

Analogs of biologically active compounds IX.¹ Synthesis of several new uracil and pteridine 6-azaanalogs based on cyclization of arylhydrazones derived from mesoxalic acid

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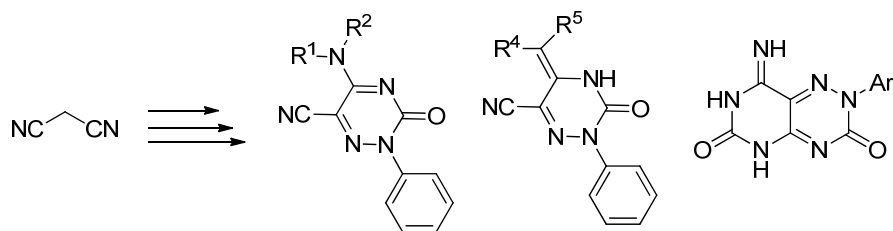
Received 04-03-2017

Accepted 06-12-2107

Published on line 08-06-2017

Abstract

A series of 5C and 5N substituted 3-oxo-2-phenyl-1,2,4-triazine-6-carbonitriles and 8-imino-2-arylpyrimido[4,5-e][1,2,4]triazine-3,6(2H,5H)-diones (6-aryl-6-azapteridines) are described via a cyclocondensation reaction from the corresponding aryliminohydrazones with CDI. The required aryliminohydrazones were obtained from the starting compound – malononitrile. These prepared compounds were tested for cytotoxic activity on cancer cell lines.



Keywords: cyclization of arylhydrazones, 1,2,4-triazine-6-carbonitrile, pyrimido[4,5-e][1,2,4]triazine, 6-azapteridine

Introduction

Certain 1,2,4-triazines and pyrimido[1,2,4]triazines can be considered as analogues of naturally occurring *N*-heterocycles, especially of pyrimidine bases, purines and pteridines. Many 1,2,4-triazine derivatives are well known to possess biological activities.^{2,3} Over the last two decades asymmetric triazines have been screened *in vitro* supporting their anticancer,⁴⁻⁶ anti-HIV⁴⁻⁶ and tuberculostatic activities.⁷ Pyrimido[1,2,4]triazines show interesting biological activities, such as the antibiotics toxoflavine, reumycine, fervenuline and methylfervenuline all possessing a pyrimido[5,4-*e*][1,2,4]triazine (7-azapteridine) system. Isomeric pyrimido[4,5-*e*][1,2,4]triazines (6-azapteridine) have also been shown to exhibit biological activity^{8,9} such as antiviral¹⁰ and antibacterial activity.^{10,11} To date, 5*C* and 5*N* substituted 3-oxo-1,2,4-triazine-6-carbonitriles and 6-aryl-6-azapteridines have not been investigated in detail, this communication serves to document these derivatives.

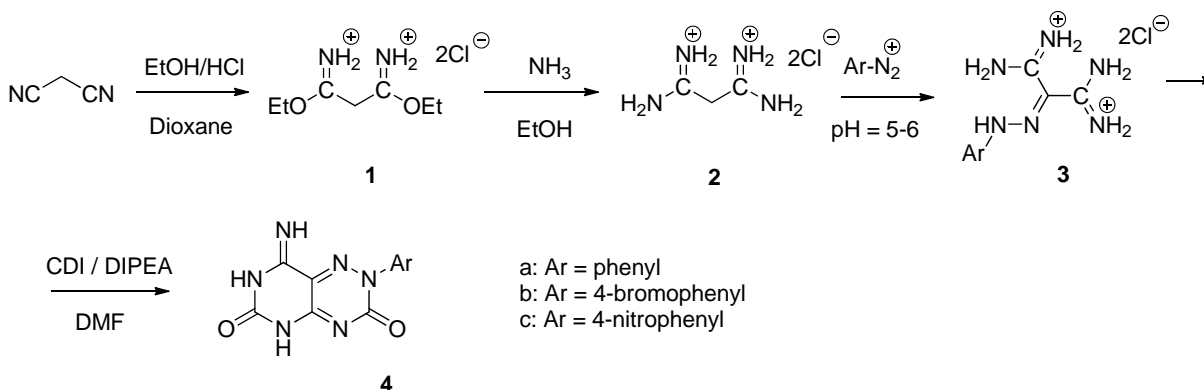
Results and Discussion

In this paper we utilize a cyclization principle based on the cyclization of arylhydrazones leading to 2-aryl-derivatives of 1,2,4-triazines. The most usual and proven syntheses of this type include the cyclization of ethyl arylhydrazono-cyanoacetyl carbamates resulting in 1-aryl-6-azauracils¹²⁻¹⁴ and the analogous cyclization of arylhydrazones of ethoxycarbonylated amidines of the mesoxalic acid providing 1-aryl-6-azacytosines.¹ On a modified principle we cyclized aryliminohydrazones of last type by direct cyclocondensation reaction with 1,1'-carbonyldiimidazole (CDI) which had been used earlier for the formation of 6-azalumazin-7(6*H*)ones.¹⁵

The starting compound utilized for the synthesis of both uncondensed and condensed 1,2,4-triazines was malononitrile. In the case of the formation of 6-azapteridine derivatives **4**, malononitrile was converted *via* reaction with diethyl malonimidate dihydrochloride **1** to malonimidamide dihydrochloride **2** as according to the previously described literature procedure.^{16,17} This compound possessing a reactive methylene group was coupled with various diazonium salts to give corresponding 2-arylhydrazonomalonimidamides **3a-3c** which were isolated as dihydrochloride salts. The purity and yields of hydrazones were very dependent on the pH and method of preparation. The optimal method found was to add the diazonium salt to an aqueous solution of malonimidamide **2** and adjust the pH to 5-6 with sodium acetate with vigorous stirring. If the pH of the reaction mixture was higher then the formed hydrazones were less pure due to the formation of gummy products making separation of the compounds difficult. Additional to this, a lower pH was found to be insufficient for initiation of the azo-coupling reaction. Three arylhydrazones differing in substitution of the *para* position (**3a-3c**) were obtained in good yields. From the literature only phenyl derivative **3a** is known and it was described as an azocompound,^{17,18} however herein we have proven it exists in a hydrazono form on the basis of the NMR spectra measured in DMSO-*d*₆. In fact, the molecule is an asymmetric compound including the diamidine moiety. Apart from the aromatic hydrogens, five different hydrogen atoms were present. One sharp signal at 11.85-12.34 ppm with integral intensity one belongs to HN-aryl hydrogen and four signals with integral intensity two belong to the diamidine functionality. Additionally from the ¹³C NMR spectroscopy, using APT method, it was evident that the methine hydrogen was missing; and so the other suggested structures are not probable.

The prepared arylhydrazones **3** were converted into corresponding 8-imino-2-arylpyrimido[4,5-*e*][1,2,4]triazine-3,6(2*H*,5*H*)-diones **4** by a direct double cyclocondensation reaction with an excess of CDI in DMF and in the presence of DIPEA (Scheme 1). Interestingly, we did not succeed in preparing of compounds

where only one ring would be formed by cyclization with one equivalent of CDI. A mixture of intermediates, starting hydrazone **3** and corresponding 6-azapteridine derivative **4** were always present in the reaction mixture. Other possible cyclization reagents such as ethyl chloroformate and phosgene used for similar formation of six-membered ring from amidines¹ failed to cyclize smoothly. Using CDI, the most readily formed compounds were derivatives **4a** and **4b**. In case of nitro derivative **4c** the yield was half as expected. These low soluble derivatives exist in their 8-imino form indicative by the three different hydrogen atoms are present in ¹H NMR spectrum, which is typical for these type of compounds.

