Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 157–162

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis, Spectroscopic Characterization and Biological Properties of New Natural Aldehydes Thiosemicarbazones

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Received 27 July 1999; accepted 8 September 1999

Abstract—As part of a research programme aimed at the synthesis of compounds with antiviral, antibacterial and antitumor properties and their spectroscopic characterization, new thiosemicarbazones deriving from natural aldehydes have been investigated. These substances contain in the same molecule both a chain with nucleophilic centres N, S with tubercolostatic activity, and a glycosidic or alkyl moiety (modified glycosides and nucleosides have recently received a great deal of attention in the fields of neoplastic diseases and viral infections). In this paper the synthesis and the characterization of these compounds by means of ^{1}H NMR, IR, and MS techniques is reported. Biological studies have involved both inhibition of cell proliferation and apoptosis tests on human leukemia cell line U937.

Introduction

Compounds potentially useful for their pharmacological properties have been often obtained by combining, in the same organic molecule, chemical species biologically active that already possess, by themselves, different reaction selectivity or different action mechanism.[1–4] A proper choice of these interacting molecules[5] should make easier the creation of non-covalent interactions with DNA, so that the resulting compounds could play an important role for the biological activity.

Recently, the antiviral and antineoplastic activity of glycosides and modified nucleosides has been discovered.[6–8] Since the biological activity carried out in vitro by thiosemicarbazones is widely known in the pharmacological field,[1–4] we have synthesized compounds that bear in the same molecule different chemical species, possessing biological properties, with the aim to examine their biological cooperative effect.

Then, as part of a research programme aimed at the synthesis of compounds with antiviral, antibacterial and antitumor properties,[9–11] new thiosemicarbazones deriving from natural aldehydes, such as citronellal, citral, xylopentadialdofuranose, glyceraldehyde, and from a nucleoside as uridine were synthesized, and subsequently characterized by IR and NMR spectroscopies. Moreover, trying to correlate experimental data and biological activity of these new compounds, assays on inhibition of proliferation and on apoptosis were made on cellular hystiocytic lines U937, in collaboration with the Institute of Medical Special Pathology of the University of Parma. All compounds have demonstrated to inhibit cell growth and one of them, the (3*R*)-(+)-citronellal thiosemicarbazone (**1**; Scheme 1), to induce apoptosis.

Results and Discussion

Synthesis of ligands 1–5

All compounds were obtained by an improved condensation reaction of the aldehyde with thiosemicarbazide, under ultrasonic irradiation to increase the

Keywords: natural products; antitumor compounds; leukemia; DNA.
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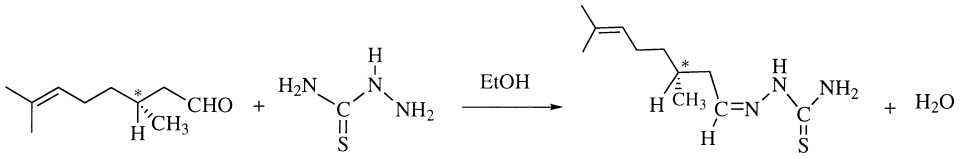
0968-0896/00/$ - see front matter
PII: S0968-0896(99)00260-6



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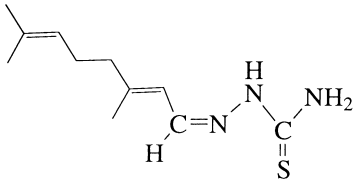
Scheme 1.

reagents solubility, to afford the corresponding thiosemicarbazones in a quantitative yield: the case of (3*R*)-(+)-citronellal (95% yield) is reported in Scheme 1.

