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# Synthesis and biological action of 5-oxo-1,2,4-triazine derivatives

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In this study, the biological activity of the 5-oxo-1,2,4-triazine derivatives **12**–**17** obtained in a reaction of $N^3$-substituted amidrazones **1**–**6** with dimethyl acethylenedicarboxylate (DMAD) has been examined.

## 1. Introduction

In the previously reported reactions of cyclic amidrazones (containing amide type nitrogen build-up into the ring) DMAD gives the derivatives of 5-hydroxy-1,2-pyrrole and 5-oxo-1,2,4-triazine [1–3]. Depending on the type of substituent these compounds show different biological e.g. fungi- and bacteriostatic activity [4, 5].

## 2. Investigations, results and discussion

### *2.1. Synthesis*

The reaction of the $N^3$-substituted amidrazones **1**–**5** [3, 6, 7] with DMAD in absolute ethanol at the temperature of −10 °C led to the formation of derivatives of dimethyl 2-[(1-aryl-amino-1-arylmethylidene)hydrazono]succinate **7**–**11**. $^1$H NMR spectra of compounds **7**–**11** revealed the presence of single proton signals of NH hydrogen atom at 9.7–9.9 ppm. The high value of their chemical shift suggests that they are the hydrogen atoms of the amidrazon amide moiety. Exceptional behavior was observed in the case of amidrazone (**6**), which gave product **17** under the above-mentioned conditions.

Cyclization of compounds **7**–**11** carried out in methanol solution in the presence of triethylamine led to the formation of methyl 2-(5-oxo-3,4-diaryl-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene)-acetates **12**–**16**. $^1$H NMR spectra of compounds **12**–**16** revealed single proton signals of a NH hydrogen atom at 10.9–11.6 ppm. The higher value of their chemical shift in comparison to the respective chain derivatives **7**–**11** was probably caused by an anisotropic effect of two neighboring double N=C and C=C bonds and could suggest rigid, cyclic structure. In the IR spectra of compounds **12**–**16** the presence of absorption bands at 1680–1735 $cm^{-1}$, attainable to the CO group of the amide moiety confirmed as well the formation of cyclic products. The analysis of MS and $^1$H NMR spectra shows that the formation of cyclic compounds is accompanied by liberation of a methanol molecule.

The course of reaction is presented in the Scheme.

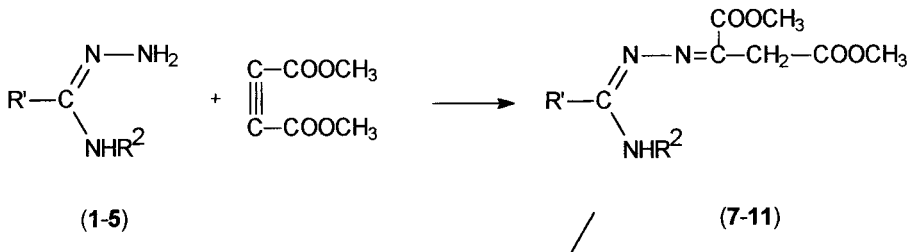
### *2.2. Microbiological investigations*

The *in vitro* activity of compounds **12**–**17** was determined by a standard dilution method in Müller-Hinton medium for bacteria and in the same medium with 4% glucose for fungi. For the minimum inhibitory concentration (MIC) the inoculum size of $10^4$ CFU/$cm^3$ was used. The bacterial cultures were incubated for 18 h and fungi cultures for 48 h at 37 °C.

### *2.3. Pharmacological tests*

Acute toxicity by accessing the $LD_{50}$ dose according to the Wilcoxone and Litchfield method was determined in the Department of Pharmacology and Toxicology, Medical University, Lublin. The acute toxicity of compounds **12**–**17** in mice was low (>2000 mg/kg i.p.).

**Scheme**




| | $R^1$ | $R^2$ |
|---|---|---|
| **1, 7, 12** | $C_6H_5$ | $C_6H_5$ |
| **2, 8, 13** | $C_6H_5$ | p-$CH_3C_6H_4$ |
| **3, 9, 14** | 2-$C_5H_4N$ | $C_6H_5$ |
| **4, 10, 15** | 2-$C_5H_4N$ | p-$CH_3C_6H_4$ |
| **5, 11, 16** | 2-$C_5H_4N$ | p-$NO_2C_6H_4$ |
| **6, 17** | 2-$C_5H_4N$ | 2-$C_5H_4N$ |