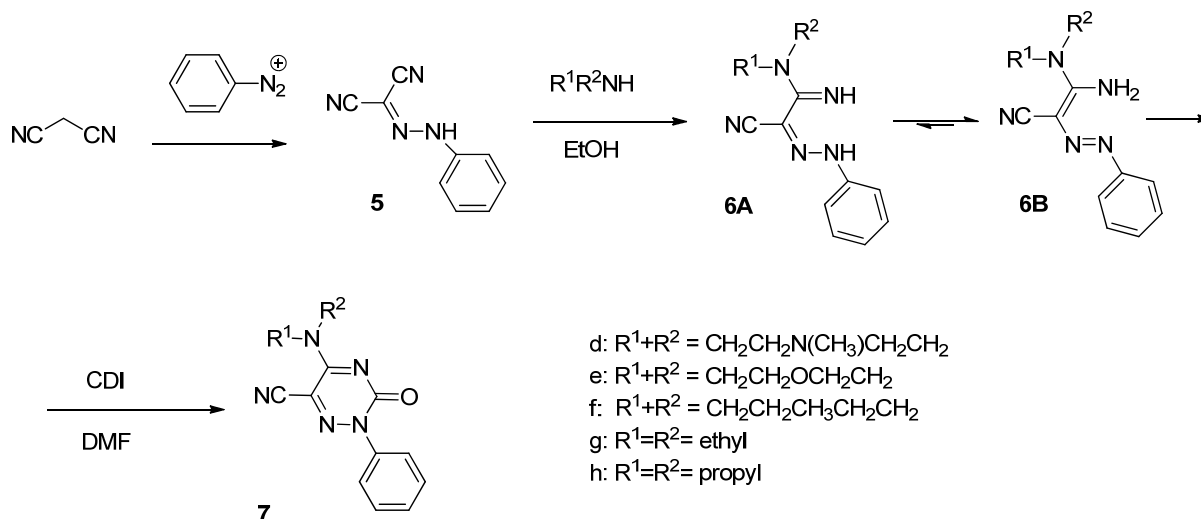


Scheme 1. Synthesis of 8-imino-2-arylpyrimido[4,5-*e*][1,2,4]triazine-3,6(2*H*,5*H*)-diones (6-aryl-6-azapteridines) **4a-4c**.

Malononitrile was also used for the preparation of uncondensed 1,2,4-triazines. Both 5*C* and 5*N* substituted 3-oxo-2-phenyl-1,2,4-triazine-6-carbonitrile derivatives were prepared from common precursor – 2-phenylhydrazonomalononitrile (**5**) which is easily accessible by azo coupling reaction of the corresponding benzenediazonium salt with malononitrile.¹⁹

In case of the preparation of 5*N* substituted 3-oxo-2-phenyl-1,2,4-triazine-6-carbonitrile derivatives **7** the phenylhydrazone **5** was converted to *N,N*-disubstituted 3-amino-2-(phenyldiazenyl)acrylonitriles **6d-6h** using a described literature procedure.²⁰ These compounds can exist in two tautomeric forms **6A-6B**. Although some literature articles have described similar derivatives as hydrazones,^{21,22} our results, supported by NMR spectra, are in accordance with the published literature where the azo form was reported.²⁰ We also observed no NH proton signal in ¹H NMR spectra of compounds **6** which would correspond to the hydrazono group. Only a two-proton singlet at 7.3-7.7 ppm is apparent from the spectra of the newly prepared compounds. The azo-amino form **6B** was observed in both the ¹H NMR spectra in DMSO-*d*₆ and CDCl₃. No mixture of the possible geometric isomers of **6B** has been observed.

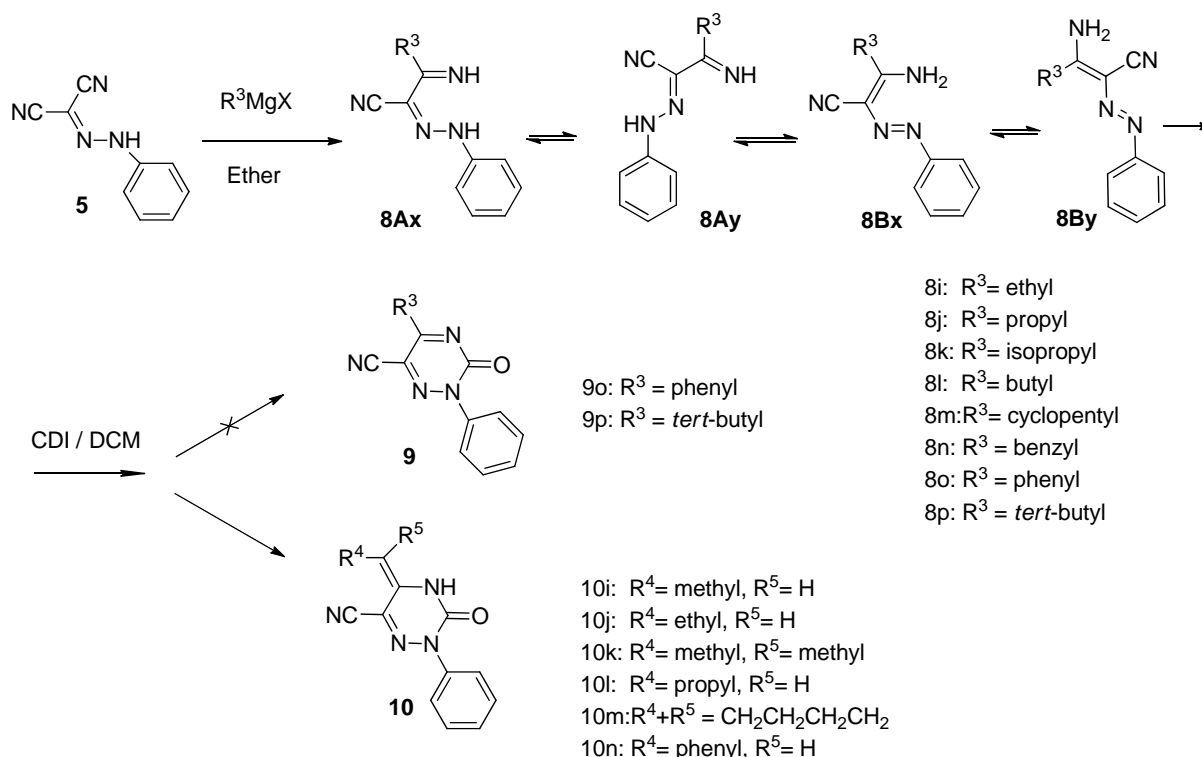
The cyclization of 2-(phenyldiazenyl)acrylonitriles **6** was performed with CDI in a solution of DMF at 50°C (Scheme 2). We have found that the temperature influences the purity of cyclized 1,2,4-triazines **7**. If the temperature was higher (around 90 °C) more by-products are formed and the yields are lowered. At 50°C the reaction proceeds slowly but the yields are much better, exceeding 75 %. However, this method is not suitable for the cyclization of *N*-monosubstituted 2-(phenyldiazenyl)acrylonitriles **6** (R₁ = methyl R₂ = H²³; R₁ = butyl R₂ = H²³) in the cases that we have tried. Several by-products were presented in the reaction mixture making their separation difficult. The preparation of 5*N*-monosubstituted 1,2,4-triazine-6-carbonitriles **7** is possible using another method wherein substitution of chlorine atom in position 5 is carried out.²⁴



Scheme 2. Synthesis of 5N substituted 3-oxo-2-phenyl-1,2,4-triazine-6-carbonitriles **7d-7h**.