By following the same procedure, also citral reacted with thiosemicarbazide to give thiosemicarbazone **2** (92% yield) (Fig. 1). We chose the natural aldehydes (3*R*)-(+)-citronellal and citral to be transformed into the corresponding thiosemicarbazones because, in the course of our studies about porphyrins bearing natural aldehydes residues in the *meso* positions, we found that lipophylic compounds were more effective than the hydrophylic ones as far as the biological activity was concerned (apoptosis tests on human leukemia cell line U937).[12]

Compounds **3** and **4** (Fig. 1) were synthesized in the same way as the previous products (90% yield), but for **4**, derived from glyceraldehyde, synthesis needed an intermediate step, in fact 2,3-*O*-isopropylidene-D-glyceraldehyde was obtained from 1,2;5,6-di-*O*-isopropylidene-D-mannitol according to literature.[13]



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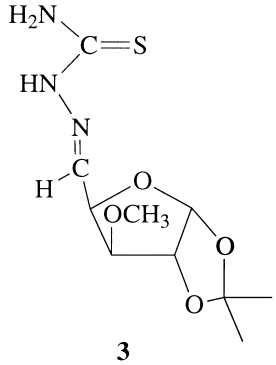

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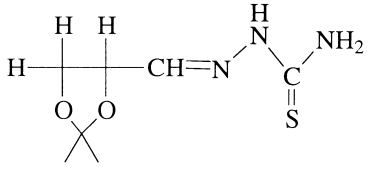

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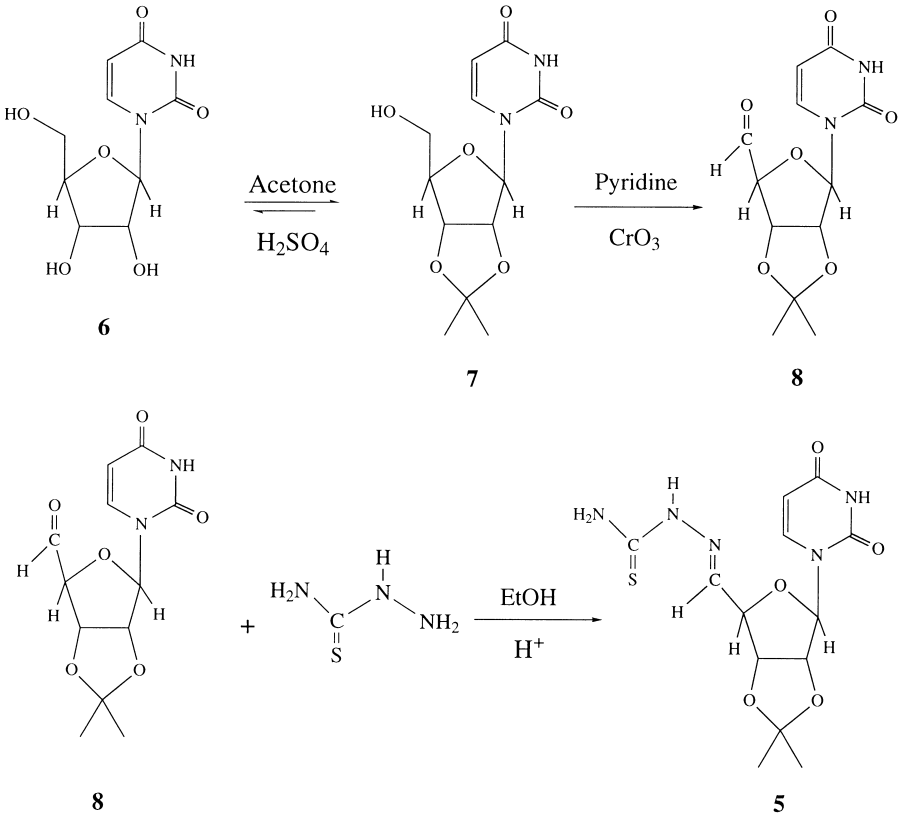
Figure 1.