**Table: Physicochemical and spectral properties of compounds 7–17**

| Compd. | $R^1$ | $R^2$ | Formula m.w. | M.p. (°C) | Yield (%) | IR ($cm^{-1}$) KBr | $^1H$ NMR (ppm, TMS) MS m/e [%] |
|---|---|---|---|---|---|---|---|
| **7** | $C_6H_5$ | $C_6H_5$ | $C_{19}H_{19}N_3O_4$ 353.2 | 94 | 65 | 3300 NH; 3007 CH arom.; 2860 CH aliph.; 1703, 1740 CO | 3.4 (s, 3 H, $OCH_3$); 3.5 (s, 3 H, $OCH_3$); 4.2 (s, 2 H, $CH_2$); 6.7–9.1 (m., 10 H, arom.); 9.9 (s, 1 H, NH) |
| **8** | $C_6H_5$ | p-$CH_3$–$C_6H_4$ | $C_{20}H_{21}N_3O_4$ 367.2 | 96 | 80 | 3300, 3280 NH; 3008 CH arom.; 2890 CH aliph.; 1728, 1710 CO | 2.4 (s, 3 H, $CH_3$); 3.4 (s, 3 H, $OCH_3$); 3.5 (s, 3 H, $OCH_3$); 4.2 (s, 2 H, $CH_2$); 6.8–9.2 (m., 9 H, arom.); 9.9 (s, 1 H, NH) |
| **9** | 2-$C_5H_4N$ | $C_6H_5$ | $C_{18}H_{18}N_4O_4$ 354.2 | 90 | 75 | 3262, 3200 NH; 3081 CH arom.; 1712, 1728 CO | 3.5 (s, 3 H, $OCH_3$); 3.6 (s, 3 H, $OCH_3$); 3.8 (s, 2 H, $CH_2$); 7.0–8.7 (m., 9 H, arom.); 9.7 (s, 1 H, NH–Ph) |
| **10** | 2-$C_5H_4N$ | p-$CH_3$–$C_6H_4$ | $C_{19}H_{20}N_4O_4$ 368.2 | 97 | 80 | 3260, 3200 NH; 3052 CH arom.: 2950 CH aliph.; 1710, 1699 CO | 2.5 (s, 3 H, $CH_3$); 3.5 (s, 3 H, $OCH_3$); 3.7 (s, 3 H, $OCH_3$); 4.0 (s, 2 H, $CH_2$); 7.0–8.5 (m., 8, H, arom.); 10.1 (s, 1 H, NH–Ph) |
| **11** | 2-$C_5H_4N$ | p-$NO_2$–$C_6H_4$ | $C_{18}H_{17}N_5O_6$ 399.2 | 106 | 70 | 3330, 3290 NH; 3050 CH arom.; 2950 CH aliph.; 1719, 1700 CO; 1301 $NO_2$ | 3.4 (s, 3 H, $OCH_3$); 3.6 (s, 3 H, $OCH_3$); 4.0 (s, 2 H, $CH_2$); 7.0–8.3 (m., 8 H, arom.); 10.2 (s, 1 H, NH) |
| **12** | $C_6H_5$ | $C_6H_5$ | $C_{18}H_{15}N_3O_3$ 321.2 | 166 | 70 | 3120 NH; 3052 CH arom.; 1735, 1699 CO | 3.6 (s, 3 H, $OCH_3$); 5.3 (s, 1 H, = CH); 7.2–7.4 (m., 10 H, arom.); 11.5 (s, 1, H, NH) |
| **13** | $C_6H_5$ | p-$CH_3$–$C_6H_4$ | $C_{19}H_{17}N_3O_3$ 335.2 | 182 | 65 | 3050 CH arom.; 2900 CH aliph.; 1732, 1711 CO | 2.4 (s, 3 H, $CH_3$); 3.6 (s, 3 H, $OCH_3$); 5.3 (s, 1 H, = CH); 7.1–7.3 (m., 9 H, arom.); 11.7 (s, 1 H, NH) |
| **14** | 2-$C_5H_4N$ | $C_6H_5$ | $C_{17}H_{14}N_4O_3$ 322.2 | 140 | 70 | 3252 NH; 3060 CH arom.; 2900 CH aliph.; 1703, 1731 CO | 3.3 (s, 3 H, $OCH_3$); 5.2 (s, 1 H, = CH); 7.1–8.2 (m., 9 H, arom.); 11.2 (s, 1 H, NH) 322 (92.3, $M.^+$), 291 (13.6), 290 (29.9), 223 (44.8), 222 (100), 181 (43.3), 119 (30), 78 (23.9), 77 (30.8) |
| **15** | 2-$C_5H_4N$ | p-$CH_3$–$C_6H_4$ | $C_{18}H_{16}N_4O_3$ 336.2 | 192 | 70 | 3005 CH arom.; 2980 CH aliph.; 1720, 1699 CO | 2.5 (s, 3 H, $CH_3$); 3.3 (s, 3 H, $OCH_3$); 5.0 (s, 1 H, = CH); 7.0–8.2 (m., 8 H, arom.); 11.0 (s, 1 H, NH) |
| **16** | 2-$C_5H_4N$ | p-$NO_2$–$C_6H_4$ | $C_{17}H_{13}N_5O_5$ 367.2 | 168 | 72 | 3021 Ch arom.; 2970 Ch aliph.; 1730, 1705 CO; 1322 $NO_2$ | 3.5 (s, 3 H, $OCH_3$); 4.8 (s, 1 H, = CH); 7.0–8.1 (m., 8 H, arom.); 10.9 (s, 1 H, NH) |
| **17** | 2-$C_5H_4N$ | 2-$C_5H_4N$ | $C_{16}H_{13}N_5O_3$ 323.2 | 138 | 80 | 3022 CH arom.; 2900 CH aliph.; 1709, 1740 CO | 3.6 (s, 3 H, $OCH_3$); 5.7 (s, 1 H, = CH); 7.0–8.3 (m., 8 H, arom.); 11.6 (s, 1 H, NH) |