For the preparation of 5C substituted 1,2,4-triazine derivatives **10**, the required 2-(2-phenylhydrazono)propanenitriles **8i-8p** were prepared in good yields by addition of Grignard reagents to phenylhydrazone **5** according to the literature procedure.²⁵ In this reference only phenyl derivative **8o** was prepared and referred to as azo compound **8B**. On basis of NMR spectroscopy we found that the newly prepared compounds **8i-8p** in either DMSO-*d*₆ or CDCl₃ solutions exist in several tautomeric forms **8Ax-y** and **8Bx-y** in an equilibrium. In the case of DMSO-*d*₆ solution there is a molar ratio of approximately 5:2 while in CDCl₃ the ratio is 6:1 (results from ¹H NMR). It is difficult to say exactly which forms predominate, however it is apparent that azo-amino forms **8Bx-y** predominates in the CDCl₃ solution, where broad signals at 5.5-5.9 ppm indicates an amino group. On the other hand, in DMSO-*d*₆, four N-H proton signals were present at 8-9 ppm. These results show that the hydrazono form predominates in their two geometric isomers **8Ax-y**. Additionally the ¹³C NMR spectra indicated that compound **8** exists as an equilibrium mixture of more than one of the geometric isomeric forms in the aforementioned solvents.

The prepared phenylhydrazones **8** were also cyclized with CDI to the corresponding 1,2,4-triazines **10** (Scheme 3). These reactions were performed in DCM as a solvent instead of DMF. Probably due to the equilibrium of tautomeric forms in a polar aprotic solvent mentioned above, we observed more by-products when using DMF instead of DCM under the same conditions. Surprisingly, on the basis on NMR spectra it was apparent that prepared 1,2,4-triazines **10** contain an alkylidene arrangement in position 5. In the case of compounds **10i**, **10j**, **10l** and **10n** only one proton at 4.88-5.87 ppm was detected on the first carbon of the aliphatic chain connected to 1,2,4-triazine ring. The second proton of these compounds was observed as a broad signal corresponding to one proton of N-H group. We were not successful to cyclize phenyl derivative **8o**²⁵ to 1,2,4-triazine **9o**. No conditions (higher temperature, solvent, time) were found for the cyclization with CDI. It is possible to conclude that if starting hydrazone **8** cannot create an alkylidene arrangement on the 1,2,4-triazine ring then no cyclization can take place. The same results were observed for *tert*-butyl derivative **8p**.



Scheme 3. Synthesis of 5C substituted 3-oxo-2-phenyl-1,2,4-triazine-6-carbonitriles **10i-10n**.

The prepared compounds were tested for biological activity. Human breast adenocarcinoma cell line *MCF7* and myelogenous leukemia line *K-562* were used for cytotoxicity determination by the MTT assay²⁶. The tested compounds showed poor cytostatic activity (IC₅₀ = 40–190 µmol/l), with the exception of the moderately active 6-azapteridine **4a** (*MCF7*: IC₅₀ = 8.3 µmol/l; *K-562*: IC₅₀ = 12.9 µmol/l), **4b** (*MCF7*: IC₅₀ = 7.2 µmol/l; *K-562*: IC₅₀ = 14.6 µmol/l) and 1,2,4-triazine **10i** (*MCF7*: IC₅₀ = 8.6 µmol/l; *K-562*: IC₅₀ = 7.5 µmol/l), **10j** (*MCF7*: IC₅₀ = 16.3 µmol/l; *K-562*: IC₅₀ = 11.3 µmol/l).

Conclusions

We have successfully developed a method for the preparation of several new 5C and 5N substituted 3-oxo-2-phenyl-1,2,4-triazine-6-carbonitriles and 8-imino-2-arylpyrimido[4,5-*e*][1,2,4]triazine-3,6(2*H*,5*H*)-diones, which represent uracil and pteridine 6-azaanalogs. The principle for the preparation of aforementioned compounds was based on the cyclization of corresponding arylhydrazones derived from mesoxalic acid using 1,1'-carbonyldiimidazole (CDI). Additionally an azo hydrazono tautomerism mechanism has been established with arylhydrazones used for the cyclization reactions. The prepared compounds were tested for cytotoxic activity on the Human breast adenocarcinoma (*MCF7*) and myelogenous leukemia (*K-562*) cancer cell lines.

Experimental Section

General. All starting materials are commercially available. Commercial reagents were used without any purification. Melting points were determined with a Boetius stage apparatus and are uncorrected. Reactions

were monitored by LC/MS analyses with a UHPLC-MS system consisting of a UHPLC chromatography with photodiode array detector and a triple quadrupole mass spectrometer using a C18 column at 30 °C and flow rate of 800 μ L/min. The mobile phase was (A; 0.01 M ammonium acetate in water) and (B; CH₃CN), linearly programmed from 10 to 80% B over 2.5 min, kept for 1.5 min. The column was reequilibrated with 10% B for 1 min. The APCI source operated at a discharge current of 5 mA, a vaporizer temperature of 400 °C, and a capillary temperature of 200 °C. High-resolution mass spectrometer based on the orbitrap mass analyzer was equipped with Heated Electrospray Ionization (HESI). The spectrometer was tuned to obtain a maximum response for m/z 70-700. The source parameters were set to the following values: HESI temperature 30 °C, spray voltage +3.5kV, -3kV; transfer capillary temperature 270 °C, sheath gas/aux gas (nitrogen) flow rates 35/10. The HRMS spectra of target peaks allowed evaluating their elemental composition with less than 3 ppm difference between experimental and theoretically calculated value. The ¹H and ¹³C NMR spectra were measured in DMSO-*d*₆, CDCl₃ at 25 °C using 400 MHz spectrometer. Chemical shift (δ) is given in ppm.

General procedure A. 2-Arylhydrazonomalonimidamide dihydrochlorides (3). The corresponding aniline derivative (5.76 mmol) was dissolved in 35% HCl (10 mL) and diazotized with a solution of sodium nitrite (397mg, 5.76 mmol) in water (10 mL) at 0-5°C. The mixture was stirred in an ice bath for 15 min and then, in one portion, it was added to a solution of malonimidamide dihydrochloride (990.7 mg, 5.76 mmol) **2** in water (20 mL) which was pre-cooled to 0-5 °C. After 15 minutes, with efficient stirring, a saturated solution of sodium acetate was added to reach pH = 5-6 and the reaction mixture was left at 2 °C for 18 h. A small amount of precipitated solid was filtered off and the filtrate concentrated under reduced pressure to one fifth of its original volume, when hydrazone started to crystalize. The product was filtered and dried.

2-(2-Phenylhydrazono)malonimidamide dihydrochloride (3a). Following the **General procedure A**, the reaction was performed with aniline (536 mg, 5.76 mmol) to afford **3a** as a yellow solid (1.30 g, 82%); mp 224-228 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.86 (s, 1HN), 9.99 (s, 2HN), 9.66 (s, 2HN), 9.26 (s, 2HN), 8.86 (s, 2HN), 7.67 (d, *J* 7.9 Hz, 2H), 7.36 (t, *J* 8.1 Hz, 2H), 7.08 (t, *J* 7.9 Hz, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 158.7, 156.0, 142.3, 129.1, 124.0, 117.0, 115.8; HRMS (HESI, m/z) calcd for C₉H₁₂N₆·2HCl (276.06) [M-2HCl+H]⁺ 205.1196, found 205.1197.