We used the following strategy to obtain the uridine thiosemicarbazone (**5**; Scheme 2): a few more steps were needed before the condensation with thiosemicarbazide. The uridine thiosemicarbazone (**5**) was afforded starting from uridine (**6**), after protecting the C-2′ and C-3′ carbons (**7**), to enhance its solubility in organic apolar aprotic solvents in which the subsequent synthetic steps took place, and contemporaneously avoid the oxidation of the hydroxylic secondary functions. The reaction of protection was practically quantitative if carried out in dry acetone in the presence of sulphuric acid.[14] Compound **7** was oxidized to aldehyde (**8**), with a mixture of CrO_3 and pyridine in CH_2Cl_2:DMF 4:1, according to our precedent optimization studies.[15] Then the aldehyde function was condensed with thiosemicarbazide in MeOH, under ultrasonic irradiation for 2 h, to get after TLC purification with ethylacetate:MeOH (80:20) the product **5** (90% yield).

The choice of uridinil-5′-carboxyaldehyde was made in agreement with the recent results reported in the literature[6–8] any modifications in the nucleosides are of great interest in the biomedical field because they can lead to compounds with antitumor and/or antiviral properties. Moreover, we believe that the isopropylidene protected hydroxyls could favour the passage of the molecule through the cell membrane. In fact compound **5** showed a good cell proliferation inhibition (see also the Biological tests paragraph, Fig. 2, lane 3), but did not result active in the apoptosis induction. At this point it could be important to understand whether the whole nucleoside molecule or just the sugar moiety was responsible for the biological activity, thus we synthesized and tested a few thiosemicarbazones derived from sugars, such as the protected cyclic carbohydrate xylopentadialdofuranose (**3**) and the less hindered 2,3-*O*-isopropylidene-D-glyceraldehyde (**4**). Both these compounds did not show remarkable activities, and this feature made us consider that the whole nucleobase would act as to determine the biological activity.

Characterization of compounds 1–5

The ^1H NMR spectrum of compound **1** (300 MHz, $CDCl_3$) shows, at room temperature, a singlet at 9.76 ppm relative to the NH next to C=S, very deshielded as expected, while the signal of the proton on the C=N double bond appears at 7.29 ppm. It is interesting



Scheme 2.

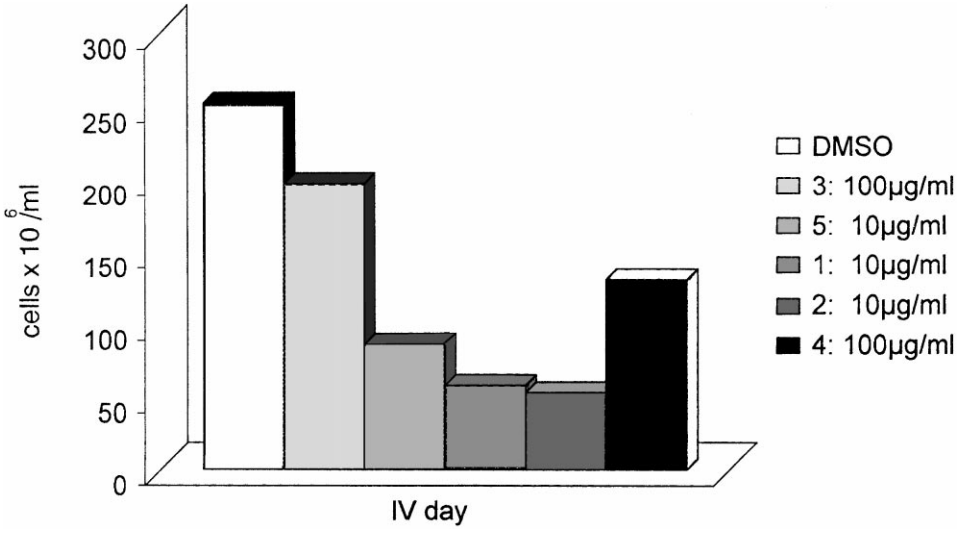


Figure 2. Effects of compounds **1**–**5** on U937 proliferation on the 4th day.

