried at 115 °C for 1 h; IR: 3471, 3270, 1723, 1680, 1572, 1487, 1335, 1123,755; ^{1}H NMR (360 MHz, DMSO-d_6): δ 5.50-6.50 (bs, 2H, NH); 6.87-6.93 (m, 2H, arom.); 7.27-7.34 (m, 3H, arom.); 7.45-7.49 (m, 2H, arom.); 7.56-7.60 (m, 2H, arom.); 9.26 (s, 1H, NH); 9.62 (bs, 1H, NH); 10.79 (bs, 1H, OH); ^{13}C NMR (300 MHz, DMSO-d_6): δ 116.3, 119.1, 120.9, 125.5, 127.3, 128.9, 129.4, 130.9, 131.2, 141.0, 144.2, 147.2, 155.9, 156.8; MS (ESI, *m/z* (rel %)): 361.2 (30) $[M+Na]^+$, 339.3 (100) $[M+H]^+$; negative mode: 337.2 (100) $[M-H]^-$.

Anal. Calcd. for $C_{16}H_{14}N_6O_3$ (338.3): C, 56.80; H, 4.17; N, 24.84. Found C, 56.72; H, 4.23; N, 24.70.

8-(2-Hydroxyphenyl)-6-phenyl[1,2,4]triazolo[4,3-*d*][1,2,4]triazin-5(6*H*)-one (**6a**).

A solution of compound **5a** (60.0 mg, 0.203 mmol) in formic acid (2 ml) was refluxed for 75 min. Upon cooling, the solution was diluted with water (10 ml) and the precipitate was collected on a filter and washed with water. Sample for analysis was prepared by crystallization from methanol (1 ml per 8 mg); Yield 56.2 mg (90.6 %), mp 240-242 °C; IR: 3445, 3144, 3097, 1728, 1494, 1467, 1363, 1281, 1179, 778, 680; ^{1}H NMR (360 MHz, DMSO-d_6): δ 7.03-7.08 (m, 2H, arom.); 7.46 (t, 1H, arom., J = 8.0 Hz); 7.51 (t, 1H, arom., J = 7.2 Hz); 7.59-7.66 (m, 2H, arom.); 7.71-7.76 (m, 2H, arom.); 7.94 (d, 1H, arom., J = 8.0 Hz); 9.77 (s, 1H, arom.); 10.15 (bs, 1H, OH); MS (ESI, *m/z* (rel %)): 306.3 (50) $[M+H]^+$.

Anal. Calcd. for $C_{16}H_{11}N_5O_2$ (305.3): C, 62.95; H, 3.63; N, 22.94. Found C, 62.76; H, 3.25; N, 22.79.

2-(3-Methyl-5-oxo-6-phenyl-5,6-dihydro[1,2,4]triazolo[4,3-*d*][1,2,4]triazin-8-yl)phenyl acetate (**6b**).

A mixture of **5a** (74.1 mg, 0.251 mmol) and acetic anhydride (0.75 ml) in anhydrous pyridine (2 ml) was heated on a boiling water bath for 1 h. Upon cooling to 2 °C, the solution was diluted with water to 40 ml. The precipitate was collected by filtration, washed with water and (for analysis) crystallized from an ethanol-water mixture; Yield 71.2 mg (78.5 %), mp 154-155 °C; IR: 3073, 1746, 1728, 1488, 1334, 1196, 913, 773; ^{1}H NMR (360 MHz, DMSO-d_6): δ 1.91 (s, 3H, $COCH_3$); 2.91 (s, 3H, CH_3); 7,37 (d, 1H, arom., J = 8.1 Hz); 7.50-7.54 (m, 2H, arom.); 7.58-7.63 (m, 2H, arom.); 7.65-7.71 (m, 3H, (arom.); 8.12 (d, 1H, arom., J = 7.9 Hz); MS (ESI, *m/z* (rel %)): 384.2 (45) $[M+Na]^+$, 362.1 (35) $[M+H]^+$.

Anal. Calcd. for $C_{19}H_{15}N_5O_3$ (361.4): C, 63.15; H, 4.18; N, 19.38. Found C, 62.98; H, 4.25; N 19.35.

8-(2-Hydroxyphenyl)-6-phenyl-3-thioxo-2,3-dihydro[1,2,4]triazolo[4,3-*d*][1,2,4]triazin-5(6*H*)-one (**6c**).