## 3. Experimental

### *3.1. Chemistry*

Melting points measured on a Boetius apparatus are given uncorrected. $^1H$ NMR spectra were recorded on a Tesla BS 567A (100 MHz) apparatus in $D_6$-DMSO with TMS as an external standard. IR spectra were recorded on a Specord IR-74 spectrometer. MS fragmentation was recorded as EI MS (15 eV) on an AMD apparatus. Chemicals were purchased from Merck Co. or Fluka Ltd. and used without further purification. Results of elemental analysis for C, H, N by method of microanalysis obtained in Department of Organic Chemistry, Medical University in Lublin, were in acceptable accordance with calculated values (±0.7% for C, 0.9% for N and 1.2% for H).

The purity of the obtained compounds was examined by TLC performed on 10 × 20 cm pre-coated plates Si 60 $F_{254}$ (E. Merck). The silica plates were activated for 30 min at 110 °C. The compounds were dissolved in ethanol or acetone (1 mg/ml) and samples were spotted on the plates. The evolution was carried out in a Chromdes (Lublin, Poland) horizontal sandwich DS-chamber [8, 9] with binary eluent comprising a mixture of chloroform and ethyl acetate (70 : 30 or 75 : 25). The plates were dried and spots were observed under UV light at λ = 254 nm.

*3.1.1. Synthesis of dimethyl 2-[(1-arylamino-1-arylmethylidene)hydrazono] succinates* **7**–**11** *(general procedure)*

Amidrazones **1**–**5** 0.01 mol were dissolved in 25 ml of absolute ethanol and the solution was cooled to −5 °C. DMAD (1.45 g, 0.01 mol) was added dropwise and the whole mixture was left for 5–10 days at −10 °C. After this time, a precipitate was formed and the solution turned to dark red. The precipitate was filtered off and purified by crystallization from ethanol.

*3.1.2. Synthesis of methyl 2-(5-oxo-3,4-diaryl-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene)-acetates* **12**–**16** *(general procedure)*

Compounds **7**–**11** (0.01 mol) were suspended in 25 ml of methanol and 1 ml of triethylamine was added. The mixture was left at room temperature for 24 h (in the case of compound **11** the mixture was refluxed for 3 h). The yielded precipitate was filtered off and purified by crystallization from ethanol.

*3.1.3. Synthesis of methyl 2-[5-oxo-3,4-di-(-2-pyridyl)-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene)acetate* (**17**)

Amidrazone **6** (2.4 g, 0.01 mol) was dissolved in 25 ml of absolute ethanol and cooled to a temperature of −5 °C. DMAD (1.45 g, 0.01 mol) was added dropwise. The whole mixture was left for 14 days at −10 °C. The formed precipitate was filtered off and purified by crystallization from ethanol. More detailed data is given in the Table.

***3.2. Antimicrobial screening***

Most of the tested compounds **12**–**17** did not influence the growth of microorganisms. The highest activity exhibited compound **17**, bearing two 2-pyridyl substituents, which inhibited the growth of *Staphylococcus aureus, Escherichia coli, Brucella abortus, Mycobacterium smegmatis* and *Candida albicans* in a concentration of 125 and 250 μg/ml. The structurally similar compound **14** (with only one 2-pyridyl substituent) was less active. Its MIC were 200–300 μg/ml for *Escherichia coli*, *Brucella abortus*, *Mycobacterium smegmatis*, *Candida albicans* and *Epidermophyton floccosum*.

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