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Improved synthesis, antibacterial activity and potential carcinogenicity of 5-amino-1,2,4-thiadiazol-3(2*H*)-one

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This paper reports on the preparation of 5-amino-1,2,4-thiadiazol-3(2*H*)-one, a sulfur-containing analogue of cytosine with the −CH=CH− group between the positions 5 and 6 of the pyrimidine ring replaced by the divalent sulfur (−S−). Improved procedures for the preparation of thiobiuret, some of its methyl derivatives and 5-amino-1,2,4-thiadiazol-3(2*H*)-one are documented. Thiobiuret and its N-methyl derivatives were obtained by addition of hydrogen sulfide to the respective 1-cyanoureas. Subsequent oxidation of thiobiuret with hydrogen peroxide in alkaline medium produced 5-amino-1,2,4-thiadiazol-3(2*H*)-one. This substance was traced back converted to the starting thiobiuret by reaction with cysteine hydrochloride. Alkaline degradation of thiadiazol led to the formation of 1-cyanourea isolated as its silver salt. An investigation of the thiadiazol biological activities has shown that it inhibits the growth of *E. coli* by 10% at 8.5 μM concentrations, but exhibited no cytostatic activity in L1210, HeLa S3 and HL-60 cell lines. Potential carcinogenicity of the prepared compounds was determined by a DC polarographic method. While the values of the parameter of carcinogenicity for all intermediates indicate only marginal carcinogenic potential, the value of the parameter of carcinogenicity for the thiadiazole indicates possible carcinogenicity of this compound.

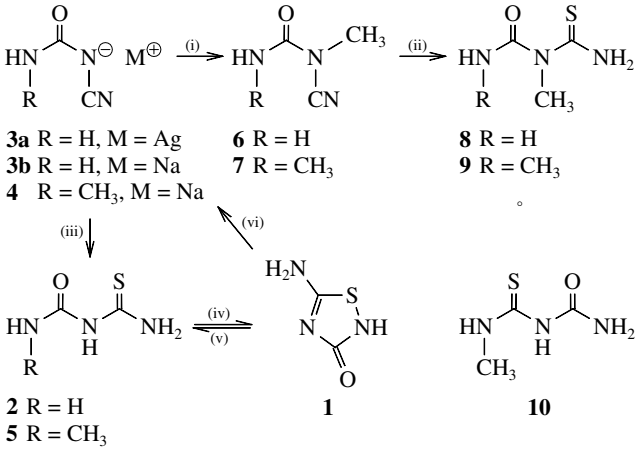
1. Introduction

In connection with the study of potential antimetabolites of pyrimidine components of nucleic acids, our effort has been devoted to the preparation and study of various biological effects of aza analogues related to natural pyrimidines. At the present time, the most clinically important compounds of this group are 5-azacytidine (azacitidine) (Píškala and Šorm 1964, 1978) and 5-aza-2′-deoxycytidine (decitabine) (Pliml and Šorm 1964; Šorm and Píškala 1966; Píškala and Šorm 1978). They are used as antileukemic agents (Glover et al. 1987; Rivard et al. 1981; Kantarjian et al. 1997). An azacitidine containing drug was registered by The Upjohn Company under the name Mylosar® many years ago and the American company SuperGen announced recently its product Dacogen® containing decitabine. Additionally, 1-β-D-arabinofuranosyl-5-azacytosine (Fazarabine) (Beisler et al. 1979; Mertes et al. 1984) exhibits remarkable antileukemic activity and is investigated in clinical trials (Grem et al. 1987). However, it has not shown a beneficial effect in the therapy of acute leukemia (Wilhelm et al. 1999). Moreover, a substantial interest in evaluating the potential carcinogenicity of new synthetic antimetabolites and related compounds is seen in connection with their possible therapeutic activity. The *in vitro* polarographic method was used in this regard (i.e. Vachálková et al. 1993; Novotný et al. 1994, 1995, 1996a, 1996b, 1999a, 1999b.

The preparation of 5-amino-1,2,4-thiadiazol-3(2*H*)-one (**1**) (Scheme), a sulfur-containing analogue of cytosine with the −CH=CH− group between the positions 5 and 6 of the pyrimidine ring replaced by divalent sulfur (−S−) is reported. It is known that such isoelectronic replacements can lead to biologically active antimetabolites (Revankar and Robins 1976, 1978; Chwang et al. 1980; Párkányi et al. 1989). The syntheses of **1** and its ribonucleoside were reported for the first time by Revankar and Robins (1976, 1978) but the final step of the synthesis of the ribonucleoside of **1** was later improved and its structure perfectly determined by Chwang, Němec and Welch (Chwang et al. 1980). However, the latter authors have found that the physical properties of their product were in all respects different from those described by Revankar and Robins. It is clear that the structure of the ribonucleoside as prepared by Revankar and Robins (Revankar and Robins 1976, 1978) is not correct, however, the real structure of this product has not been elucidated yet. Eventually, the thiadiazol **1** has been prepared also by Párkányi et al. (1989) using a modified procedure based on that of Revankar and Robins (1978).

This paper reports on improved procedures for the preparation of thiobiuret **2**, some of its methyl derivatives and 5-amino-1,2,4-thiadiazol-3(2*H*)-one (**1**) and on reductive cleavage, alkaline degradation course and antibacterial activity of compound **1**. Additionally, the potential carcino-

Scheme



(i) Me_2SO_4; (ii) H_2S, NaHS; (iii) H_2S; (iv) H_2O_2, NaOH: (v) L-cysteine, HCl; (vi) NaOH, 100 °C

genicity of these compounds was investigated by DC polarography and the results are also reported.