A mixture of **5a** (64.9 mg, 0.219 mmol) and carbon disulfide (0.2 ml) in pyridine (3 ml) was stirred in a sealed glass vial at room temperature. After 18 h, the vial was heated on a water bath (70 °C) for 10 min and evaporated to dryness *in vacuo*. The residue was mixed with water (5 ml), collected on a filter, washed with water and crystallized from ethanol. Yield 68.1 mg (91.8 %), mp 293-296 °C; IR: 3505, 3140, 3076, 2906, 1746, 1490, 1314, 1252, 1157, 631; ^{1}H NMR (360 MHz, DMSO-d_6): δ 6.96-7.00 (m, 2H, arom.); 7.40 (t, 1H, arom., J = 8.2 Hz); 7.48 (t, 1H, arom.); 7.53-7.59 (m, 3H, arom.); 7.62-7.65 (m, 2H, arom.); 9.86 (s, 1H, OH); 14.71 (bs, 1H, NH); ^{13}C NMR (300 MHz, DMSO-d_6): δ 116.9, 117.7, 119.4, 126.5, 128.6, 129.3, 130.9, 132.2, 138.1, 140.5, 141.3, 142.6, 143.2, 156.1; MS (ESI, *m/z* (rel %)): 360.3 (100) $[M+Na]^+$, 338.3 (30) $[M+H]^+$; negative mode: 336.1 (100) $[M-H]^-$.

Anal. Calcd. for $C_{16}H_{11}N_5O_2S$ (337.4): C, 56.96; H, 3.29; N, 20.76. Found C, 56.83; H, 3.60; N, 20.52.

8-(2-Hydroxyphenyl)-6-phenyl[1,2,4]triazolo[4,3-*d*][1,2,4]triazine-3,5(2*H*,6*H*)-dione (**6d**).

Hydrate of **5c** (14.93 mg, 0.042 mmol) was heated at 200 °C in a weighed glass vial for 30 min and the resulting melt was crystallized from ethanol (10 ml); Yield 13.4 mg (99.5 %), mp 345-347 °C (dec.); IR: 3144, 3067, 1772, 1685, 1492, 1334, 1298, 768; ^{1}H NMR (360 MHz, DMSO-d_6): δ 6.95-7.00 (m, 2H, arom.); 7.40 (t, 1H, arom., J = 8.5 Hz); 7.46 (t, 1H, arom., J = 7.0 Hz); 7.53-7.58 (m, 2H, arom.); 7.61-7.63 (m, 3H, arom.); 9.93 (bs, 1H, OH); 12.90 (bs, 1H, NH); MS (ESI, *m/z* (rel %)): 322.3 (20) $[M+H]^+$, negative mode: 320.2 (100) $[M-H]^-$.

Anal. Calcd. for $C_{16}H_{11}N_5O_3$ (321.3): C, 59.81; H, 3.45; N, 21.80. Found C, 59.81; 3.45; N, 20.60.

8-(2-Hydroxyphenyl)-6-phenyltetrazolo[1,5-*d*][1,2,4]triazin-5(6*H*)-one (**7**).

Compound **5a** (50.0 mg, 0.169 mmol) was dissolved in solution of 35% hydrochloric acid (0.2 ml) and water (15 ml) at 60-70 °C and then cooled to 0-5 °C. To this mixture, a solution of sodium nitrite (11.68 mg, 0.169 mmol) in water (2 ml) was added. After 3 h stirring at 0-5 °C, the precipitate was collected by filtration and washed with water. Sample for analysis was prepared by crystallization from methanol (1 ml per 1 mg); Yield 45.5 mg (87.7 %), mp 222-225 °C (dec.); IR: 3450, 3175, 1761, 1748, 1463, 1217, 773, 709; ^{1}H NMR (360 MHz, DMSO-d_6): δ 7.04-7.10 (m, 2H, arom.); 7.49 (t, 1H, arom., J = 8.3 Hz); 7.57-7.71 (m, 4H, arom.); 7.76-7.78 (m, 2H, arom.); 10.18 (s, 1H, OH); ^{13}C NMR (300 MHz, DMSO-d_6): δ 117.0, 118.1, 119.8, 126.5, 129.5, 129.6, 131.3, 132.8, 137.2, 140.1, 141.6, 143.3, 156.4; MS (ESI, *m/z* (rel %)): 307.1 (100) $[M+H]^+$.

Anal. Calcd. for $C_{15}H_{10}N_6O_2$ (306.3): C, 58.82; H, 3,29; N, 27.44. Found C, 58.89; H, 3.20; N, 27.29.

6-(2-Hydroxyphenyl)-2-phenyl-5-thioxo-4,5-dihydro-1,2,4-triazin-3(2*H*)-one (**8**).

