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Synthesis and root growth activity of some new acetylhydrazinecarbothioamides and 1,2,4-triazoles substituted with the 5*H*-dibenzo[*a,d*][7]annulene moiety

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Abstract: New hydrazinecarbothioamides **5a–d** bearing 5*H*-dibenzo[*a,d*][7]annulene moiety were synthesized using classical procedures. ¹H-NMR analysis indicated the existence of two conformational isomers, a major axial (about 75 %) and a minor equatorial one (25 %), which are interconvertible by middle ring inversion. Cyclization of compounds **5a–d** in NaOH solution produced the corresponding 4*H*-1,2,4-triazole-3-thiols (**6a–d**) that proved to be pure axial isomers. All the new compounds were extensively characterized by elemental analysis, IR, UV, ¹H-NMR and ¹³C-NMR spectroscopy and were biologically investigated using phytobiological tests.

Keywords: carbothioamide; 1,2,4-triazole; 5*H*-dibenzo[*a,d*][7]annulene; plant growth regulation activity.

INTRODUCTION

The 5*H*-dibenzo[*a,d*][7]annulene ring is present in the structure of many compounds used in therapeutics as antibacterial, anticonvulsive, anticholinergic, myorelaxant, antihistaminic, antifungal, carbonic anhydrase inhibitors, anti-inflammatory, analgesic, anti-arrhythmia, antiparasitic agents, but mostly they are used as antidepressant drugs.^{1–4}

Hydrazinecarbothioamides are compounds with various pharmacological activities: anti-inflammatory, antimicrobial, tuberculostatic, anticonvulsive and antiviral.^{5–8}

The synthesis of compounds containing the 1,2,4-triazole ring in their structure has attracted widespread attention, mainly in connection with their wide range of pharmacological properties. It is well documented that 1,2,4-triazole

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derivatives possess antimicrobial, antifungal, tuberculostatic, analgesic and carbonic anhydrase inhibitor activities.^{9–13}

Motivated by these facts and as a continuation of previous work,^{2,14–18} the synthesis, characterization and action on vegetal cell division of new hydrazine-carbothioamides and 1,2,4-triazole derivatives containing the 5*H*-dibenzo[*a,d*]-[7]annulene moiety are presented herein.

The structures of these new compounds were elucidated by elemental analysis and IR, UV, ¹H-NMR and ¹³C-NMR spectroscopy. The newly synthesized products were tested using phytochemical tests.^{19–21}

The *Triticum* bioassay (Constantinescu method)^{19,21} revealed concentration-dependent mitoinhibitory effects for the investigated substances.

EXPERIMENTAL

Materials, methods and instruments

All chemicals used in this study were supplied by Sigma–Aldrich and Merck.

The melting points were determined using a Bötius apparatus and are uncorrected. Elemental analyses were realized using a Perkin–Elmer CHNS/O Analyzer Series II 2400 instrument and the results were within ±0.4 % of theoretical values. The infrared spectra were recorded on a Vertex 70 Bruker spectrometer using the KBr pellet technique and the results are expressed in wave number (cm⁻¹). The ¹H-NMR and ¹³C-NMR spectra were registered on a Varian Gemini 300 BB spectrometer working at 300 MHz for ¹H and 75 MHz for ¹³C, using DMSO-*d*₆ as the solvent for hydrazinecarbothioamides and CDCl₃ for 1,2,4-triazole compounds. The chemical shifts are expressed in δ (ppm) using TMS as the internal standard. The UV–Vis spectra were recorded on a SPECORD 40 Analytik Jena spectrometer in methanol (2.5×10⁻⁵ M) in the wavelength range 200–600 nm.

The plant bioassay (*Triticum* bioassay) used embryonic roots from *Triticum vulgare* Mill. as the biological reagent. A Labophot II Nikon microscope (ob. 10× and 100×) was used.^{20,21}

*Procedure for the preparation of 2-(5*H*-dibenzo[*a,d*][7]annulen-5-ylacetyl)hydrazinecarbothioamide 5a*

The mixture of 2-(5*H*-dibenzo[*a,d*][7]annulen-5-yl)acetohydrazide (**3**, 0.004 mol), and KSCN (0.008 mol) in 40 mL water was refluxed in the presence of 1 mL of concentrated HCl for 10 h. On cooling the reaction mixture to room temperature, a white solid appeared. This was filtered off and recrystallized from methanol–water (1:3, v:v) to obtain the desired compound.