2. Investigations, results and discussion

The synthesis of 5-amino-1,2,4-thiadiazol-3(2*H*)-one (**1**) (Scheme 1) started from the silver salt (Bieling et al. 1964) of 1-cyanourea **3a** which was converted into the sodium salt with sodium iodide (Bieling et al. 1964) (**3b**). Reaction of an aqueous solution of **3b** with hydrogen sulfide at elevated temperature gave a 73% yield of thiobiuret **2**. Similar procedures for the preparation of **2** from 1-cyanourea were described earlier (Wunderlich 1886; Hecht 1892) and gave in our hands only 3% yield. Compound **1** was obtained in 71% yield by oxidative cyclization of **2** with hydrogen peroxide in an alkaline medium using a modification of the known procedure (Revankar and Robins 1978; Párkányi et al. 1989; Kurzer 1965). Compound **1** was tested for its antibacterial activity. It inhibited the growth of *E. coli* by 10% at 8.5 μM. However, this inhibition was not proportional to the concentration. At 85 μM, only 40% inhibition was observed. Compounds **1** and **2** exhibited no cytostatic activity in L 1210, HeLa S3 and HL-60 cell lines. A preliminary biochemical examination of **1** shows that its antibacterial activity is not reversed in the presence of natural pyrimidines indicating that this compound is not an antimetabolite of pyrimidine components of nucleic acids but that it interferes with bacterial growth at a different metabolic step, presumably by interfering with proteins. This result could be explained by the absence of enzymes transforming the nucleobase to nucleosides and nucleotides of **1**. A similar situation was observed with 5-fluorocytosine, which in contrast to 5-fluorouracil is active only when applied in the form of its nucleosides (Koechlin et al. 1966). 5-Fluorocytosine itself lacks any significant cytostatic activity in mammals. In addition, it exhibits practically no bacteriostatic activity. However, it was rather selectively active against *Candida albicans* that is capable of enzymatic transformation of this analogue to nucleosides and nucleotides (Koechlin et al. 1966). Also, 5-azacytosine (Škoda et al. 1962) has shown a negligible antibacterial activity in contrast to 5-azacytidine (Šorm et al. 1964), which was highly active against *Escherichia coli* and exhibited a pronounced antileukemic activity.

The assumed interference of compound **1** with proteins led to an investigation of the reaction of this compound with some amino acids. It was found, that the reaction of L-cystine hydrochloride with **1** in water at room temperature afforded L-cystine and thiobiuret **2**. This result shows that the presumed interference of **1** is based on its ability to function as a weak oxidative agent capable of transforming thiol groups to disulfides. Some heterocyclic compounds which can oxidize glutathione (G-SH) to the respective disulfide (G-S-S-G) were recently studied as modulators of resistance to cytostatics, particularly to cisplatin (Osmak et al. 2000). The thiadiazol **1** may represent a suitable chemical agent that should also be investigated as a resistance modulator.

To evaluate the stability of **1**, the compound was hydrolyzed with 2.5 M sodium hydroxide at 100 °C. The major product of the degradation was 1-cyanourea, isolated as its silver salt **3a** in a yield of 80%. Additionally, the presence of cyanamide formed by further hydrolysis of 1-cyanourea was documented by TLC. Earlier, it was reported (Chwang et al. 1980) that the ribonucleoside of **1** is very unstable under alkaline conditions even at room temperature. The instability of this nucleoside was followed at ambient temperature by UV spectrophotometry, which exhibited decreasing absorbance with time in alkaline solutions. Because the ribosyl residue has no substantial influence on the UV spectra, both the thiadiazole **1** as well as its ribonucleoside exhibit maxima at about 250 (pH 7.0) or 260 nm (pH 13.0), however, the spectra of the thiadiazole **1** have not shown decreasing absorbance in alkaline solutions. These results indicate that the decomposition concerns the heterocyclic part of the molecule and not the C−N ribosyl bond. Although the structure of the decomposition product has not been cleared, it is assumed that the hydrolysis of the ribonucleoside of thiadiazole **1** proceeds in analogy to the abovementioned hydrolysis of the unsubstituted thiadiazole **1** with the formation of 1-cyano-3-ribosylurea. Because cyanoureas have no absorption in the range of 250–260 nm [cyanourea exhibits a maximum at 216 nm (pH 11)], the decrease in absorbance with time at 260 nm in alkaline solutions is well understandable and it supports the proposed course of degradation of the ribonucleoside. In this connection it is of interest to note that at room temperature the C−N glycosyl bond of glycosylureas is rather stable in alkaline media; a substantial cleavage of

this bond occurs only at higher temperatures and in strong alkaline solutions. High yields of 1-amidino-3-glycosylureas were obtained by hydrolysis of 1-glycosyl-5-azacytosines with ammonia solutions at room temperature (Píška-la et al. 2003). The much lower stability of the ribonucleoside in comparison with the unsubstituted base is due to the replacement of the acid hydrogen by the ribosyl group. This prevents the reaction rate-lowering anionization of thiadiazol in alkaline solutions.