to notice the presence of two broad singlets for the two NH_2 protons, respectively at 7.05 and 6.38 ppm: it means that the free rotation around the C–N bond is blocked because of its partial double bond character.[16] In the IR spectrum of **1** the bands in the region 3407–3261 cm^{-1} are due to the stretching frequencies ν (NH_2), while ν (NH) is present at 3162 cm^{-1}. The typical bands of the aliphatic CH appear in the frequency range 3026–2915 cm^{-1}, while ν (CN) and ν (C=C) give just one band, by superimposition, at 1595 cm^{-1}. No band due to the SH group is observed between 2600 and 2500 cm^{-1}, in agreement with the thionic form of ligand **1** and with the presence of bands ν (CS) at 928 and 820 cm^{-1}.

The ^1H NMR spectrum of compound **2** (300 MHz, $CDCl_3$) shows at 10.48 ppm the singlet relative to the NH next to C=S, at 7.94 ppm the signal of the proton on the C=N double bond, and, in the same way as compound **1**, two broad singlets for the two NH_2 protons, respectively at 7.03 and 6.77 ppm.[16] In the IR spectrum of **2** the bands at 3381 and 3264 cm^{-1} are due to the stretching frequencies ν (NH_2), while ν (NH)

is present at 3161 cm^{-1}. The spectrum is very similar to the previous one: in the frequency range 3030–2945 cm^{-1} appear the typical bands of the aliphatic CH, the ν (CN) is present at 1641 cm^{-1}, the ν (C=C) at 1607 cm^{-1}, and the ν (CS) at 835 cm^{-1}.

For compound **3** (300 MHz, CD_3COCD_3) the singlet of the NHCS appears at 11.39 ppm, very deshielded; the two broad singlets of the NH_2 protons are observed at 8.12 and 7.74 ppm, and the signal of the proton on the C=N double bond at 7.28 ppm.

Thiosemicarbazones **4** and **5** (300 MHz, DMSO) behave in a similar way, giving rise to signals in the same ranges as found for **1**–**3**. For compound **4** the singlet of the NHCS appears at 9.86 ppm, the two broad singlets of NH_2 at 7.95 and 7.48 ppm, and the CH=N signal at 7.06 ppm. In the IR spectrum of **4** the bands in the region 3396–3280 cm^{-1} are due to the stretching frequencies ν (NH_2), while ν (NH) is observed at 3197 cm^{-1}. In the frequency range 3064–2926 cm^{-1} appear the typical bands of the aliphatic CH, while the ν (CO) and ν (CN) produce two bands, respectively, at 1605 and 1562 cm^{-1}. Finally, in analogy with the behaviour shown by products **1** and **2**, no band due to the SH group is observed between 2600 and 2500 cm^{-1}, in agreement with the thionic form of ligand **4** and with the presence of bands ν (CS) at 935 and 828 cm^{-1}. For compound **5** the ^1H NMR spectrum is quite complicated. A broad singlet relative to the two NH, the one next to C=S and the heterocyclic NH-3, appear at 11.3 ppm; the NH_2 gives rise to two broad singlets at 8.15 and 7.96 ppm, while the doublet of the CH=N proton is observed at 7.45 ppm.

Biological tests: effects on cell proliferation and apoptosis induction

Compounds **1**–**5** have been tested in vitro on human leukemic cell lines U937, focusing our attention on their activity with respect to *cell proliferation inhibition* and *apoptosis induction*. It has been reported that a number of anticancer drugs may exert their activity by inducing *apoptosis*.[17] The mechanism underlying the effects varies depending on the initiating stimulus but a common feature is the activation of the endonuclease leading to DNA fragmentation. Thiosemicarbazones **1**, **2** and **5** inhibited remarkably *cell proliferation* when used at 10 μg/mL, while **4** (100 μg/mL) was less effective, and ligand **3**, even at larger concentrations (100 μg/mL), did not show any effect (Fig. 2).