2-(2-(4-Bromophenyl)hydrazono)malonimidamide dihydrochloride (3b). Following the **General procedure A**, the reaction was performed with 4-bromoaniline (990 mg, 5.76 mmol) to afford **3b** as a yellow solid (1.65 g, 81%); mp 207-208°C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.95 (s, 1HN), 9.97 (s, 2HN), 9.66 (s, 2HN), 9.27 (s, 2HN), 8.92 (s, 2HN), 7.66 (d, *J* 8.8 Hz, 2H), 7.52 (d, *J* 9.2 Hz, 2H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 158.5, 155.8, 141.7, 131.8, 118.0, 117.8, 115.8; HRMS (m/z) calcd for C₉H₁₁N₆Br·2HCl (353.97) [M-2HCl+H]⁺ 283.0301 and 285.0286, found 283.0302 and 285.0278.

2-(2-(4-Nitrophenyl)hydrazono)malonimidamide dihydrochloride (3c). Following the **General procedure A**, the reaction was performed with 4-nitroaniline (795 mg, 5.76 mmol) to afford **3c** as a red solid (1.44 g, 78%); mp 280-282°C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.34 (s, 1HN), 10.09 (s, 2HN), 9.72 (s, 2HN), 9.43 (s, 2HN), 9.14 (s, 2HN), 8.22 (d, *J* 9.2 Hz, 2H), 7.90 (d, *J* 9.6 Hz, 2H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 158.2, 155.4, 148.0, 142.6, 125.3, 121.6, 115.8; HRMS (m/z) calcd for C₉H₁₁N₇O₂·2HCl (321.05) [M-2HCl+H]⁺ 250.1047, found 250.1048.

General procedure B. 8-Amino-2-arylpyrimido[4,5-*e*][1,2,4]triazine-3,6(2*H*,5*H*)-diones (4). The corresponding 2-arylhydrazonomalonimidamide dihydrochloride **3** (0.46 mmol), CDI (302 mg; 1.86 mmol) and DIPEA (323 μ L; 1.86 mmol) were dissolved in DMF (2 mL). The reaction mixture was stirred at room temperature for 12 h. After that the reaction mixture was adjusted with acetic acid (0.5 mL) and 35% HCl (0.4 mL) and stirred for 10

minutes. Then ethanol (1 mL) and ether (12 mL) were added to the solution, when compound **4** started to crystallize upon cooling. The product was filtered off, washed with water and dried at 95 °C.

8-Amino-2-phenylpyrimido[4,5-*e*][1,2,4]triazine-3,6(2*H*,5*H*)-dione (4a). Following the **General procedure B**, the reaction was performed with **3a** (127 mg, 0.46 mmol) to afford **4a** as a yellow solid (76.5 mg, 65%); mp above 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.60 (brs, 1H), 8.45 (brs, 1H), 8.28 (brs, 1H), 7.67 (d, *J* 7.8 Hz, 2H), 7.53 (t, *J* 7.6 Hz, 2H), 7.46 (t, *J* 7.3 Hz, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 160.5, 155.5, 154.7, 152.6, 141.0, 128.6, 128.5, 125.6, 120.4; HRMS (*m/z*) calcd for C₁₁H₈N₆O₂ (256.07) [M+H]⁺ 257.0781, found 257.0776.

8-Amino-2-(4-bromophenyl)pyrimido[4,5-*e*][1,2,4]triazine-3,6(2*H*,5*H*)-dione (4b). Following the **General procedure B**, the reaction was performed with **3b** (163 mg, 0.46 mmol) to afford **4b** as a yellow solid (115 mg, 75%); mp above 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm; 11.64 (brs, 1H), 8.47 (brs, 1H), 8.31 (brs, 1H), 7.71 (d, *J* 9.3 Hz, 2H), 7.66 (d, *J* 8.8 Hz, 2H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 160.5, 155.5, 154.7, 152.4, 140.2, 131.5, 127.6, 121.4, 120.7; HRMS (*m/z*) calcd for C₁₁H₇N₆O₂Br (333.98) [M+H]⁺ 334.9887 and 336.9872, found 334.9888 and 336.9865.

8-Amino-2-(4-nitrophenyl)pyrimido[4,5-*e*][1,2,4]triazine-3,6(2*H*,5*H*)-dione (4c). Following the **General procedure B**, the reaction was performed with **3c** (148 mg, 0.46 mmol) to afford **4c** as a yellow solid (58.3 mg, 42%); mp above 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.73 (brs, 1NH), 8.56 (s, 1NH), 8.44 (s, 1NH), 8.37 (d, *J* 9.3 Hz, 2H), 8.05 (d, *J* 8.8 Hz, 2H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 160.3, 155.4, 154.7, 152.2, 146.4, 145.7, 126.2, 124.0, 121.4; HRMS (*m/z*) calcd for C₁₁H₇N₇O₄ (301.05) [M+H]⁺ 302.0632, found 302.0628.

General procedure C. 3-Amino-3-(*N*-substituted)-2-(phenyldiazenyl)acrylonitriles (6). A mixture of 2-phenylhydrazonomalononitrile (0.75 g, 4.4 mmol) and corresponding amine (0.5 mol) in 50 mL ethanol was heated with stirring at 70 °C for 10 h. Then the reaction mixture was poured onto crushed ice. The precipitated product was filtered off and washed with water. A sample for analysis was prepared by crystallization from a mixture of ethanol/water.

3-Amino-3-(4-methylpiperazin-1-yl)-2-(phenyldiazenyl)acrylonitrile (6d). Following the **General procedure C**, the reaction was performed with 1-methylpiperazine (55 mL, 0.5 mol) to afford **6d** as a yellow solid (899 mg, 76%); mp 168-169 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.66 (s, 2NH), 7.48 (d, *J* 7.3 Hz, 2H), 7.33 (t, *J* 7.8 Hz, 2H), 7.09 (t, *J* 7.3 Hz, 1H), 3.58 (t, *J* 4.8 Hz, 4H), 2.41 (t, *J* 4.8 Hz, 4H), 2.21 (s, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 162.3; 153.5, 128.6, 125.5, 120.4, 117.0, 92.7, 54.4, 48.2, 45.5; HRMS (HESI, *m/z*) calcd for C₁₄H₁₈N₆ (270.16) [M+H]⁺ 271.1666, found 271.1669.

3-Amino-3-morpholino-2-(phenyldiazenyl)acrylonitrile (6e). Following the **General procedure C**, the reaction was performed with morpholine (44 mL, 0.5 mol) to afford **6e** as a yellow solid (781 mg, 69%); mp 172-173 °C (174-177 °C²¹); ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.70 (s, 2NH), 7.50 (d, *J* 7.8 Hz, 2H), 7.32 (t, *J* 7.8 Hz, 2H), 7.12 (t, *J* 7.2 Hz, 1H), 3.70-3.68 (m, 4H), 3.60-3.59 (m, 4H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 162.4, 153.5, 128.7, 125.6, 120.5, 117.0, 92.8, 66.0, 48.8; HRMS (HESI, *m/z*) calcd for C₁₃H₁₅N₅O (257.12) [M+H]⁺ 258.1349, found 258.1351.