In an attempt to obtain model compounds of nucleosides of **1**, methyl derivatives of thiobiuret **2** were prepared by addition of hydrogen sulfide to the respective 1-cyanoureas. The sodium salt of 1-cyano-3-methylurea **4** in the form of its solvate with *N*,*N*-dimethylformamide (DMF) was obtained in quantitative yield by the reaction of methyl isocyanate with sodium salt of cyanamide in DMF. The solvate of **4** reacted with hydrogen sulfide in analogy to the sodium salt of 1-cyanourea **3b** and provided an 87% yield of the known (Hecht 1892) 5-methyl-2-thiobiuret **5**. Methylation of sodium salts **3b** and **4** with dimethyl sulfate resulted in 1-cyano-1-methylurea (**6**) and 1-cyano-1,3-dimethylurea (**7**), respectively. These intermediates reacted with hydrogen sulfide in the presence of sodium hydrogensulfide even at room temperature to give the respective 3-methyl-2-thiobiuret (**8**) and 3,5-dimethyl-2-thiobiuret (**9**) which have not been described yet. The different reaction rates of the sodium salts **3b** or **4** and the neutral methyl derivatives **6** or **7** can be explained by repulsions between the negative charge of the anions of **3b** or **4** and the negative charge of the HS-nucleophile. In the case of the neutral molecules **6** or **7**, the reaction rate-lowering action of anionization is absent. Unfortunately, all attempts to achieve cyclization of the methyl derivatives **5**, **8** or **9** to the respective methyl derivatives of **1** by hydrogen peroxide in alkaline medium failed and only decomposition products of the particular compounds were detected. The reason for the much lower stability of these compounds under alkaline conditions in comparison with the unsubstituted thiadiazol **1** is the same as given for the aforementioned ribonucleoside of **1**. For spectral and polarographic measurements, 1-methyl-2-thiobiuret (**10**) was also prepared by a known procedure (Kurzer and Taylor 1958).

The chemical structures of all the prepared compounds were confirmed by MS, UV, ^{1}H and ^{13}C NMR spectra. It is of interest to note that fast atom bombardment mass spectra (FAB MS) of thiobiurets **2**, **5** and **10** resulted also in the formation of dimers. By contrast, the respective 3-methyl and 3,5-dimethyl derivatives of thiobiuret (**8**) and **9** have shown only the presence of monomers at comparable conditions. We hypothesized that the formation of non-covalent dimers of thiobiurets **2**, **5** and **10** is hydrogen bond mediated with the involvement of $N^3-H...O=C^4$ hydrogen bonds.

In continuation of our previous studies on potential carcinogenicity of pyrimidine analogues and their intermediates (Vachálková et al. 1993; Novotný et al. 1994, 1995, 1996a, 1996b, 1999a, 1999b) we were also interested in the polarographic evaluation of the potential carcinogenicity of 5-amino-1,2,4-thiadiazol-3(2*H*)-one (**1**), thiobiuret **2**, its methyl derivatives **5** and **8**–**10** as well as of the sodium salt of 1-cyanourea **3b** (Table). All compounds shown in the Table possess polarographic activity. Polarographic reduction of biuret and related compounds and determination of their potential carcinogenicity were reported previously (Novotný et al. 1996b). For comparison, these results are also included in the Table. The sulfur-containing cytosine analogue, 5-amino-1,2,4- thiadiazol-3(2*H*)-one (**1**) is reduced in anhydrous DMF at the mercury dropping electrode in two one-electron steps with values of the half-wave potential $E_{1/2}$ −1.530 V and −2.740 V vs. SCE, respectively. In comparison to cytosine (Vachálková et al. 1993), which is reduced under the same experimental conditions at $E_{1/2} = -2.410$ V vs. SCE, the displacement of the CH=CH group of the pyrimidine ring of cytosine for the bivalent sulfur not only changed the reduction mechanism but also shifted the half-wave potential $E_{1/2}$ by more than 900 mV towards the positive values.

In our experiments, all the investigated compounds were polarographed in the presence of α-lipoic acid (LA) for determination of their carcinogenic potential. This is based on the fact that LA or more probably its reactive reduction intermediate forms a complex with a polarographed compound, in our case with the thiadiazol **1**, and a new polarographic wave is formed at a different half-wave potential ($E_{1/2} = -1.230$ V vs. SCE) (Fig.). This new polarographic wave is of a diffuse and reversible nature and its height is directly related to the concentration of LA in the polarographed solution. The value of the diffuse current of the newly formed polarographic wave increases linearly with the concentration of LA at the expense of the first wave of 5-amino-1,2,4-thiadiazol-3(2*H*)-one (**1**), recorded without LA, with simultaneous disappearance of the second wave of the tested compound (Fig.). At equimolar concentrations of LA and thiadiazol **1**, only the new wave, representing the complex, is recorded. The nature of this complex has not been clarified. We suppose that a covalent interaction of a reactive radical anion formed as an intermediate of the reduction of LA with the labile N-S bond of **1**. The formation of radical anions in course of electrochemical reduction of disulfides in DMF was recently established (Antonello et al. 2002). The fact that the complex is formed in the course of the polarographic reduction and not before has been confirmed by the measurement of mass- and UV spectra of equimolar mixtures of the thiadiazole **1** or thiobiuret **2** with LA. These meas-

Table: Half-wave potentials $E_{1/2}$ and the potential carcinogenicity parameters, tg α, of the investigated compounds