Compound **1** (84.0 mg, 0.319 mmol) was suspended in a solution of NaOH (*ca*. 400 mg of NaOH in 5 ml of water) saturated with H_2S at room temperature. The mixture was stirred for 3 h at room temperature to obtain a solution. After 18 h, the solution was diluted with water (10 ml) and carefully neutralized with dilute acetic acid to pH 7-8. The precipitate was collected on filter and washed with water. Sample for analysis was obtained by crystallization from a heptane-toluene mixture; Yield 40.0 mg (42.1 %), mp 157-160 °C (dec.); IR: 3031, 1725, 1694, 1605, 1487, 1454, 1319, 762; ^{1}H NMR (360 MHz, DMSO-d_6): δ 6.84-6.90 (m, 2H, arom.); 7.22 (d, 1H, arom., J = 7.5 Hz); 7.27 (t, 1H, arom., J = 7.3 Hz); 7.44 (t, 1H, arom., J = 7.2 Hz); 7.50-7.55 (m, 2H, arom.); 7.60-7.66 (m, 2H, arom.); 9.48 (s, 1H, OH); 13.81 (bs, 1H, NH); MS (ESI, *m/z* (rel %)): negative mode: 296.4 (100) $[M-H]^-$.

Anal. Calcd. for $C_{15}H_{11}N_3O_2S$ (297.3): C, 60.59; H, 3.73; N, 14.13. Found C, 60.33; H, 3.45; N, 14.42.

6-(2-Hydroxyphenyl)-2-phenyl-4,5-dihydro-1,2,4-triazin-3(2*H*)-one (**9**).

A mixture of a solution of NaOH (*ca*. 200 mg of NaOH in 3.5 ml of water) saturated with hydrogen selenide (prepared from MgSe and dilute HCl in a stream of nitrogen) and compound **1** (97.1 mg, 0.369 mmol) in a sealed glass vial under nitrogen was stirred for 12 h at room temperature. The content of the vial was diluted with water (5 ml) and carefully neutralized with dilute acetic acid to pH 7-8. The precipitate was collected on filter and washed with water. The dry product was suspended in chloroform (5 ml) allowed to stand overnight (red Se changed to metallic Se) and collected by filtration. Purification by elution with chloroform through a short column (4 cm i.d.) filled with silica gel 60 (230-400 mesh, 40 g) gave **9**. Sample for analysis was prepared by crystallization from heptane-toluene-methanol mixture (2:1:0.1 v/v/v); Yield 37.5 mg (38.0 %), mp 137-140 °C (dec.); IR: 2928, 1665, 1497, 1466, 1256, 1197, 695; ^{1}H NMR (360 MHz, $CDCl_3$): δ 3.38 (s, 2H, CH_2); 5,81 (s, 1H, NH); 6,90 (t, 1H, arom., J = 8.2 Hz); 6,97 (d, 1H, arom., J = 8.2 Hz); 7,29-7,57 (m, 7H, arom.); 11,02 (bs, 1H, OH); MS (ESI, *m/z* (rel %)): negative mode: 266.3 (100) $[M-H]^-$.

Anal. Calcd. for $C_{15}H_{13}N_3O_2$ (267.3): C, 67.40; H, 4.90; N, 15.72. Found C, 67.52; H, 4.86; N, 15.88.

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REFERENCES AND NOTES

[1] Part I in the series "The Cleavage of Heterocyclic Compounds in Organic Synthesis".

[2] A. M. Gold, *J. Org. Chem.*, **26**, 3991 (1961).

[3] D. Starr and R. M. Hixon, *Org. Synth. Coll. Vol.* **II**, 571 (1943).

[4] H. Stone and H. Shechter, *Org. Synth. Coll. Vol.* **IV**, 321 (1963).

[5] K. Hafner and K. P. Meinhardt, *Org. Synth. Coll. Vol.* **VII**, 15 (1990).

[6] P. Baumgarten, *Ber. Dtsch. Chem. Ges.*, **59**, 1166 (1926).

[7] G. W. Fisher, *Ber. Dtsch. Chem. Ges.*, **103**, 3489 (1970).

[8] R. Koenig, *Ber. Dtsch. Chem. Ges.*, **63**, 2823 (1930).

[9] J. Stýskala, J. Slouka, M. Hejsek and V. Bekárek, *Collect. Czech. Chem. Commun.*, **62**, 1754 (1997).

[10] J. Stýskala and J. Slouka, *Acta Univ. Palacki. Olomuc., Fac. Rerum Nat., Chemica*, **37**, 73 (1998); *Chem. Abstr.*, 131, 102259 (1999).

[11] J. Stýskala and J. Slouka, *Heterocycl. Commun.*, **5**, 349 (1999).

[12] Z. Trávníček, M. Maloň, Z. Šindelář, K. Doležal, J. Rolčík, V. Kryštov, M. Strnad and J. Marek, *J. Inorg. Biochem.*, **84**, 23 (2001).