Using these products at the minimum concentration values at which the maximum inhibition was observed, only compound **1**, derived from (3*R*)-(+)-citronellal, was able to cause the typical DNA fragmentation at 10 g/mL, inducing *apoptosis* (Fig. 3). A striking feature is that, notwithstanding the similarity between compounds **1** and **2** and their analogous behaviour with respect to cell inhibition, only compound **1** showed apoptotic activity. Further studies are now in progress in our laboratories to ascertain a possible different mechanism of action between the two.

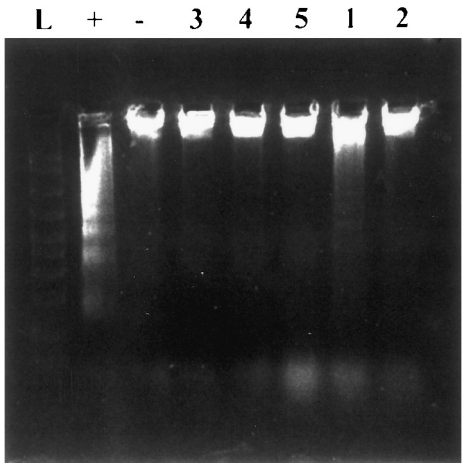


Figure 3. Agarose gel electrophoresis of cellular DNA. The ethidium bromide-stained agarose gel shows DNA from U937 cells treated as follows: lane L ladder; lane + positive control; lane − untreated control cells; lane 3 compound **3**; lane 4 compound **4**; lane 5 compound **5**; lane 1 compound **1**; lane 2 compound **2**.

Experimental

General

All the materials and solvents were obtained from commercial suppliers and used without further purification. Thiosemicarbazide was purchased from Janssen, citral from Sigma, while (3*R*)-(+)-citronellal, 1,2-*O*-isopropylidene-3-*O*-methyl- α-D-xylopentodialdofuranose, uridine and 1,2;5,6-di-*O*-isopropylidene-D-mannitol from Fluka. 2,3-*O*-Isopropylidene-D-glyceraldehyde was obtained from 1,2;5,6-di-*O*-isopropylidene-D-mannitol according to literature.[13] TLC were performed on E. Merck plates precoated with SiO_2 F_{254}. Sonication was performed by a Branson Type 2200 instrument (120 W, 220–240 V, 50–60 Hz) operating at a 30–50 kHz frequency. ^1H NMR spectra were obtained at room temperature on a Brucker AMX-300 spectrometer, and chemical shifts are given in units of δ relative to TMS as an internal reference. For compounds **4** and **5**, dissolved in DMSO-d_6, the two signals of DMSO and water arose respectively at 2.5 and 3.4 ppm. Optical rotations were measured on a Rudolph Autopol III polarimeter. FT–IR spectra were recorded on a Nicolet 5PCFT–IR infrared spectrophotometer as KBr pellets, in the frequency range 4000–400 cm^{-1}. CI–MS (*m*/*z*, 70 eV) were obtained on a Finnegan 1020 6c mass spectrometer. Elemental analyses were performed by the Microanalytical Laboratory of the University of Parma (Dipartimento di Chimica Generale ed Inorganica).

Synthesis of the natural aldehydes thiosemicarbazones (1–5)

(3*R*)-(+)-Citronellal thiosemicarbazone (1): [(3*R*)-3,7-dimethyl-6-octenyl]-1-carboxyaldehydethiosemicarbazone (1). To a solution of thiosemicarbazide (178 mg, 1.95 mmol) in EtOH 95% (10 mL) was added, with stirring, a solution of (3*R*)-(+)-citronellal (0.35 mL, 1.95 mmol) in EtOH 95% (10 mL). After 60 min under