Compound	$E_{1/2}$ I [V] (SCE)	$E_{1/2}$ II [V] (SCE)	$E_{1/2\ comp\cdot LA}$ [V] (SCE)	tg α	Reference
Hydroxyurea	−2.760	–	−1.290	0.290	Novotný et al. 1996b
Biuret	−2.040	−2.350	−1.250	0.100	Novotný et al. 1996b
5-Amino-1,2,4-thiadiazolin-3(2*H*)-one (**1**)	−1.530	−2.740	−1.230	0.236	This work
2-Thiobiuret (**2**)	−2.650	–	−1.290	0.030	This work
3-Methyl-2-thiobiuret (**8**)	−2.420	–	−1.280	0.030	This work
1-Methyl-2-thiobiuret (**10**)	−2.680	–	−1.360	0.030	Kurzer and Taylor 1958
5-Methyl-2-thiobiuret (**5**)	−2.610	–	−1.260	0.030	This work
3,5-Dimethyl-2-thiobiuret (**9**)	−2.410	–	−1.260	0.030	This work
N-cyanourea sodium salt (**3b**)	−1.940	–	−1.290	0.277	This work

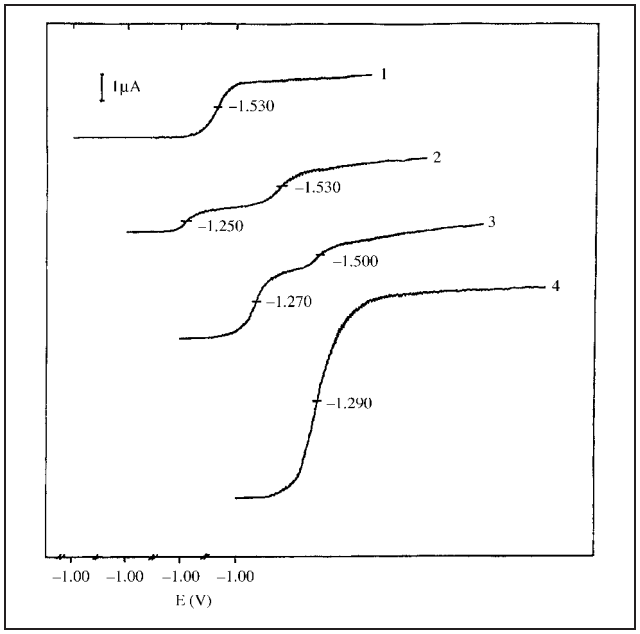


Fig.: Effect of α-lipoic acid (LA) on reduction of 5-amino-1,2,4-thiadiazol-3(2 H)-one ($c = 5 \times 10^{-4}$ mol·l^{-1}) in anhydrous DMF. Concentration of α-lipoic acid c_{LA}: 1: 0 mol·l^{-1}; 2: 8×10^{-5} mol·l^{-1}; 3: 2×10^{-4} mol·l^{-1}; 4: 6×10^{-4} mol·l^{-1}.

urements have shown that LA is not able to form either covalent or non-covalent complexes with these compounds. The structure of the reduction intermediate of LA requires further investigation. However, the study of this problem as well as that of the basis for the correlation of the parameter of potential carcinogenicity, tg α, with carcinogenicity of a respective compound was not the aim of the present paper. It is well known that carcinogenicity is based on covalent interactions of nucleophilic centers of nucleic acids, particularly oncogenes or tumor suppressor genes, with electrophilic centers of carcinogens (Stiborová 2002). We assume that the nucleophilic reactive intermediate of the polarographic reduction of LA forms a covalent adduct with the studied compound and the parameter tg α is a measure of its electrophilicity and hence also potential carcinogenicity. The connection of electrophilicity of chemical compounds with their carcinogenicity is generally accepted (Stiborová 2002). The parameter tg α indicates the ability of the respective compound to react with nucleophilic centers of nucleobases in DNA, which are important for the hydrogen-bond mediated pairing with complementary nucleobases and hence for the correct transfer of genetic information. The radical anion of lipoic acid is considered as a nucleophilic center similar (although more reactive) to nucleophilic centers of nucleic acids (e.g. N^1 of adenine or N^3 of cytosine), which have partial negative charges and can interact with electrophilic centers of carcinogens. The parameter tg α for **1** is 0.236. On the basis of our results already published (Vachálková et al. 1993; Novotný et al. 1994, 1995, 1996a, 1996b, 1999a, 1999b), this value indicates a moderate carcinogenic potential. Additionally, it should be mentioned that the values of the parameter tg α of the compounds determined earlier are in a good agreement with results of biological tests. As an example for the correlation of the parameter of carcinogenicity, tg α, with biological tests could serve a comparison of 5-azacytidine, which exhibites a high value of tg α (Novotný et al. 1994) and has shown an apparent increase in the incidence of tumors in rats treated with this agent (Carr et al. 1984), with 5-aza-2′-deoxycytidine, which exhibites a lower value of tg α (Novotný et al. 1994) and was shown to be non-tumorigenic (Carr et al. 1988). These results also illustrate the connection of carcinogenicity with electrophilicity, because 5-azacytidine is more electrophilic than 5-aza-2′-deoxycytidine. The electrophilic center of these nucleosides [C_6] of the 5-azapyrimidine ring and its electrophilicity, which is connected with low electron density, depends on the inductive effect of the glycosyl residue in position 1. The ribosyl residue, which involves more oxygen atoms than the deoxy derivative, has a higher inductive effect, and for this reason the ribonucleoside is more reactive against nucleophilic agents than its desoxy congener (Benjamin 1980).