3-Amino-3-(piperidin-1-yl)-2-(phenyldiazenyl)acrylonitrile (6f). Following the **General procedure C**, the reaction was performed with piperidine (49 mL, 0.5 mol) to afford **6f** as a yellow solid (890 mg, 79%); mp 159-161 °C (165-167 °C²¹); ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.57 (s, 2NH), 7.47 (d, *J* 7.3 Hz, 2H), 7.31 (t, *J* 7.8 Hz, 2H), 7.08 (t, *J* 7.1 Hz, 1H), 3.55 (brs, 4H), 1.63 (brs, 6H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 162.2, 153.6, 128.6, 125.3, 120.4, 117.0, 93.7, 49.3, 25.6, 23.6; HRMS (HESI, *m/z*) calcd for C₁₄H₁₇N₅ (255.15) [M+H]⁺ 256.1557, found 256.1564.

3-Amino-3-(diethylamino)-2-(phenyldiazenyl)acrylonitrile (6g). Following the **General procedure C**, the reaction was performed with diethylamine (52 mL, 0.5 mol) to afford **6g** as a yellow solid (674 mg, 63%); mp 128-130 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.48 (d, *J* 6.9 Hz, 2H), 7.42 (s, 2NH), 7.29 (t, *J* 7.8 Hz, 2H), 7.09 (t, *J*

7.3 Hz, 1H), 3.56 (q, *J* 7.0 Hz, 4H), 1.20 (t, *J* 7.1 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-*d*₆) δ 160.6, 153.7, 128.7, 125.2, 120.4, 117.1, 92.1, 44.1, 13.1; HRMS (HESI, *m/z*) calcd for C₁₃H₁₇N₅ (243.14) [M+H]⁺ 244.1557, found 244.1558.

3-Amino-3-(dipropylamino)-2-(phenyldiazenyl)acrylonitrile (6h). Following the **General procedure C**, the reaction was performed with dipropylamine (68 mL, 0.5 mol) to afford **6h** as a yellow solid (758 mg, 63%); mp 120-124 °C; ^1H NMR (400 MHz, DMSO-*d*₆) δ 7.49 (d, *J* 7.8 Hz, 2H), 7.45 (s, 2NH), 7.30 (t, *J* 7.8 Hz, 2H), 7.10 (t, *J* 7.1 Hz, 1H), 3.46 (t, *J* 7.8 Hz, 4H), 1.65 (td, *J* 14.9 Hz, 7.3 Hz, 4H), 0.86 (t, *J* 7.1 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-*d*₆) δ 161.4, 153.6, 128.6, 125.3, 120.4, 117., 92.4, 51.4, 20.5, 10.7; HRMS (HESI, *m/z*) calcd for C₁₅H₂₁N₅ (271.18) [M+H]⁺ 272.1870, found 272.1872.

General procedure D. 3-Oxo-2-phenyl-5-(N-substituted)-2,3-dihydro-1,2,4-triazine-6-carbonitriles (7). The corresponding 2-(phenyldiazenyl)acrylonitrile **6** (0.75 mmol) and CDI (243 mg, 1.5 mmol) were dissolved in dry DMF (2 mL) and stirred at 50 °C for 12 h. The reaction mixture was diluted with water (20 mL) and stirred at room temperature for 30 min. The resulting precipitate was filtered off, washed with water, and dried. A sample for analysis was prepared by crystallization from a mixture of ethanol/water.

5-(4-Methylpiperazin-1-yl)-3-oxo-2-phenyl-2,3-dihydro-1,2,4-triazine-6-carbonitrile (7d). Following the **General procedure D**, the reaction was performed with **6d** (203 mg, 0.75 mmol) to afford **7d** as a white solid (114 mg, 51%); mp 102-104 °C; ^1H NMR (400 MHz, DMSO-*d*₆) δ 7.53-7.43 (m, 5H), 3.90 (brs, 4H), 2.46 (brs, 4H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*₆) δ 154.3, 150.0, 140.7, 129.3, 128.8, 125.4, 115.0, 112.0, 54.1, 48.8, 45.5; HRMS (HESI, *m/z*) calcd for C₁₄H₁₃N₅O₂ (296.13) [M+H]⁺ 297.1458, found 297.1457.

5-Morpholino-3-oxo-2-phenyl-2,3-dihydro-1,2,4-triazine-6-carbonitrile (7e). Following the **General procedure D**, the reaction was performed with **6e** (193 mg, 0.75 mmol) to afford **7e** as a beige solid (175 mg, 82%); mp 116-118 °C; ^1H NMR (400 MHz, DMSO-*d*₆) δ 7.54-7.43 (m, 5H), 3.93 (t, *J* 4.6 Hz, 4H), 3.74 (t, *J* 5.0 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-*d*₆) δ 154.1, 149.8, 140.4, 128.8, 128.6, 125.2, 114.8, 111.9, 65.6, 46.1; HRMS (HESI, *m/z*) calcd for C₁₄H₁₃N₅O₂ (283.11) [M+H]⁺ 284.1142, found 284.1139.

3-Oxo-2-phenyl-5-(piperidin-1-yl)-2,3-dihydro-1,2,4-triazine-6-carbonitrile (7f). Following the **General procedure D**, the reaction was performed with **6f** (192 mg, 0.75 mmol) to afford **7f** as a white solid (156 mg, 74%); mp 134-136 °C; ^1H NMR (400 MHz, DMSO-*d*₆) δ 7.53-7.41 (m, 5H), 3.88 (brss, 4H), 1.67 (brs, 6H); ^{13}C NMR (100 MHz, DMSO-*d*₆) δ 153.6, 149.8, 140.5, 128.7, 128.4, 125.2, 114.9, 111.7, 47.1, 25.4, 23.4; HRMS (HESI, *m/z*) calcd for C₁₅H₁₅N₅O (281.13) [M+H]⁺ 282.1349, found 282.1341.

5-(Diethylamino)-3-oxo-2-phenyl-2,3-dihydro-1,2,4-triazine-6-carbonitrile (7g). Following the **General procedure D**, the reaction was performed with **6g** (183 mg, 0.75 mmol) to afford **7g** as a white solid (145 mg, 72%); mp 120-122 °C; ^1H NMR (400 MHz, DMSO-*d*₆) δ 7.53-7.41 (m, 5H), 3.72 (brs, 4H), 1.25 (brs, 6H); ^{13}C NMR (100 MHz, DMSO-*d*₆) δ 152.9, 149.5, 140.5, 128.7, 128.5, 125.2, 115.0, 111.0, 43.6; 13.5 (brs); HRMS (HESI, *m/z*) calcd for C₁₄H₁₅N₅O (269.13) [M+H]⁺ 270.1349, found 270.1352.

5-(Dipropylamino)-3-oxo-2-phenyl-2,3-dihydro-1,2,4-triazine-6-carbonitrile (7h). Following the **General procedure D**, the reaction was performed with **6h** (204 mg, 0.75 mmol) to afford **7h** as a white solid (164 mg, 74%); mp 78-80 °C; ^1H NMR (400 MHz, DMSO-*d*₆) δ 7.51-7.40 (m, 5H), 3.60 (brs, 4H), 1.67 (brs, 4H), 0.89 (t, *J* 7.3 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-*d*₆) δ 153.1, 149.4, 140.6, 128.7, 128.5, 125.2, 115.1, 111.0, 50.8, 22.1 (brs), 10.6 (brs); HRMS (HESI, *m/z*) calcd for C₁₆H₁₉N₅O (297.16) [M+H]⁺ 298.1662, found 298.1659.