*General procedure for the preparation of 2-(5*H*-dibenzo[*a,d*][7]annulen-5-ylacetyl)-*N*-alkyl-(aryl)hydrazinecarbothioamides 5b–d*

The mixture of 2-(5*H*-dibenzo[*a,d*][7]annulen-5-yl)acetohydrazide (**3**, 0.004 mol) and the corresponding isothiocyanate **4b–d** (0.004 mol) in absolute ethanol (30–50 mL) was refluxed for 6–12 h. On cooling the reaction mixture to room temperature, a solid appeared. This was filtered off and recrystallized from ethanol to obtain the desired compound.

General procedure for the preparation of compounds 6a–d

A solution of the corresponding carbothioamide **5a–d** (1 mmol) in 8 mL of 8 % NaOH solution was refluxed for 3–9 h and then filtered. After cooling, the filtrate was neutralized

with acetic acid. The obtained white precipitate was filtered and recrystallized from CHCl_3 :petroleum ether (1:2, v/v).

Triticum bioassay – Constantinescu method

The effects of the title compounds on root growth of wheat were determined according to the bioassay – Triticum test. The method is based on the study of the influence of substances at various dilutions on root elongation and mitotic film, depending on their duration of action on wheat (*Triticum* sp., Poaceae) caryopses germinated under well-defined laboratory conditions.

All the compounds were tested at 0.5, 0.25 and 0.05 mM concentrations. The solutions were placed in 10 cm diameter Petri dishes and then the wheat caryopses with a main root of 1 cm were introduced.

The dishes were covered with their lids and the caryopses were left in contact with the solutions for 5 days. In parallel, a control sample was prepared, in which the test solutions were replaced by distilled water. Root elongation was evaluated at the same time for 5 days. Observations were made on the morphological changes, as well as on the aspect, the number of radicles and the length of the main radicle.

For the microscopic study, after 24 h, the embryonic root of two caryopses from each Petri dish was sectioned at a distance of 5 mm from the tip and stained with dilute acetic orcein, a dye with a great affinity for chromatin in acetic medium (an acid pH is required for the hydrolysis of the chromatin) which will be stained red. The stained sections were microscopically examined using a LaboPhot II Nikon microscope (ocular 10 \times , object-glass 100 \times) by immersion in cedar oil.

The data represent the average values from two independent experiments and the results were processed statistically by the Student's *t*-test (Table I).

TABLE I. Plant growth regulation activities of compounds **5a–d** and **6a–d** (the root length is the mean value of measurements made on day five of the treatment)

Compd.	Concentration, mM	Root length \pm SD ^a , mm	<i>p</i> -Value ^b	Effect, %
M	–	108.4 \pm 3.3	–	–
5a	0.5	33.2 \pm 3.0	<0.00001	76.42 ^c
5a	0.25	42.0 \pm 4.1	<0.00001	67.48
5a	0.05	64.0 \pm 6.0	<0.00001	45.12
5b	0.5	68.7 \pm 5.8	<0.0001	40.34
5b	0.25	85.5 \pm 7.0	<0.001	23.27
5b	0.05	92.0 \pm 8.7	<0.001	16.66
5c	0.5	52.0 \pm 5.2	<0.00001	57.31
5c	0.25	75.3 \pm 7.0	<0.001	33.63
5c	0.05	82.1 \pm 8.7	<0.001	26.72
5d	0.5	102.8 \pm 1.9	NS ^d	5.69
5d	0.25	96.5 \pm 9.1	<0.001	12.09
5d	0.05	89.6 \pm 9.0	<0.001	19.10
6a	0.5	56.5 \pm 5.6	<0.00001	52.74
6a	0.25	61.3 \pm 4.0	<0.0001	47.86
6a	0.05	103.7 \pm 2.1	NS	4.77
6b	0.5	70.7 \pm 7.7	0.0001	38.31
6b	0.25	79.1 \pm 8.0	0.001	29.77
6b	0.05	92.5 \pm 8.2	<0.001	16.15