Thiobiurets **2**, **5** and **8**–**10** were reduced at the mercury-dropping electrode by the same mechanism involving a single irreversible two-electron step at highly negative values of their half-wave potentials (Table 1). We suggest that the polarographic reduction proceeds at the C=S double bond. When compared with biuret (Novotný et al. 1996b) which is reduced by a two-step process, the $E_{1/2}$ values of thiobiurets are more negative (Table). The new polarographic waves of the complexes formed in presence of LA appear at much more positive potentials characterized by the $E_{1/2}$ values. The new waves moderately increased with increasing concentration of LA. Also in these cases, the structure of the complexes formed in the course of the polarographic reduction of thiobiurets in presence of LA has not been established. We assume a covalent interaction of the C=S groups of thiobiurets with the reactive reduction intermediate of LA. The values of the parameters of potential carcinogenicity, tg α, for thiobiurets (**2**, **5** and **8**–**10**; Table) are much lower when compared with the value determined for biuret (Table), indicating a negligible carcinogenic potential of these substances.

In anhydrous DMF the sodium salt of 1-cyanourea (**3b**) is reduced in a single two-electron irreversible step at $E_{1/2} = -1.940$ V. We suggest that the polarographic reduction proceeds at the cyano group in this case. This is supported by the fact that unsubstituted urea under the same experimental conditions is not reduced on the dropping mercury electrode and does not provide any polarographic wave (Novotný et al. 1996b). When compared with hydroxyurea, the reduction of the sodium salt of 1-cyanourea (**3b**) takes place at a potential that is by 800 mV more positive than the reducing potential of hydroxyurea (Novotný et al. 1996b). In the presence of LA, a new polarographic wave is formed at $E_{1/2} = -1.290$ V. As stated previously, the structure of the complex formed in presence of LA has not been determined. However, we assume that a covalent interaction of the reactive reduction intermediate of LA with the cyano group is formed. The parameter of potential carcinogenicity tg α determined on the basis of the formation of the new polarographic wave is 0.277. The $E_{1/2}$ value of the complex formed in presence of LA is the same as in the cases of hydroxyurea and thiobiuret **2**. However, the value of the parameter of potential carcinogenicity tg α determined for compound **3b** is significantly higher as compared to the value determined for thiobiuret **2** and it is similar to that estimated earlier for hydroxyurea. The parameter tg α value indicates that compound **3b** is, similarly to hydroxyurea, a potential carcinogen and differs in this respect from thiobiuret **2** (Table).

The results obtained by polarographic measurements allow the conclusion that thiobiuret **2** as well as its methyl derivatives **5** and **8**–**10**, hydroxyurea and the sodium salt of 1-cyanourea (**3b**) are reduced on a dropping mercury electrode in anhydrous DMF by the same mechanism invol-

ving a single irreversible two-electron step. Out of all the investigated compounds, only the sulfur-containing cytosine analogue, 5-amino-1,2,4-thiadiazol-3(2*H*)-one (**1**), is reduced under defined experimental conditions in two one-electron steps. Moreover, the mechanism of its reduction is different in comparison to the mechanism of reduction of cytosine (Novotný et al. 1994). The values of tg α determined for thiobiuret **2** and its methyl derivatives **5** and **8**–**10** are marginal. This indicates that these compounds are not carcinogens. However, the value of the parameter tg α determined for 5-amino-1,2,4-thiadiazol-3(2*H*)-one (**1**) is similar to the same parameter of hydroxyurea and the sodium salt of 1-cyanourea (**3b**). These values are significantly higher when compared with thiobiuret **2** and some other compounds and indicate a possible carcinogenicity. The parameter tg α denotes that hydroxyurea is the strongest carcinogen among the three above-mentioned substances. The values of tg α indicate a decrease in the carcinogenic activity in the order hydroxyurea > 1-cyanourea sodium salt (**3b**) > 5-amino-1,2,4-thiadiazol-3(2*H*)-one (**1**).

3. Experimental

3.1. Melting points

Melting points were determined using a heated microscope stage (Kofler block) and were not corrected.

3.2. Thin-layer chromatography

Thin-layer chromatography was performed on Silufol UV 254 plates (Kavalier, Votice, Czech Republic) in the solvent system butan-1-ol/acetic acid/water (5 : 2 : 3) (I) or ethyl acetate (II). The sulfur-containing compounds were detected visually in UV light (254 nm). Cyanoureas and cyanamides were detected with an alkaline solution of sodium nitroprusside and potassium ferricyanide. Dimethylcyanourea was detected with a saturated solution of silver nitrate and heating to 100 °C for 10–20 min.

3.3. UV spectrometry

UV spectra were measured on a Unicam SP 8000 spectrophotometer (Pye Unicam, Cambridge, UK) in buffer solutions of ionic strength 0.01 prepared according to the reference (Perrin 1963). λ values are given in nm and ε in $m^2 \cdot mol^{-1}$.

3.4. NMR spectrometry

NMR spectra were measured on a Varian UNITY 500 instrument (^1H at 500 MHz, ^{13}C at 125.7 MHz) in DMSO-d_6 with the solvent signals as internal references [$\delta(^1H) = 2.50$ ppm, $\delta(^{13}C) = 39.7$ ppm]. The chemical shifts (δ) are given in ppm and coupling constants (J) in Hz.

3.5. Mass spectrometry

Mass spectra (m/z) were measured on a ZAB-EQ (VG Analytical Ltd., Manchester, UK) spectrometer using the EI (electron energy 70 eV) or FAB (ionization by Xe, accelerating voltage 8 kV, matrices glycerol and thioglycerol) techniques.

