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1,2-Dithiolan-3-ones and derivatives structurally related to leinamycin. Synthesis and biological evaluation

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Abstract

Leinamycin, an antitumor antibiotic isolated from *Streptomyces* sp., shows a 1,2-dithiolan-3-one 1-oxide heterocycle that appears to be involved in the biological activity. Several derivatives related to 1,2-dithiolan-3-one 1-oxide have been prepared and their activity as antineoplastic agents have been investigated. The synthesized compounds did not display a significative antitumor or cytotoxic activity in vitro.

Keywords: Leinamycin; 1,2-Dithiolan-3-ones-1-oxide; Antineoplastic activity

1. Introduction

Many antibiotics are routinely used as antineoplastic agents. Leinamycin (Fig. 1), an antitumor antibiotic isolated from *Streptomyces* sp. [1], displays potent antitumor and cytotoxic activities and an interesting activity against Gram-positive bacteria [2].

The antibiotic contains a number of interesting features, including a 5-(thiazol-4-yl)penta-2,4-dieneone system embedded in its 18-membered macrolactam and a 1,2-ditholan-3-one 1-oxide heterocycle that is unique to this natural product. It has been reported that, due to its intrinsec high reactivity, the 1,2-dithiolan-3-one 1-oxide heterocycle is likely to play a crucial role in the events that culminate in DNA damage [3]. Leinamycin is bioactivated by reaction with cellular thiols. Studies of the reaction between thiols and simplified leinamycin models (Fig. 1, compounds **1** and **2**) performed by Gates' group [3] revealed both a thiol-triggered DNA damage mechanism and a thiol-independent mode of DNA alkylation by this antibiotic. The similarities between thiol-independent and thiol-triggered DNA damage by leinamycin suggested that a common alkylating agent might be involved in both processes [4]. Particularly, the 1,2-dithiolan-3-one 1-oxide is unstable in aqueous solution and the attack of water was proved to initiate a cascade of chemical reactions leading to DNA alkylation. Indeed, the hydrolysis of the unstable 1,2-dithiolan-3-one 1-oxide heterocycle seems to unmask leinamycin's latent alkylating abilities.

According to this, we have synthesized some 1,2-dithiolan-3-one and 3-thione derivatives **a–d** and their corresponding bioisosters **e**–**h** (Fig. 2) as simplified structures of the leinamycin hypothesized pharmacophore in order to investigate their potential activity as antitumor agents. Compounds **a–h** have been tested according to the NCI program of screening in vitro against 60 different human tumor cell lines [5].

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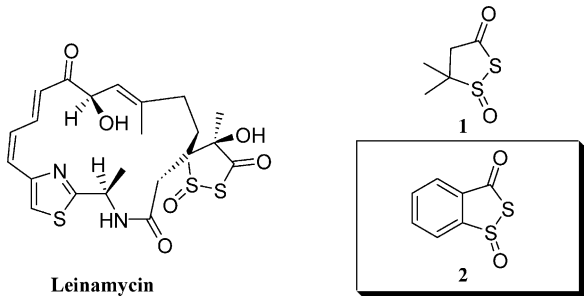


Fig. 1. Leinamycin and structures discussed in the text.

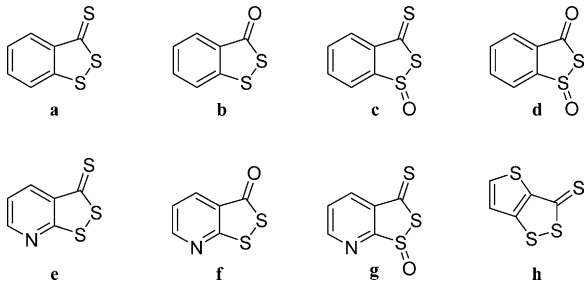


Fig. 2. Structures of compounds described in the text (**a**–**h**).

2. Chemistry

*2.1. Synthesis of 3H-1,2-benzodithiol-3-thione (**a**) and -3-one (**b**) and their corresponding 1-oxide derivatives (**c** and **d**)*

The intramolecular cyclization of 2,2′-dithiosalicylic acid (**3**, X = CH) (Scheme 1) with phosphorus pentasulfide in anhydrous pyridine afforded 3*H*-1,2-benzodithiol-3-thione (**a**) which, by oxidation with H_2O_2 (30%) in acetic acid, gave 3*H*-1,2-benzodithiol-3-thione 1-oxide (**c**). The oxidative desulfuration of 3*H*-1,2-benzodithiol-3-thione (**a**) with mercuric acetate, afforded the 3*H*-1,2-benzodithiol-3-one (**b**) which was then oxidized to (**d**) by the same procedure described above.