TABLE I. Continued

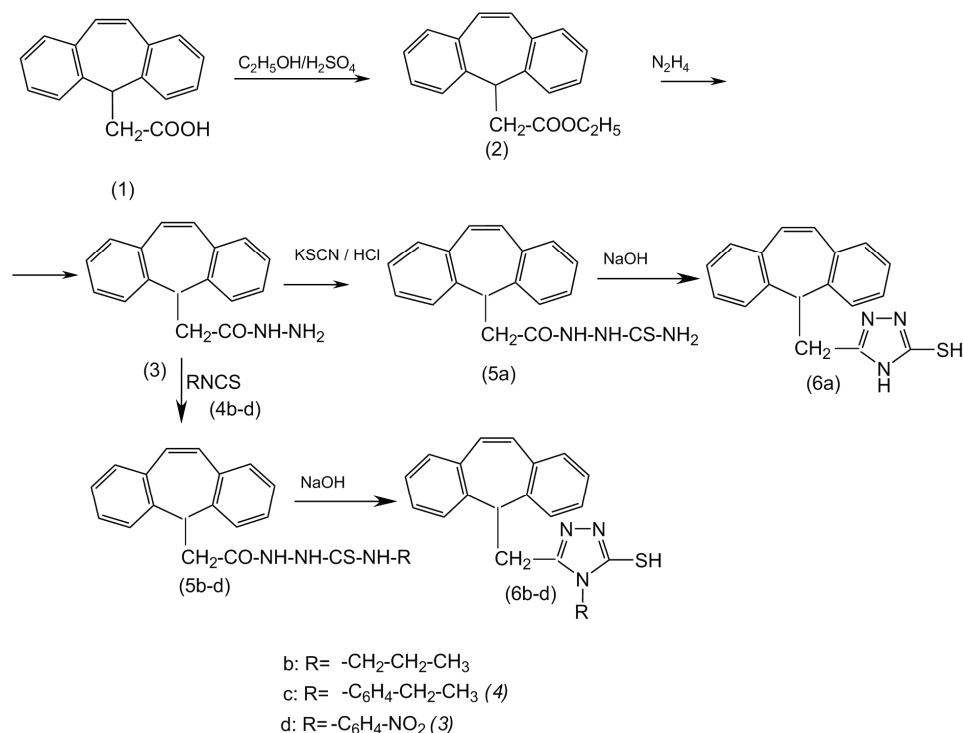
Compd.	Concentration, mM	Root length \pm SD ^a , mm	<i>p</i> -Value ^b	Effect, %
6c	0.5	63.3 \pm 6.1	<0.00001	45.83
6c	0.25	65.3 \pm 5.9	<0.0001	40.75
6c	0.05	98.8 \pm 3.9	0.004	9.75
6d	0.5	63.0 \pm 5.8	<0.00001	46.13
6d	0.25	83.2 \pm 7.9	<0.001	25.60
6d	0.05	109.7 \pm 3.9	NS	-1.32 ^e

^astandard deviation; ^bsignificant level (statistical significance in the two-population (independent) Student's *t*-test); ^cpositive value of effect = inhibition effect on wheat root growth; ^dthe two means are not significantly different; ^enegative value of effect = stimulating effect on wheat root growth

RESULTS AND DISCUSSION

Chemistry

The synthesis pathway used for the preparation of the title compounds is shown in Scheme 1.

Scheme 1. Reaction pathway to the target compounds **5a–d** and **6a–d**.

The synthesis of the new compounds was realized in several steps, starting from 5H-dibenzo[*a,d*][7]annulen-5-ylacetic acid (**1**) that was transformed into the

ethyl ester **2**. Ethyl 5*H*-dibenzo[*a,d*][7]annulen-5-ylacetate (**2**) was reacted with hydrazine hydrate according to a literature method^{14,22,23} affording 2-(5*H*-dibenzo[*a,d*][7]annulen-5-yl)acetohydrazide (**3**).

2-(5*H*-Dibenzo[*a,d*][7]annulen-5-ylacetyl)hydrazinecarbothioamide (**5a**) was obtained by refluxing **3** with KSCN and concentrated HCl.

The new 2-(5*H*-dibenzo[*a,d*][7]annulen-5-ylacetyl)-*N*-alkyl/arylhydrazinecarbothioamides **5b–d** were obtained by nucleophilic addition of **3** to different alkyl or aryl isothiocyanates **4b–d**.^{14,15,24,25}

The new 5-(5*H*-dibenzo[*a,d*][7]annulen-5-ylmethyl)-4-alkyl/aryl-4*H*-1,2,4-triazole-3-thiols **6a–d** were synthesized by subjecting **5a–d** to intramolecular cyclization in 8 % sodium hydroxide solution under reflux.^{14–16,26,27}

Analytical and spectral data of the newly prepared compounds

The spectral data of all the newly synthesized hydrazinecarbothioamides and 1,2,4-triazoles are given in the Supplementary Material to this paper. The data are in accordance with the proposed structures. The general structure of **5** with atom numbering is given Fig. 1.