3.6. Determination of bacteriostatic and cytostatic activity

Stationary cultivation of E. coli was performed at 37 °C in a mineral medium with glucose (Čihák and Šorm 1965). The tested compound was added before inoculation and the growth of bacteria was measured 16 h later. The cytostatic activity was evaluated in L1210, HeLa S3 and HL-60 cell lines according to Hocek et al. (2000).

3.7. DC polarographic experiments

Polarographic experiments were performed on a polarographic analyzer PA4 equipped with a two-line recorder XY 4106 (Laboratorni pristroje, Prague, the Czech Republic). As the indicating electrode, a dropping mercury electrode was used with a drop time of 3 s and a flow rate of 2.27 mg/s at a mercury column height h_{Hg} of 81 cm. As the reference electrode, a saturated calomel electrode (SCE) modified for the work in anhydrous conditions was used. A platinum electrode OH 9377 (Radelkis, Hungary) was used as the auxiliary electrode.
All polarographic measurements were carried out at 25 °C in a stream of dry nitrogen. DMF, used as a solvent, was purified by twice-repeated distillation in vacuum under a stream of dry nitrogen (Riddick and Bunger 1970). The water content in the purified DMF, which was checked periodically by Karl Fischer dead-stop titrations, never exceeded 0.1%. Electrochemically pure tetrabutylammonium perchlorate (TBAP) was used as a supporting electrolyte at 0.15 mM concentration. DMF and TBAP were commercial products of Fluka, Switzerland.
The carcinogenic potential of the synthesized compounds was determined in the presence of a modulator of the carcinogenic process, α-lipoic acid (LA) (Aldrich, Germany). The number of electrons participating in the reduction processes was determined by the logarithmic analysis of polarographic curves as the log $I_d/(I_l - I_d)$ vs. E plots (I_d represents the value of the diffusion current at the potential E, I_l represents the limiting current.)

3.8. Synthesis of the compounds investigated

3.8.1. Sodium salt of 1-cyanourea (**3b**)

A suspension of the silver salt of 1-cyanourea (**3a**, 190.0 g, 1 mol) in a solution of sodium iodide (157 g, 1.05 mol) in water (1000 ml) was stirred at room temperature for 24 h. The precipitate of silver iodide was filtered off with suction, washed with water, the filtrate concentrated at 35 °C (bath temperature) to a small volume (ca. 200 ml) and diluted with acetone (2000 ml). The precipitate was filtered off with suction and washed with acetone to give 99.5 g (93%) of the sodium salt (Bieling et al. 1964) of 1-cyanourea (**3b**). UV spectrum, λ_{max} (log ε): (MeOH), 212 (3.95); (pH 2.32), 224 (2.47); (pH 6.93), 208 (4.07); (pH 10.93), 216 (3.83). ^1H NMR spectrum: 4.87 brs, 2H (NH_2). ^{13}C NMR spectrum: 169.92 s (CO); 124.36 s (CN). MS (FAB): 108 (7) $[M + H]^+$.
$C_2H_2N_3ONa$ (107.1).

3.8.2. Thiobiuret (**2**)

Hydrogen sulfide was bubbled into a solution of the sodium salt of 1-cyanourea (**3b**, 10.7 g, 0.1 mol) in water (50 ml) at 95–100 °C (bath temperature) and the temperature of the reaction mixture was kept for 3 h at 75–80 °C. The mixture was kept overnight in a refrigerator and the deposited crystals were filtered off by suction to give the first crop (3.40 g) of monohydrate of thiobiuret **2**. The mother liquor was reacted again with hydrogen sulfide under the given conditions for 3 h to give a second crop (3.35 g) of the product; a further treatment of the mother liquor with hydrogen sulfide for 4 h gave a third crop. Overall yield: 10.05 g (73%) of monohydrate of thiobiuret **2**, m.p. 192–193 °C (dec.). Recrystallization from water (50 ml) raised the m.p. to 194–195 °C (dec.) (Hecht 1892, reports the m.p. 186 °C (dec.)). The last mother liquor contained predominantly thiourea formed by hydrolysis of **2** as determined by TLC. When the reaction was performed with continuous heating for 12 h, the yield of the product dropped to 44% and the mother liquor contained more thiourea. Drying of the monohydrate at 100 °C/40 Pa for 4 h afforded the water-free product, m.p. 194–195 °C (dec.). UV spectrum, λ_{max} (log ε): (EtOH), 260 (4.38), 213 (3.99); (pH 2.36), 256 (4.19), 219 (3.71); (pH 6.86), 256 (4.22), 208 (4.25); (pH 10.96), 257 (4.15), 217 (3.93). ^1H NMR spectrum: 9.48 brs, 1 H and 8.95 brs, 1 H (1-NH_2); 9.69 s, 1 H (3-NH); 6.95 brs, 1 H and 6.29 brs, 1 H (5-NH_2). ^{13}C NMR spectrum: 187.87 s (C=S); 155.30 s (C=O). MS (FAB): 120 (100) $[M + H]^+$, 239 (5) $[2M + H]^+$.
$C_2H_5N_3OS$ (119.2)

3.8.3. 5-Amino-1,2,4-thiadiazol-3(2H)-one (**1**)