*2.2. Synthesis of 3H-1,2-pyridindithiol-3-thione (**e**) and -3-one (**f**) and 3H-1,2-pyridindithiol-3-thione 1-oxide (**g**)*

These compounds have been prepared by the same procedure described above (Scheme 1) using 2-mercaptonicotinic acid (**3**, X = N) as starting material.

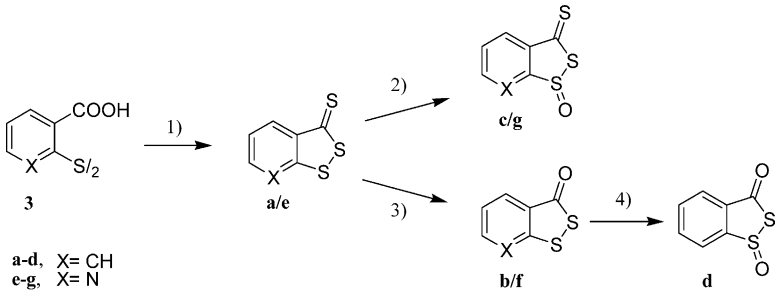
*2.3. Synthesis of 3H-1,2-thiophendithiol-3-thione (**h**)*

This compound was prepared as previously described [6] starting from 2-chloroacrylonitrile (**4**) (Scheme 2) and ethyl thioglycolate (**5**) which, by cyclization in presence of sodium ethoxide, gave 3-amino-2-ethoxycarbonylthiophene (**6**). By treatment with $NaNO_2$/HCl and ethylxanthogenate, 3-mercapto-2-ethoxycarbonylthiophene (**7**) was easily obtained. Cyclization of compound **7** with phosphorus pentasulfide in anhydrous pyridine afforded 3*H*-1,2-thiophendithiol-3-thione (**h**).

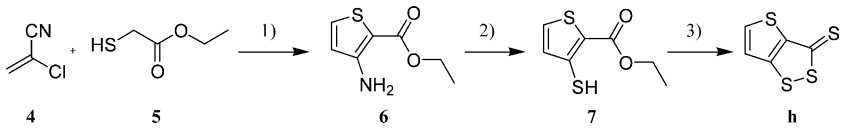
Several attempts to obtain the bioisoster of 3*H*-1,2-benzodithiol-3-one 1-oxide bearing a pyridine ring instead of a phenyl were unsuccessful (data not shown). All compounds were characterized by ^{1}H NMR, IR and mass spectrometry. Analytical data for known compounds were in agreement with those previously reported.

2.4. Antitumor activity

The anticancer assays were performed at the National Cancer Institute (NCI, Bethesda, USA) according to the program of screening in vitro against 60 different human tumor cell lines [5,7,8]. Generally, all compounds displayed poor or absent antineoplastic activity (data not reported). A weak cell grow inhibition was observed for 3*H*-1,2-benzodithiol-3-thione-1-oxide (**c**) (NSC 706184) against leukemia cell lines CCRF-CEM and HL-60 (TB) (Fig. 3).



Scheme 1. Reagents: 1) P_4S_{10}, anhydr. pyridine; 2) and 4) H_2O_2 30%, AcOH; 3) $Hg(OAc)_2$, AcOH/$CHCl_3$



Scheme 2. Reagents: 1) EtONa; 2) $NaNO_2$/HCl, HSCSOEt/Na_2CO_3; 3) P_4S_{10}, anhydr. pyridine

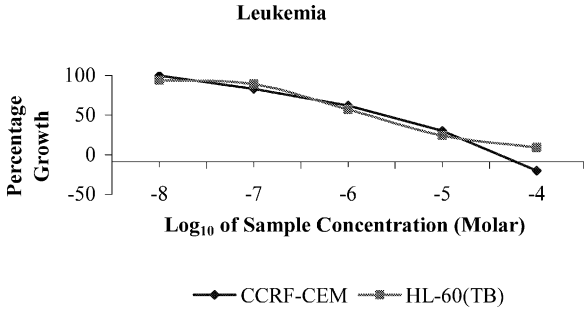


Fig. 3. Dose–response curve for leukemia cell lines CCRF-CEM and HL-60 (TB).