ultrasonic irradiation (40 °C) the mixture was cooled, and compound **1** was isolated as a white powder (95% yield). $[\alpha]^{20}_{546}$ + 30.9; $[\alpha]^{20}_{589}$ + 46.5 (c = 0.082, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.76 (1H, s, NHCS), 7.29 (1H, t, J = 6.0 Hz, CH=N), 7.05 and 6.38 (2H, 2 br s, 1H each, NH_2), 5.05 (1H, m, C*H*=C(CH_3)$_2$), 2.24 (1H, m, CH_2 in C-2, H-α), 1.94 (2H, m, CH_2 in C-5), 1.73 (1H, m, CH_2 in C-2, H-β), 1.66 and 1.57 (6H, 2s, 3H each, C*H*$_3$C=C), 1.35 (1H, m, C*H*CH_3), 1.32 and 1.23 (2H, 2m, 1H each, CH_2 in C-4, H-α + H-β), 0.92 (3H, d, J = 7.0, CHC*H*$_3$). FT IR (KBr, cm^{-1}) ν (NH_2) 3407 (s), 3261 (s, br); ν (NH) 3162 (s); ν (CH_3) 3026 (m); ν (CH_2) 2962 (s), 2915 (s); ν (CN) + ν (C=C) 1595 (s); ν (CS) 928 (w), 820 (m). MS (CI, CH_4) 228 (M + 1). Anal. calcd for $C_{11}H_{21}N_3S$: C 58.11, H 9.32, N 18.49, S 14.08; found: C 58.02, H 9.22, N 18.61, S 14.15.

Citral thiosemicarbazone (2): [3,7-dimethyl-2,6-octadienyl]-1-carboxyaldehydethiosemicarbazone (2). To a solution of thiosemicarbazide (178 mg, 1.95 mmol) in EtOH 95% (10 mL), was added, with stirring, a solution of citral (0.33 mL, 1.95 mmol) in EtOH 95% (10 mL). After 60 min under ultrasonic irradiation (40 °C) the mixture was cooled, and compound **2** was isolated as a yellow powder (92% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.48 (1H, s, NHCS), 7.94 (1H, d, J = 12.1 Hz, CH=N), 7.03 and 6.77 (2H, 2 br s, 1H each, NH_2), 5.80 (1H, dm, C*H*=C in C-2), 4.98 (1H, m, C*H* = C(CH_3)$_2$ in C-6), 2.15 (2H, m, CH_2 in C-4), 1.82 (2H, m, CH_2 in C-5), 1.73 (3H, d, J = 3.0, CH_3 in C-3), 1.58 and 1.49 (6H, 2s, 3H each, C*H*$_3$C=C). FT IR (KBr, cm^{-1}) ν (NH_2) 3381 (s, br), 3264 (s); ν (NH) 3161 (s); ν (CH_3) 3030 (m); ν (CH_2) 2971 (s), 2945 (m); ν (CN) 1641 (m); ν (C=C) 1607 (s); ν (CS) 835 (m). MS (CI, CH_4) 226 (M + 1). Anal. calcd for $C_{11}H_{19}N_3S$: C 58.63, H 8.51, N 18.66, S 14.2; found: C 58.51, H 8.41, N 18.78, S 14.3.

Xylopentadialdofuranose thiosemicarbazone (3): 1′,2′-*O*-isopropylidene-3′-*O*-methyl-5′-deoxy-α-D-xylo-pentafuranosil-5′-carboxyaldehydethiosemicarbazone (3). To a solution of thiosemicarbazide (50 mg, 0.55 mmol) in EtOH 95% (15 mL), under stirring, was added 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylopentodialdofuranose (111 mg, 0.55 mmol) in EtOH 95% (5 mL). After 2 h under ultrasonic irradiation (40 °C) the mixture was cooled, and compound **3** was obtained as a colourless oil (90% yield). $[\alpha]^{20}_{546}$ + 57.2; $[\alpha]^{20}_{589}$ + 112.5 (c = 0.070, CH_3COCH_3). ^{1}H NMR (300 MHz, CD_3COCD_3) δ 11.39 (1H, s, NHCS), 8.12 and 7.74 (2H, 2 br s, 1H each, NH_2), 7.28 (1H, d, J = 7.0 Hz, CH=N), 5.89 (1H, d, J = 4.0 Hz, H-1′), 4.77 (1H, dd, J = 7.0, 3.0 Hz, H-4′), 4.62 (2H, m, H-2′ + H-3′), 3.32 (3H, s, OCH_3), 1.39 and 1.26 (6H, 2s, 3H each, CH_3). MS (CI, CH_4) 276 (M + 1). Anal. calcd for $C_{10}H_{17}N_3O_4S$: C 43.62, H 6.23, N 15.27, S 11.62; found: C 43.5, H 6.14, N 15.38, S 11.77.