To a stirred solution of thiobiuret (**2**, 0.595 g, 5 mmol) in 2.5 M NaOH (4 ml, 10 mmol) a 6% solution of hydrogen peroxide (4 ml) was added. Due to an exothermic reaction, the mixture was cooled (ice bath) so that the temperature of the reaction mixture did not exceed 50 °C. The solution was kept at 45–50 °C for 5 min, chilled and acidified with a 25% solution of acetic acid in water (4 ml). The mixture was kept at 0 °C for 10 min, filtered off with suction and washed with cold water to give 0.410 g (71%) of 5-amino-1,2,4-thiadiazol-3(2*H*)-one (**1**). The product did not have a defined melting point but when heated above 230 °C, it decomposes without melting (Párkányi et al. 1989 report m.p. 219–221 °C (dec)). UV spectrum, λ_{max} (log ε): (MeOH), 250 (3.68), 221 (4.07); (pH 2.32), 248 (3.75), 224 (3.89); (pH 6.93), 250 (3.64), 218 (4.01); (pH 10.93), 259 (3.64), 217 (3.85). ^1H NMR spectrum: 9.39 brs, 1 H (NH); 8.07 brs, 2 H (NH_2). ^{13}C NMR spectrum: 176.43 s (C=O); 168.49 s (C=N). MS (EI): 117 (72) $[M]^+$.
$C_2H_3N_3OS$ (117.1)

3.8.4. Reduction of 5-amino-1,2,4-thiadiazol-3(2H)-one (**1**) *with L-cysteine hydrochloride*

To a solution of **1** (0.234 g, 2 mmol) in water (80 ml), L-cysteine hydrochloride monohydrate (0.878 g, 5 mmol) was added and the mixture was kept at room temperature for 3 days. The precipitate of L-cystine (0.224 g; 93 % based on **1**; m.p. > 240 °C (dec.)) was filtered off with suction, washed with water and the filtrate evaporated to a syrup that was dissolved in water (1 ml) and which kept overnight at room temperature to give 0.151 g (58%) of monohydrate of thiobiuret **2**, m.p. 192–194 °C (dec), undepressed as admixture with an authentic sample. The mother liquor contained predominantly compound (**2**) as determined by TLC.

3.8.5. Alkaline degradation of 5-amino-1,2,4-thiadiazol-3(2H)-one (**1**)

A solution of **1** (0.234 g, 2 mmol) in 2.5 M NaOH (5 ml) was heated at 100 °C (bath temperature) for 1 h. TLC of the reaction mixture indicated the presence of 1-cyanourea (major product) and cyanamide (minor product). The cooled solution was neutralized with diluted nitric acid (1 : 1) (hydrogen sulfide evolves) and the precipitate of elemental sulfur was filtered off by suction (5 mg, 8%). The filtrate was acidified with nitric acid to pH 3 and than reacted with a solution of silver nitrate (1 g) in water (5 ml). The black precipitate was filtered off by suction, washed with water and suspended in dilute ammonia (1 : 1) (4 ml). The insoluble black precipitate of silver sulfide was filtered off [0.150 g (54%)] and the filtrate was acidified with dilute nitric acid to give the white precipitate of the silver salt of 1-cyanourea (**3a**, 0.304 g, 80%).
$C_2H_2N_3OAg$ (189.9)

3.8.6. 5-Methyl-2-thiobiuret (**5**)

To a stirred suspension of sodium salt of cyanamide (6.40 g, 100 mmol) in anhydrous DMF (50 ml), methyl isocyanate (6.5 ml) was added dropwise excluding atmospheric moisture and cooling with ice. The mixture was kept at room temperature for 1 h. The voluminous precipitate was filtered off by suction and washed with ether to give 19.42 g (100%) of the DMF solvate of the sodium salt of 1-cyano-3-methylurea (**4**) as fine hygroscopic needles. This product (9.70 g, 50 mmol) was reacted with hydrogen sulfide in analogy to the preparation of 2-thiobiuret (**2**) to give 5.82 g (87%) of 5-methyl-2-thiobiuret (**5**), m.p. 208–210 °C (dec.). TLC of the mother liquor indicated also the presence of thiourea formed by decomposition of **5**. Recrystallization from water raised the m.p. to 210–211 °C (dec.) (Hecht 1892, reports m.p. 194 C (dec.)). UV spectrum, λ_{max} (log ε): (EtOH), 259 (4.31), 215 (4.04); (pH 2.36), 255 (4.27), 223 (3.96); (pH 6.86), 255 (4.35), 211 (4.40); (pH 10.96), 256 (4.22), 218 (4.11). ^{1}H NMR spectrum: 9.42 brs, 1 H and 8.93 brs, 1 H (5-NH_2); 9.77 s, 1 H (3-NH); 6.67 q, 1 H, J(NH, CH_3) = 4.6 (1-NH); 2.62 d, 3 H, J(CH_3, NH) = 4.6 (CH_3). ^{13}C NMR spectrum: 181.51 s (C=S); 154.85 q, ^{3}J(CO, CH_3) = 3.9 (C=O); 25.92 qd, ^{1}J(C, H) = 137.7, ^{2}J(CH_3,NH) = 2.9 (CH_3). MS (FAB): 134 (100) $[M+H]^+$, 267 (8) $[2M+H]^+$.
$C_3H_7N_3OS$ (133.2)

3.8.7. 3-Methyl-2-thiobiuret (**8**)