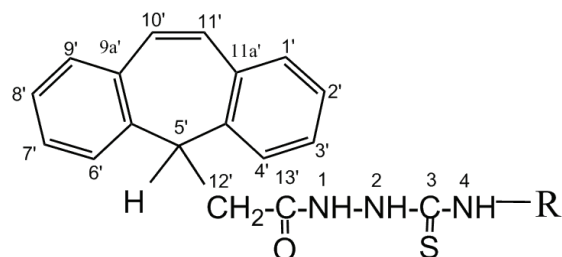
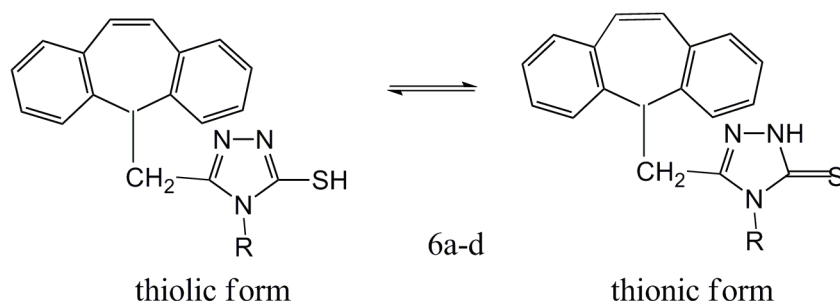


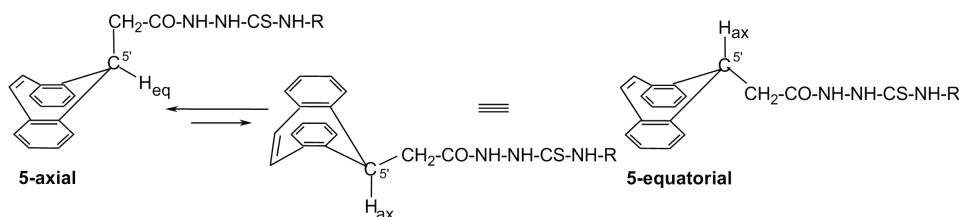
Fig. 1. The general structure of **5** with atom numbering.

The nucleophilic addition of 2-(5*H*-dibenzo[*a,d*][7]annulen-5-yl)acetohydrazide to KSCN and various isothiocyanates was confirmed in the infrared spectra of the new acylhydrazinecarbothioamides **5a–d** by the appearance of new absorption bands due to the stretching vibration of the C=S group (1256–1259 cm⁻¹), the NO₂ group (1566, 1350 cm⁻¹) and the stretching vibration of the CH₃ group. The C=O and N–H stretching bands were present at 1686–1674 and 3419–3184 cm⁻¹, respectively.

The structures of the 1,2,4-triazole-3-thiols **6a–d** were also proved based on their spectral data. The IR spectra confirmed the cyclization of **5a–d** to **6a–d** by the disappearance of the ν_{C=O} band. Moreover, instead of two or three NH bands of compound **5**, compound **6** exhibited only one band in KBr at 3419–3092 cm⁻¹, characteristic for the –NH–CS group. It seems that in KBr, the mercaptotriazole **6** existed in the form of their thionic tautomers (Scheme 2).

Scheme 2. Tautomeric forms of the mercaptotriazoles **6a-d**.

The $^1\text{H-NMR}$ spectra of carbothioamides **5a-d** indicated the presence of two isomers, 5'-axial and 5'-equatorial in a ratio of about 3:1, interconvertible by middle ring inversion (Scheme 3).

Scheme 3. Interconversion of the acylcarbothioamides **5a-d** between the 5'-axial and 5'-equatorial isomers by middle ring inversion.

The NH protons signals appear as singlets between 8.13–10.30 ppm, the double bond protons $\text{H}^{10'}$ and $\text{H}^{11'}$ appear as singlets at 7.01–7.07 ppm. The aliphatic side chains protons also exhibited normal δ values.

In the $^{13}\text{C-NMR}$ spectra of **5a-d**, the dibenzo[*a,d*][7]annulene moiety appears in a narrow δ domain (123–140 ppm), with $\text{C}^{10'}$ and $\text{C}^{11'}$ easily recognizable at δ 130.9–131.7 ppm. The signal at $\delta \approx 181$ ppm could be attributed to the C=S carbon atom. The remaining carbon atoms afforded signals that partially overlapped with those of the side chains. However, the assignments of these signals could be made using incremental calculations for the substituents and the results were very similar to those of the triazoles **6a-d**.