2′,3′-*O*-Isopropylidene-D-glyceraldehyde thiosemicarbazone (4): 2′,3′-*O*-isopropylidene-2′,3′-dihydroxyethyl-1-carboxyaldehydethiosemicarbazone (4). An aqueous solution (10 mL) of $NaIO_4$ (462 mg, 2.16 mmol) was added to SiO_2 (4282 mg, 71.28 mmol) and CH_2Cl_2 (80 mL), under stirring and at room temperature. Then 1,2;5,6-di-*O*-isopropylidene-D-mannitol (569 mg, 2.16 mmol) and CH_2Cl_2 (10 mL) were poured in the reaction vessel.[13] After 10 min the solution was filtered on Buchner and dried over $MgSO_4$ (5 g) to give, after filtering and concentrating, 2,3-*O*-isopropylidene-D-glyceraldehyde (325 mg, 2.5 mmol) (70% yield), that was subsequently added, in EtOH 95% (5 mL), to a solution of thiosemicarbazide (228 mg, 2.5 mmol) in EtOH 95% (20 mL), under stirring and sonication (40 °C). After 3 h the mixture was cooled, and compound **4** was obtained as a yellow powder (90% yield). $[\alpha]^{20}_{546}$ + 56.4; $[\alpha]^{20}_{589}$ + 87.6 (c = 0.093, DMSO). ^{1}H NMR (300 MHz, DMSO) δ 9.86 (1H, s, NHCS), 7.95 and 7.48 (2H, 2 br s, 1H each, NH_2), 7.06 (1H, br s, CH=N), 4.05 (1H, m, H-2′), 3.95 (1H, m, CH_2 in C-3′, H-α), 3.80 (1H, m, CH_2 in C-3′, H-β), 1.29 and 1.25 (6H, 2s, 3H each, CH_3). FT IR (KBr, cm^{-1}) ν (NH_2) 3396 (s), 3280 (s, br); ν (NH) 3197 (s); ν (CH) 3064 (m), 3013 (m), 2926 (m); ν (CO) + ν (CN) 1605 (s), 1562 (s); ν (CS) 935 (w), 828 (m). MS (CI, CH_4) 204 (M + 1). Anal. calcd for $C_7H_{13}N_3O_2S$: C 41.36, H 6.45, N 20.69, S 15.74; found: C 41.25, H 6.38, N 20.77, S 15.89.