General procedure E. 3-Substituted-3-imino-2-(2-phenylhydrazono)alkanenitriles (8). The corresponding commercially available Grignard reagent was slowly added dropwise into the solution of 2-phenylhydrazonomalononitrile (0.25 g, 1.46 mmol) in dry Et₂O (10 mL). The reaction mixture was stirred at room temperature for 18 h. The reaction was quenched with saturated solution of ammonium chloride (15 mL) and extracted with ethyl acetate (3 x 5 mL). The organic phase was washed with brine, dried over MgSO₄

and concentrated under reduced pressure. The crude product was crystallized from a mixture of hexane/DCM. NMR spectra of compounds **8** are given as a mixture of isomers; for integral intensity of protons see Supplementary material. Due to dynamic equilibrium not all carbon atoms are apparent from CDCl₃ spectra.

3-Imino-2-(2-phenylhydrazono)pentanenitrile (8i). Following the **General procedure E**, the reaction was performed with 2.0 M solution of ethylmagnesium chloride (4.4 mL, 8.8 mmol) to afford **8i** as a yellow solid (225 mg, 77%); mp 108-110 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.76 (brs, NH), 8.70 (s, NH), 8.33 (s, NH) 8.11 (s, NH), 7.72 (d, *J* 7.3 Hz, arom), 7.50 (d, *J* 7.3 Hz, arom), 7.40 (t, *J* 7.8 Hz, arom), 7.23-7.31 (m, arom), 2.86 (q, *J* 7.5 Hz, CH₂), 2.50 (q, *J* 7.6 Hz, CH₂), 1.26 (t, *J* 7.7 Hz, CH₃), 1.22 (t, *J* 7.6 Hz, CH₃); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 173.0, 169.5, 152.8, 152.5, 129.0, 128.9, 128.1, 127.5, 121.6, 120.8, 117.7, 114.2, 103.0, 102.2, 26.5, 24.5, 13.4, 12.7; ¹H NMR (400 MHz, CDCl₃) δ 8 – 9 (brs, NH), 7.64 (d, *J* 8.5 Hz, arom), 7.41 (d, *J* 8.4 Hz, arom), 7.32 (t, *J* 8.4 Hz, arom), 5.69 (brs, NH₂), 3.03 (q, *J* 7.6 Hz, CH₂), 2.70 (q, *J* 7.6 Hz, CH₂), 1.37 (t, *J* 7.6 Hz, CH₃), 1.37 (t, *J* 7.6 Hz, CH₃); HRMS (HESI, *m/z*) calcd for C₁₁H₁₂N₄ (200.11) [M+H]⁺ 201.1135, found 201.1136.

3-Imino-2-(2-phenylhydrazono)hexanenitrile (8j). Following the **General procedure E**, the reaction was performed with 1.0 M solution of *n*-propylmagnesium chloride (8.8 mL, 8.8 mmol) to afford **8j** as a yellow solid (212 mg, 68%); mp 56-57 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.76 (brs, NH), 8.69 (s, NH), 8.32 (s, NH) 8.11 (s, NH), 7.72 (d, *J* 7.4 Hz, arom), 7.50 (d, *J* 7.3 Hz, arom), 7.41 (t, *J* 7.8 Hz, arom), 7.31-7.23 (m, arom), 2.84 (q, *J* 7.4 Hz, CH₂), 2.52 (q, *J* 7.5 Hz, CH₂), 1.73-1.69 (m, 2 x CH₂) 0.98 (t, *J* 7.7 Hz, CH₃), 0.92 (t, *J* 7.6 Hz, CH₃); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 171.5, 168.0, 152.8, 152.5, 129.1, 128.9, 128.1, 127.5, 121.7, 120.8, 115.8, 114.2, 103.6, 102.9, 35.2, 32.6, 22.0, 21.4, 13.6, 13.5; ¹H NMR (400 MHz, CDCl₃) δ 8 – 9 (brs, NH), 7.66 (d, *J* 8.5 Hz, arom), 7.40 (d, *J* 8.4 Hz, arom), 7.32 (t, *J* 8.4 Hz, arom), 5.57 (brs, NH₂), 2.98 (q, *J* 7.6 Hz, CH₂), 2.65 (q, *J* 7.6 Hz, CH₂), 1.84-1.78 (m, 2 x CH₂), 1.09 (t, *J* 7.6 Hz, CH₃), 1.05 (t, *J* 7.6 Hz, CH₃); HRMS (HESI, *m/z*) calcd for C₁₂H₁₄N₄ (214.12) [M+H]⁺ 215.1291, found 215.1293.

3-Imino-4-methyl-2-(2-phenylhydrazono)pentanenitrile (8k). Following the **General procedure E**, the reaction was performed with 2.0 M solution of isopropylmagnesium chloride (4.4 mL, 8.8 mmol) to afford **8k** as a yellow solid (150 mg, 48%); mp 102-104 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.74 (brs, NH), 8.41 (s, NH), 8.12 (s, NH) 8.01 (s, NH), 7.49 (d, *J* 8.4 Hz, arom), 7.45-7.38 (m, arom), 7.29 (t, *J* 7.6 Hz, arom), 7.25 (t, *J* 7.6 Hz, arom), 4.00 (heptaplet, *J* 7.2 Hz, CH), 3.07 (heptaplet, *J* 7.2 Hz, CH), 1.29 (d, *J* 6.8 Hz, CH₃), 1.23 (d, *J* 6.8 Hz, CH₃); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 175.9, 172.8, 152.7, 152.5, 129.0, 128.9, 128.1, 127.5, 121.7, 120.8, 115.5, 114.2, 103.1, 101.8, 32.1, 28.7, 20.0, 19.9; HRMS (HESI, *m/z*) calcd for C₁₂H₁₄N₄ (214.12) [M+H]⁺ 215.1291, found 215.1292.

3-Imino-2-(2-phenylhydrazono)heptanenitrile (8l). Following the **General procedure E**, the reaction was performed with 2.0 M solution of *n*-butylmagnesium chloride (4.4 mL, 8.8 mmol) to afford **8l** as a yellow solid (223 mg, 67%); mp 78-80 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.75 (brs, NH), 8.68 (s, NH), 8.31 (s, NH) 8.01 (s, NH), 7.73 (d, *J* 8.4 Hz, arom), 7.50 (d, *J* 7.8 Hz, arom), 7.41 (t, *J* 8.2 Hz, arom), 7.28 (t, *J* 7.6 Hz, arom), 7.28 (t, *J* 7.6 Hz, arom), 2.86 (t, *J* 7.6 Hz, CH₂), 2.56 (t, *J* 7.6 Hz, CH₂), 1.71-1.61 (m, 2 x CH₂), 1.43-1.31 (m, 2 x CH₂), 0.92 (t, *J* 7.6 Hz, CH₃), 0.89 (t, *J* 6.8 Hz, CH₃); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 171.8, 168.3, 152.7, 152.5, 129.0, 128.9, 128.1, 127.5, 121.6, 120.8, 115.7, 114.1, 103.6, 102.7, 32.6, 30.6, 30.4, 30.1, 21.7, 14.5; HRMS (HESI, *m/z*) calcd for C₁₃H₁₆N₄ (228.14) [M+H]⁺ 229.1448, found 229.1449.