The $^1\text{H-NMR}$ spectra of triazoles **6a-d** indicated the presence of a single conformational isomer, namely the axial one.

Surprisingly, cyclization of **5** to **6** and the subsequent work-up were accompanied by the loss of the minor equatorial isomer, probably because of its higher solubility in the acidic water.

The $\text{H}^{5'(\text{eq})}$ appears at δ 4.35–4.61 ppm (triplet, $J = 7.7$ Hz) and $\text{CH}_2^{12'}$ protons as a doublet at 2.89–3.01 ppm in **6a-d**. Interestingly, the NH signals of **5b-d** totally disappeared being replaced by a singlet at δ 10.85–11.70 ppm, attri-

butable to the SH proton. In the case of triazole **6a**, a singlet appears at 9.04 ppm attributed to the NH group and another one at 10.85 ppm attributed to the SH group.

Thus, in solution, the equilibrium is shifted towards the thiolic form.

Conversion of carbothioamides **5** to triazoles **6** is also nicely supported by ^{13}C -NMR spectra in which a new quaternary carbon signal (for C_3) appears at δ 165.69–168.37 ppm simultaneously with the disappearance of the C=S signal from **5** (δ 181 ppm). Moreover, instead of C=O signal from **5** at 169–170 ppm, a new signal appears for C_5 of **6** at about 150–151 ppm.

Triticum bioassay

The effects of the title compounds **5a–d** and **6a–d** on the sprouting of wheat were investigated. After treating with solutions of 0.5, 0.25 and 0.05 mM concentrations of compounds **5a–d** and **6a–d** for 5 days, from the difference in length between the main root of caryopses treated with the title compounds and those treated with distilled water (last day), the plant growth regulating activities of the compounds were determined from the formula:

$$\text{Effect (\%)} = 100 - \frac{\text{length of sample radicle} - 10 \text{ mm}}{\text{length of reference radicle} - 10 \text{ mm}} \times 100$$

where 10 mm represents the initial length of the main root. A positive result represents inhibition, whereas a negative result implies growth stimulation.

The results of the *Triticum* bioassay – the Constantinescu method (the root elongation values for each sample tested in the day) are presented in Table I.

The compounds **5a–d** and **6a–c** inhibited root growth at all concentrations, without cytotoxicity (Table I). The inhibitory activity declined with decreasing applied concentration.

The microscopic examination of the wheat embryonic roots showed mitodepressive and mitostatic effects for 0.50 and 0.25 mM concentrations: the shape of the cell was irregular and nuclei with a hypertrophied nucleolus were observed. At 0.05 mM concentration (except for compound **5a**), phases of normal division were noticed, the aspect being generally similar to that observed for the control.

CONCLUSIONS

In this paper, the synthesis and characterization of four new acetylhydrazine-carbothioamides and four 4*H*-1,2,4-triazole-3-thiol derivatives containing 5*H*-di-benzo[*a,d*][7]annulene moiety were presented. The structures of new compounds were confirmed by spectral data (IR-, UV-, ^1H -NMR and ^{13}C -NMR).

All the compounds were investigated for their biological activities concerning regulation of the growth of wheat using the phytobiological method – the *Triticum* test. A microscopic study demonstrated mitosis inhibition activity for compounds **5a–d** and **6a–d**, without cytotoxicity.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the synthesized compounds are available electronically from <http://www.shd.org.rs/JSCS/>, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА И БИОЛОШКА АКТИВНОСТ НОВИХ АЦЕТИЛХИДРАЗИН-
КАРБОТИОАМИДА И 1,2,4-ТРИАЗОЛА СУПСТИТУИСАНИХ
5Н-ДИБЕНЗО[*a,d*][7]АНУЛЕНИМА

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Описана је синтеза нових деривата **5a-d** 5Н-добензо[*a,d*][7]анулена. Анализа ¹H-NMR спектра указује на присуство два конформациона изомера, доминантниог (око 75%) аксијалног и споредног екваторијалног (око 25%), који подлежу конформационој измени инверзијом средњег прстена. Циклизацијом деривата **5a-d** у раствору натријум-хидроксида добијају се одговарајући деривати **6a-d**, 4Н-1,2,4-триазол-3-тиоли, који су добијени као чисти аксијални изомери. Сва нова једињења окарактерисана су елементалном анализом, IR, UV, ¹H-NMR и ¹³C-NMR спектроскопијом и испитана им је биолошка активност фитобиолошким тестом.

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