Uridine thiosemicarbazone (5): 2′,3′-*O*-isopropylidene-4′-dehydroxymethyluridinil-5′-carboxyaldehydethiosemicarbazone (5). To a solution of uridine (**6**, 601 mg, 2.46 mmol) in distilled acetone (60 mL) was added dropwise H_2SO_4 96% (12 drops, pH 3) under nitrogen atmosphere and stirring. After 3 h the reaction mixture was neutralised with solid $BaCO_3$ (5 g, pH 6) and filtered on a Buchner apparatus. The resulting light yellow oil was evaporated under vacuum and purified by flash chromatography (ethyl acetate:MeOH 20%) to give the protected uridine (2′,3′-*O*-isopropylidene-uridine) **7** (83% yield) as a yellow oil. Then a mixture consisting of CrO_3 (704 mg, 7.04 mmol) and pyridine (1.14 mL, 14.08 mmol) in CH_2Cl_2:DMF 4:1 (10 mL), was stirred under nitrogen atmosphere, at room temperature: after 30 min compound **7** (500 mg, 1.76 mmol) in CH_2Cl_2: DMF 4:1 (10 mL) was added, together with acetic anhydride (0.66 mL, 7.04 mmol), and oxidized. The reaction mixture was stirred for another 15 min, and then quenched with EtOH 95% (1 mL), poured in ethyl acetate (100 mL), filtered on silica recovered with dry Na_2SO_4, and evaporated under vacuum affording **8** (2′,3′-*O*-isopropylidene-uridine-5′-carboxyaldehyde, 80% yield) as a colourless oil. **8** (401 mg, 1.42 mmol) in MeOH (10 mL) was subsequently condensed with the thiosemicarbazide (129 mg, 1.42 mmol) previously dissolved in MeOH (10 mL), under nitrogen atmosphere and stirring. After 2 h under ultrasonic irradiation (40 °C) the reaction mixture was filtered and purified by TLC purification with ethylacetate:MeOH (80:20) to obtain **5** (uridine thiosemicarbazone) as a yellow oil (361 mg, 90% yield). $[\alpha]^{20}_{546}$ + 88.3; $[\alpha]^{20}_{589}$ + 128.4 (c = 0.055, DMSO). ^{1}H NMR (300 MHz, DMSO) δ 11.30 (2H, br s, NHCS + NH-3), 8.15 and 7.96 (2H, 2s, 1H each, NH_2), 7.78 (1H, d, J = 8.1 Hz, H-6), 7.45 (1H, d, J = 4.5 Hz, CH=N), 5.85 (1H, d, J = 8.1 Hz, H-5), 5.62 (1H, m, H-4′), 5.18 (1H, d, J = 0.7 Hz, H-1′), 4.60 (1H, m, H-2′), 4.05 (1H, m, H-3′), 2.90 and 2.70 (6H, 2s, 3H each, CH_3). MS (CI, CH_4) 356 (M + 1). Anal. calcd for $C_{13}H_{17}N_5O_5S$: C 43.93, H 4.82, N 19.72, S 9.00; found: C 43.81, H 4.73, N 19.81, S 9.16.

Biological data: materials and methods

The cells U937 were seeded at 2×10^5/mL in the presence of RPMI 1640 supplemented with L-glutamine 200 mM, 10% fetal bovine serum (FBS) and with antibiotics (penicillin 100 U/mL and streptomycin 100 μg/mL), and then treated with compounds **1**–**5**. All compounds were previously stored dry at room temperature and dissolved in dimethyl sulphoxide (DMSO) just before their use. Cell mortality, evaluated on the fourth day by the trypan blue exclusion method and determined by using a hemocytometer, never exceeds 5%. For the apoptosis assay the cells U937 were seeded at 5×10^5/mL in the presence of the above mentioned products at the same concentrations as used for the proliferation inhibition. After 18 h the cells (10^6) were washed twice with PBS at 2000 rpm for 10 min at 4 °C, and then 20μL of a lysis buffer solution (EDTA 10 mM, Tris HCl 50 mM pH 8.0 and 0.5% (w/v) sodium laurylsarkosinate) were added. The pellet was subsequently redissolved and 2.5 μl of Proteinase K was added (4 mg/mL). After 1 h at 50 °C, 2.5 μg/mL of RNAse A (2 mg/mL) was added and incubated for 1 h at 40 °C and then for 10 min at 70 °C. 10 μL of loading buffer (EDTA 10 mM pH 8, 1% agarose, 0.25% bromophenol blue, 40% sucrose) were subsequently added to the extracted DNA, which was loaded on the agarose gel (2%) with 0.1 μg/mL ethidium bromide to evaluate the characteristic effects of apoptosis by means of electrophoresis.

Acknowledgements

This work was supported by Italian MURST (60%) and by AIRC (Associazione Italiana per la Ricerca sul Cancro), Italy. The authors acknowledge the Centro Interfacoltà di Misure "Giuseppe Casnati" dell'Università di Parma for NMR and MS instrumental facilities.

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