3. Experimental

3.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker ACE-300 spectrometer in deuterochloroform. ^{1}H chemical shifts (δ) were reported with tetramethylsilane ($\delta = 0.00$ ppm) as internal standard. The following abbreviations are used: s = singlet; d = doublet; dd = double doublet; t = triplet; m = multiplet. Thin-layer chromatography (TLC) was performed on 0.25 mm Merck silica gel (60 F_{254}) and visualized by UV light ($\lambda = 264$ or 365 nm); flash chromatography was performed using silica gel 60 (60–200 μm, Merck). Elemental analyses were performed on a Carlo Erba 1106 elemental analyser and were within $\pm 0.35\%$ of the theoretical values. Mass spectra (EI, 70 eV) were performed on a gc-ms Finnigan ITD instrument.

Compounds **a** [9], **b** [10], **d** [11], **e** [12], **f** [12], **h** [6] have been prepared as previously described.

3.2. H-1,2-Benzodithiol-3-thione 1-oxide (*c*)

A solution of H_2O_2 (30%) (0.296 ml, 9.78 mmol) was added to a stirred suspension of **b** (0.300 g, 1.63 mmol) in glacial acetic acid (50 ml) at room temperature. After 14 h, the reaction mixture was heated at 40 °C for 2 h. The suspension was filtered and the filtrate was added to crushed ice. The red crystalline precipitate obtained was washed with water and dissolved in dichloromethane. After extraction with dichloromethane (3 × 100 ml), the organic phases were dried over anhydrous magnesium sulfate, filtered and then evaporated under reduced pressure. The residue was purified by chromatography (hexane–ethyl acetate 1:1.5) to give 3*H*-1,2-benzodithiol-3-thione 1-oxide (**c**) as a red solid (0.280 g; yield: 86%), m.p. = 125 °C. ^{1}H NMR: δ 7.71 (d, 1H, H_A), 7.55 (dd, 1H, H_D), 7.32 (m, 2H, H_B, H_C). MS: *m*/*z* (200): 69, 76, 96, 108, 120, 140, 200, 202 (*M*+2). Elemental analysis ($C_7H_4S_3O$): C = 42.03%; H = 2.01%; Found: C = 42.29%; H = 2.36%.

3.3. H-1,2-Pyridindithiol-3-thione 1-oxide (*g*)

A solution of H_2O_2 (30%) (0.245 ml, 8.1 mmol) was added to a stirred suspension of **e** (0.250 g, 1.35 mmol) in glacial acetic acid (50 ml) at room temperature. After 14 h the suspension was filtered and the filtrate was added to crushed ice until the complete deposition of a brownish precipitate. The residue was filtered under reduced pressure, washed with water and dissolved in dichloromethane. After extraction with dichloromethane (3 × 100 ml), the collected organic phases were dried over anhydrous magnesium sulfate and evaporated sub vacuum. The residue was purified by chromatography (hexane–ethyl acetate 1:2) to give 3*H*-1,2-pyridindithiol-3-thione 1-oxide **g** as a brown–red solid (0.054 g; yield: 20%), m.p. = 163 °C; ^{1}H NMR: δ 8.60 (dd, 1H, H_A), 8.00 (dd, 1H, H_C), 7.20 (dd, 1H, H_B); MS: m/z (201): 109, 153, 169, 185, 201, 203 (M+2). Elemental analysis ($C_7H_4S_3ON$): C = 41.82%; H = 1.99%; Found: C = 41.9%; H = 2.00%.

4. Results and discussion

In this paper we reported the synthesis and the biological evaluation as antitumor agents of some compounds structurally related to leynamicin, an important antibiotic potentially useful in cancer therapy. Particularly, as the peculiar 1,2-dithiolan-3-one 1-oxide heterocycle contained in the complex structure of leynamycin was recently pointed out to be directly involved in its antitumor and citotoxic activity [3,4], we thought to prepare some simplified derivatives bearing this moyety. Our attention was focused onto 3*H*-1,2-benzodithiol-3-thione and -3-one 1-oxides (**c**–**d**) and their corresponding bioisosters 3*H*-1,2-pyridindithiol-3-thione 1-oxide (**g**) and 3*H*-1,2-thiophendithiol-3-thione (**h**). Another evidence seemed to support our hypothesis; in fact, the purposed compounds and their precursors, share the same mechanism of action of leinamycin, being both bioactivated by reaction with cellular thiols.

Unfortunately, the new substances were biologically inactive, being the high reactivity of the 1,2-dithiolan-3-one 1-oxide heterocycle in itself probably not sufficient to explain the antitumor activity of leinamycin. Nevertheless, a facile oxidation procedure has been developed affording, at least for the benzene series, S-oxide derivatives in high yields.

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