To a solution of the sodium salt of 1-cyanourea (**3b**, 10.7 g, 0.1 mol) in water (50 ml), dimethyl sulfate (9 ml) was added, and the mixture was stirred intensively at room temperature for 105 min. The mixture was concentrated to a small volume and the crystals were filtered off with suction to give 4.55 g (46%) of 1-cyano-1-methylurea (**6**), m.p. 146–149 °C. Recrystallization from acetone raised the m.p. to 149–150 °C. UV spectrum, λ_{max} (log ε): (EtOH), 208 (2.80); (pH 2.32), 229 (3.00); (pH 6.94), 228 (3.13); (pH 10.93), 215 (3.22).
A suspension of **6** (0.99 g, 10 mmol) in water (10 ml) was cooled in an ice bath and saturated with hydrogen sulfide for 15 min. Then sodium hydrogensulfide (0.56 g, 10 mmol) was added, and the mixture was saturated again with hydrogen sulfide for 30 min. The mixture was kept in a refrigerator for 5 h and the crystalline precipitate filtered off by suction to give 0.88 g (66%) of 3-methyl-2-thiobiuret (**8**), m.p. 171–173 °C (dec.). TLC of the mother liquor indicated the presence of *N*-methylthiourea formed by decomposition of **8**. Re-crystallization of **8** from water raised the m.p. to 173–175 °C (dec.). UV spectrum, λ_{max} (log ε): (EtOH), 261 (4.21), 217 (3.96); (pH 2.32), 257 (4.15), 223 (3.97); (pH 6.93), 257 (4.16), 212 (4.14); (pH 10.93) (decomposition), 235 (4.08), 218 (3.91). ^{1}H NMR spectrum: 10.07 brs, 1 H and 8.94 brs, 1 H (1-NH_2); 7.28 brs, 2 H (5-NH_2); 3.49 s, 3 H (CH_3). ^{13}C NMR spectrum: 185.085 q, ^{3}J(CS, CH_3) = 3.9 (C=S); 158.16 s (C=O); 37.18 q, ^{1}J(C,H) = 140.6 (CH_3). MS (FAB): 134 (56) $[M+H]^+$.
$C_3H_7N_3OS$ (133.2)

3.8.8. 3,5-Dimethyl-2-thiobiuret (**9**)

To a solution of the sodium salt of 1-cyano-3-methylurea (**4**, as a solvate with DMF, 5.82 g, 30 mmol) in water (15 ml), dimethyl sulfate (2.7 ml) was added and the mixture was intensively stirred at room temperature for 2 h. The product was extracted with chloroform (3 × 25 ml), the organic phase was dried with anhydrous magnesium sulfate and evaporated. Crystallization of the residue from benzene afforded 1.80 g (53%) of 1-cyano-1,3-dimethylurea (**7**), m.p. 113–114 °C (Slotta and Tschesche 1929, report m.p. 114 °C). A suspension of this product (1.13 g, 10 mmol) in water (10 ml) was reacted with hydrogen sulfide and sodium hydrogensulfide in analogy to the preparation of 3-methyl-2-thiobiuret (**8**) to give 1.25 g (85%) of 3,5-dimethyl-2-thiobiuret (**9**), m.p. 106–107 °C. TLC of the mother liquor indicated only the presence of the title product **9**, no *N*-methylthiourea was detected. Re-crystallization of the product from benzene raised the m.p. to 107–108 °C. UV spectrum, λ_{max} (log ε): (EtOH), 261 (4.21), 222 (4.04); (pH 2.32), 256 (4.14), 227 (3.98); (pH 6.93), 255 (4.14), 213 (4.05); (pH 10.93), 255 (4.14), 222 (4.03). ^{1}H NMR spectrum: 9.78 brs, 1 H and 8.87 brs, 1 H (5-NH_2); 7.74 q, 1 H, J(NH, CH_3) = 4.3 (1-NH); 3.48 s, 3 H (N–CH_3); 2.65 d, 3 H, J(CH_3, NH) = 4.3 (NH–CH_3). ^{13}C NMR spectrum: 184. 565 q, ^{3}J(CS, CH_3) = 3.9 (C=S); 157.25 okt, ^{3}J(CO, CH_3) = 2.9 (C=O); 36.75 q, ^{1}J(C, H) = 140.6 (N–CH_3); 27.41 qd, ^{1}J(C, H) = 138.7, ^{2}J(CH_3, NH) = 2.9 (NH-CH_3). MS (FAB): 148 (100) $[M+H]^+$.
$C_4H_9N_3OS$ (147.2)

3.8.9. 1-Methyl-2-thiobiuret (**10**)

1-Methyl-2-thiobiuret (**10**) was prepared by a published procedure; Kurzer and Taylor (1958) report m.p. 174–175 °C (dec.). UV spectrum, λ_{max} (log ε): (EtOH), 256 (4.37); (pH 2.32), 251 (4.21), 223 (4.07); (pH 6,93), 251 (4.21), 218 (4.27); (pH 10.94), 251 (4.19), 219 (4.17). ^{1}H NMR spectrum: 10.29 q, 1 H, J(NH, CH_3) = 4.8 (1-NH); 9.73 s, 1 H (3-NH); 6.91 brs, 1 H and 6.27 brs, 1 H (5-NH_2); 2.98 d, 3 H, J(CH_3, NH) = 4.8 (CH_3). ^{13}C NMR spectrum: 181.07 q, ^{3}J(CS, CH_3) = 4.9 (C=S); 155.68 s (C=O); 31.53 qd, ^{1}J(C, H) = 138.7, ^{2}J(CH_3, NH) = 2.9 (CH_3). MS (FAB): 134 (100) $[M+H]^+$, 267 (7) $[2M+H]^+$.
$C_3H_7N_3OS$ (133.2)

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