3-Cyclopentyl-3-imino-2-(2-phenylhydrazono)propanenitrile (8m). Following the **General procedure E**, the reaction was performed with 2.0 M solution of cyclopentylmagnesium chloride (4.4 mL, 8.8 mmol) to afford **8m** as a yellow solid (266 mg, 76%); mp 202-206 °C; ¹H NMR (400 MHz, CDCl₃) δ 8 – 9 (brs, NH), 7.66 (d, *J* 8.5 Hz, arom), 7.40 (d, *J* 8.4 Hz, arom), 7.31 (t, *J* 8.4 Hz, arom), 5.54 (brs, NH₂), 4.16 (quintet, *J* 9.2 Hz, CH), 3.30 (quintet, *J* 9.2 Hz, CH), 2.20-2.16 (m, CH₂), 1.85-1.78 (m, CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 171.2, 167.8, 167.6,

152.9, 152.8, 152.3, 129.0, 128.9, 128.7, 121.8, 121.6, 116.3, 105.8, 43.1, 39.8, 32.0, 31.7, 26.0, 25.9; HRMS (HESI, m/z) calcd for $C_{14}H_{16}N_4$ (240.13) $[M+H]^+$ 241.1448, found 241.1449.

3-Imino-4-phenyl-2-(2-phenylhydrazono)butanenitrile (8n). Following the **General procedure E**, the reaction was performed with 1.0 M solution of benzylmagnesium chloride (8.8 mL, 8.8 mmol) to afford **8n** as a yellow solid (279 mg, 73%); mp 86-88 °C; 1H NMR (400 MHz, $CDCl_3$) δ 8 – 9 (brs, NH), 7.72 (d, J 8.2 Hz, arom), 7.63 (d, J 8.2 Hz, arom), 7.42-7.30 (m, arom), 5.49 (brs, NH_2), 4.41 (s, CH_2), 4.03 (s, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.7, 152.1, 134.7, 133.5, 129.4, 129.3, 129.2, 129.1, 128.9, 128.8, 128.1, 127.8, 121.9, 121.5, 113.8, 106.0, 39.3, 36.4; HRMS (HESI, m/z) calcd for $C_{16}H_{14}N_4$ (262.12) $[M+H]^+$ 263.1291, found 263.1293.

3-Imino-4,4-dimethyl-2-(2-phenylhydrazono)pentanenitrile (8p). Following the **General procedure E**, the reaction was performed with 1.0 M solution of *tert*-butylmagnesium chloride (8.8 mL, 8.8 mmol) to afford **8n** as a yellow solid (173 mg, 52%); mp 102-106 °C; 1H NMR (400 MHz, $DMSO-d_6$) δ 8.79 (brs, NH), 8.01 (s, NH), 7.96 (s, NH), 7.74 (d, 8.8 Hz, arom), 7.67 (brs, NH), 7.48 (d, J 8.8 Hz, arom), 7.41 (t, J 7.6 Hz, arom), 7.28 (t, J 7.6 Hz, arom), 1.43 (s, *tert*-butyl CH_3); ^{13}C NMR (100 MHz, $DMSO-d_6$) δ 176.6, 175.6, 152.8, 152.5, 129.1, 128.8, 127.9, 127.4, 121.7, 120.8, 116.8, 114.6, 103.9, 101.6, 38.8, 36.1, 29.4, 27.8; 1H NMR (100 MHz, $CDCl_3$) δ 8-9 (brs, NH), 7.61 (d, J 7.6 Hz, arom), 7.39 (t, J 7.6 Hz, arom), 7.30 (t, J 7.6 Hz, arom), 5.75 (brs, NH), 1.52 (s, *tert*-butyl CH_3); HRMS (HESI, m/z) calcd for $C_{13}H_{16}N_4$ (228.13) $[M+H]^+$ 229.1449, found 229.1451.

General procedure F. 5-Alkylidene-3-oxo-2-phenyl-2,3,4,5-tetrahydro-1,2,4-triazine-6-carbonitriles (10). The corresponding 3-substituted-3-imino-2-(2-phenylhydrazono)propanenitrile **8** (0.5 mmol) and CDI (142 mg, 0.87 mmol) were dissolved in dry DCM (2 mL) and stirred at 40 °C for 2 h. The reaction mixture was diluted with DCM (10 mL) and extracted with water (2 x 5 mL). Organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to a half its volume. After adding of hexane (15 mL) and stirring for 15 min, the resulting precipitate was filtered off, washed with hexane and dried. A sample for analysis was prepared by crystallization from ethanol.

5-Ethylidene-3-oxo-2-phenyl-2,3,4,5-tetrahydro-1,2,4-triazine-6-carbonitrile (10i). Following the **General procedure F**, the reaction was performed with **8i** (100 mg, 0.5 mmol) to afford **10i** as a yellow solid (81.3 mg, 72%); mp 242-246 °C; 1H NMR (400 MHz, $DMSO-d_6$) δ 10.27 (brs, 1NH), 7.43-7.37 (m, 4H), 7.35-7.31 (m, 1H), 5.00 (q, J 7.6 Hz, 1H), 1.72 (d, J 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $DMSO-d_6$) δ 145.7, 139.9, 128.5, 128.4, 127.3, 125.3, 123.8, 112.8, 103.2, 10.6; HRMS (HESI, m/z) calcd for $C_{12}H_{10}N_4O$ (226.08) $[M-H]^-$ 225.0782, found 225.0762.

3-Oxo-2-phenyl-5-propylidene-2,3,4,5-tetrahydro-1,2,4-triazine-6-carbonitrile (10j). Following the **General procedure F**, the reaction was performed with **8j** (107 mg, 0.5 mmol) to afford **10j** as a yellow solid (84.5 mg, 70%); mp 190-193 °C; 1H NMR (400 MHz, $DMSO-d_6$) δ 10.29 (brs, 1NH), 7.43-7.36 (m, 4H), 7.35-7.31 (m, 1H), 4.90 (t, J 7.8 Hz, 1H), 2.16 (q, J 7.6 Hz, 2H), 0.97 (t, J 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $DMSO-d_6$) δ 145.8, 139.9, 128.5, 127.4, 127.2, 125.4, 123.8, 112.9, 110.1, 18.0, 13.4; HRMS (HESI, m/z) calcd for $C_{13}H_{12}N_4O$ (240.10) $[M-H]^-$ 239.0938, found 239.0930.

3-Oxo-2-phenyl-5-(propan-2-ylidene)-2,3,4,5-tetrahydro-1,2,4-triazine-6-carbonitrile (10k). Following the **General procedure F**, the reaction was performed with **8k** (107 mg, 0.5 mmol) to afford **10k** as an orange solid (85.5 mg, 71 %); mp 174-178 °C; 1H NMR (400 MHz, $DMSO-d_6$) δ 9.75 (brs, 1NH), 7.42-7.38 (m, 4H), 7.35-7.31 (m, 1H), 2.06 (s, 3H), 1.80 (s, 3H); ^{13}C NMR (100 MHz, $DMSO-d_6$) δ 147.4, 140.0, 128.5, 127.2, 125.0, 121.3, 119.9, 116.8, 115.2, 20.0, 19.2; HRMS (HESI, m/z) calcd for $C_{13}H_{12}N_4O$ (240.10) $[M-H]^-$ 239.0938, found 239.0927.

5-Butylidene-3-oxo-2-phenyl-2,3,4,5-tetrahydro-1,2,4-triazine-6-carbonitrile (10l). Following the **General procedure F**, the reaction was performed with **8l** (127 mg, 0.5 mmol) to afford **10l** as a yellow solid (78.6 mg, 62%); mp 162-166 °C; 1H NMR (400 MHz, $DMSO-d_6$) δ 10.33 (brs, 1NH), 7.45-7.39 (m, 4H), 7.35-7.31 (m, 1H),

4.92 (t, J 7.8 Hz, 1H), 2.15 (q, J 7.6 Hz, 2H), 1.39 (hex, J 7.4 Hz, 2H), 0.90 (t, J 7.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 145.7, 139.9, 128.5, 127.8, 127.4, 125.4, 123.8, 112.8, 108.4, 26.3, 21.7, 13.5; HRMS (HESI, m/z) calcd for $\text{C}_{14}\text{H}_{14}\text{N}_4\text{O}$ (254.11) $[\text{M}-\text{H}]^-$ 253.1095, found 253.1079.

5-Cyclopentylidene-3-oxo-2-phenyl-2,3-dihydro-1,2,4-triazine-6-carbonitrile (10m). Following the **General procedure F**, the reaction was performed with **8m** (120 mg, 0.5 mmol) to afford **10m** as a yellow solid (46.6 mg, 35%); mp 152-155 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 9.63 (brs, 1H), 7.42-7.35 (m, 4H), 7.34-7.30 (m, 1H), 2.64 (t, J 6.9 Hz, 2H), 2.31 (t, J 7.1 Hz, 2H), 1.73-1.74 (m, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 146.5, 139.9, 128.5, 127.2, 126.2, 125.1, 121.9, 118.4, 114.8, 31.1, 29.7, 27.0, 25.3; HRMS (HESI, m/z) calcd for $\text{C}_{15}\text{H}_{14}\text{N}_4\text{O}$ (266.11) $[\text{M}-\text{H}]^-$ 265.1095, found 265.1080.

5-Benzylidene-3-oxo-2-phenyl-2,3-dihydro-1,2,4-triazine-6-carbonitrile (10n). Following the **General procedure F**, the reaction was performed with **8n** (131 mg, 0.5 mmol) to afford **10n** as a yellow solid (57.8 mg, 40%); mp 196-200 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 10.60 (brs, 1H), 7.54-7.28 (m, 10H), 5.89 (s, 1H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 145.4, 139.9, 132.9, 128.6, 128.5, 127.7, 127.5, 127.2, 125.3, 124.2, 112.9, 106.6; HRMS (HESI, m/z) calcd for $\text{C}_{17}\text{H}_{12}\text{N}_4\text{O}$ (288.10) $[\text{M}-\text{H}]^-$ 287.0938, found 287.0921.

Acknowledgements

This work was supported by the Czech National Program for Sustainability (project LO1304), internal faculty project IGA (IGA_PrF_2017_009) and OP PIK project (CZ.01.1.02/0.0/0.0/15_019/0004431).

Supplementary Material

NMR spectra of new compounds are available as Supplementary material.

References

- For previous paper see: Stýskala J.; Slouka, J.; Švecová, V. *Arkivoc* **2006**, (i), 68.
<http://dx.doi.org/10.3998/ark.5550190.0007.107>
- Kumar, R.; Sirohi, T.S.; Singh, H.; Yadav, R.; Roy, R.K.; Chaudhary, A.; Pandeya, S.N. *Mini. Rev. Med. Chem.* **2014**, *14*, 168.
<http://dx.doi.org/10.2174/1389557514666140131111837>
- Arshad, M.; Khan, T. A.; Khan, M. A. *International Journal of Pharma Sciences and Research* **2014**, *5*, 149.
- Abdel-Rahman, R.M.; Seada, M.; Fawzy, M.; El-Edfawy, S. *Pharmazie* **1994**, *49*, 811.
- Abdel-Rahman, R.M.; Morsy, J.M.; El-Edfawy, S. *Pharmazie* **1999**, *54*, 667.
- Abdel-Rahman, R.M.; Morsy, J.M.; Hanafy, F. *Pharmazie* **1999**, *54*, 347.
- Shchegol'kov, E.V.; Khudina, O.G.; Ivanova, A.E.; Burgart, Y.V.; Sadchikova, E.V.; Kravchenko, E.V.; Saloutin, V.I. *Pharm. Chem. J.* **2014**, *48*, 383.
<http://dx.doi.org/10.1007/s11094-014-1115-6>
- Wamhoff, H.; Tzanova, M. *Coll. Czech. Chem. Commun.* **2003**, *68*, 965.
<http://dx.doi.org/10.1135/cccc20030965>

9. Wamhoff, H.; Tzanova, M. *Arkivoc* **2003**, (ii), 98.
<http://dx.doi.org/10.3998/ark.5550190.0004.212>
10. Kuchler, C.; Kuchler, W.; Heinisch, L. *Arzneim. Forsch.* **1966**, 16, 1122.
11. Taylor, E.C.; Morrison, R.W. *J. Am. Chem. Soc.* **1965**, 87, 1976.
<http://dx.doi.org/10.1021/ja01087a021>
12. Slouka, J. *Monatsh. Chem.* **1963**, 94, 258.
<http://dx.doi.org/10.1007/BF00900246>
13. Slouka, J. *Coll. Czech. Chem. Commun.* **1990**, 55, 2967.
<http://dx.doi.org/10.1135/cccc19902967>
14. Bílek, P.; Slouka, J. *J. Heter. Chem.* **2002**, 39, 357.
<http://dx.doi.org/10.1002/jhet.5570390219>
15. Yoneda, F.; Higuchi, M. *J. Heterocyclic. Chem.* **1980**, 17, 1365.
<http://dx.doi.org/10.1002/jhet.5570170702>
16. Akalay, D.; Düerner, G.; Bats, J.W.; Bolte, M.; Güebel, M.W. *J. Org. Chem.* **2007**, 72, 5618.
<http://dx.doi.org/10.1021/jo070534j>
17. Shaw, E. *J. Biol. Chem.*, **1950**, 185, 439.
18. Richter, E.; Taylor, E.C. *J. Am. Chem. Soc.* **1956**, 78, 5848.
<http://dx.doi.org/10.1021/ja01603a038>
19. Krystof, V.; Cankar, P.; Frysová, I.; Slouka, J.; Kontopidis, G.; Dzubák, P.; Hajdúch, M.; Srovnal, J.; de Azevedo W.F. Jr; Orság M.; Paprskárová, M.; J. Rolcík, Látr, A.; Fischer, P.; Strnad, M. *J. Med. Chem.* **2006**, **49**, 6500.
<http://dx.doi.org/10.1021/jm0605740>
20. Belskaya, N.P.; Demina, M.A.; Sapognikova, S.G.; Fan, Z.; Zhang, H.; Dehaen, W.; Bakulev, V.A. *Arkivoc* **2008**, (xvi), 9.
<http://dx.doi.org/10.3998/ark.5550190.0009.g02>
21. Schaefer, H.; Gewald, K.; Bellmann, P.; Gruner, M. *Monatsh. Chem.* **1991**, 122, 195.
<http://dx.doi.org/10.1007/BF00809365>
22. Jacq, J.; Pasau, P. *Chem. Eur. J.* **2014**, 20, 12223.
<http://dx.doi.org/10.1002/chem.201402074>
23. Sulo, P.; Šturdík, E.; Liptaj, T.; Jakubík, T.; Antalík, M. *Coll. Czech. Chem. Commun.* **1985**, 50, 375.
<http://dx.doi.org/10.1135/cccc19850375>
24. Slouka, J.; Švecová, V. *Acta Universitatis Palackianae Olomucensis, Facultas Rerum Naturalium* **1973**, 41, 143.
25. Abdel-Motaleb, R.M.; Makhloof, A.A.; Ibrahim, H.M.; Elnagdi, M.H. *J. Heter. Chem.* **2006**, 43, 931.
<http://dx.doi.org/10.1002/jhet.5570430418>
26. Hajdúch, M.; Mihál, V.; Minařík, J.; Faber, E.; Šafářová, M.; Weigl, E.; *Cytotechnology* **1996**, 19, 243.
<http://dx.doi.org/10.1007/BF00744219>