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Il Farmaco 56 (2001) 549–554

IL FARMACO

Synthesis and biological evaluation of some differently substituted 9,10-anthracenediones

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Received 20 July 2000; accepted 16 October 2000

Abstract

9,10-Anthracenedione derivatives are known to exhibit a quite potent anticancer activity. It has also been reported that these compounds can be effectively employed in both antibacterial and antitrypanosomal therapy. Anthraquinones also exhibit some undesirable side effects, like cardiotoxicity. So many interactions seem to demonstrate that 9,10-anthracenediones strongly interact with a number of biological sites. In this paper we wish to report on the synthesis and the pharmaceutical activity of some newly synthesised derivatives containing the anthraquinone pharmacophore. © 2001 Elsevier Science S.A. All rights reserved.

Keywords: 9,10-Anthracenediones; Amino-acid derivatives; Topoisomerase II

1. Introduction

9,10-Anthracenedione derivatives have been widely employed in anticancer therapy; some of them have also exhibited effective antibacterial properties [1].

With respect to the former aspect, the synthesis of amino-acid derivatives of 9,10-anthracenedione was recently developed in order to get a better understanding of their mechanism of action and to grant higher therapeutic specificity [2]. In fact, the aromatic planar part of the anthracenedione would be able to intercalate between the base pairs of the DNA, inhibiting the topoisomerase II, a DNA processing enzyme, while the amino-acid residue should be able to interfere with the groups protruding from the minor/major groove of the nucleic acid. In the frame of the pharmacological interest of anthracenedione-containing compounds, the synthesis and the structural characterisation of some sulfonamide derivatives of 9,10 anthracenediones has been undertaken by some of us, with the aim of obtaining new chemotherapeutic derivatives [3].

The synthesis of some D- and L-aminoacyl-9,10-anthraquinones has also been reported in order to investigate the influence of both aminoacyl substituents and chirality on biological activity [4–6].

Anthracenediones are also known to exhibit a quite potent in vitro trypanocidal activity. This is particularly evident in the case of Daunorubicin which is capable of permanently abolishing the infection of African trypanosomes at less than nanomolar concentrations [7,8].

Many biological interactions necessarily require different interaction sites.

A free radical mechanism has been proposed by some researchers [9], thus giving a possible rationale for the undesired cardiotoxicity exhibited by most of the anthracene derivatives.

In this paper we wish to report on the synthesis and the biological activity of some new derivatives containing the 9,10 anthracenedione pharmacophore.

2. Results and discussion

Seven new 9,10-anthraquinone derivatives, reported in Fig. 1, were synthesised and their antimicrobial, antifungal and antimycobacterial activity evaluated.

The activities of compounds **1**–**7** are summarised in Tables 1 and 2.

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PII: S0014-827X(01)01082-5

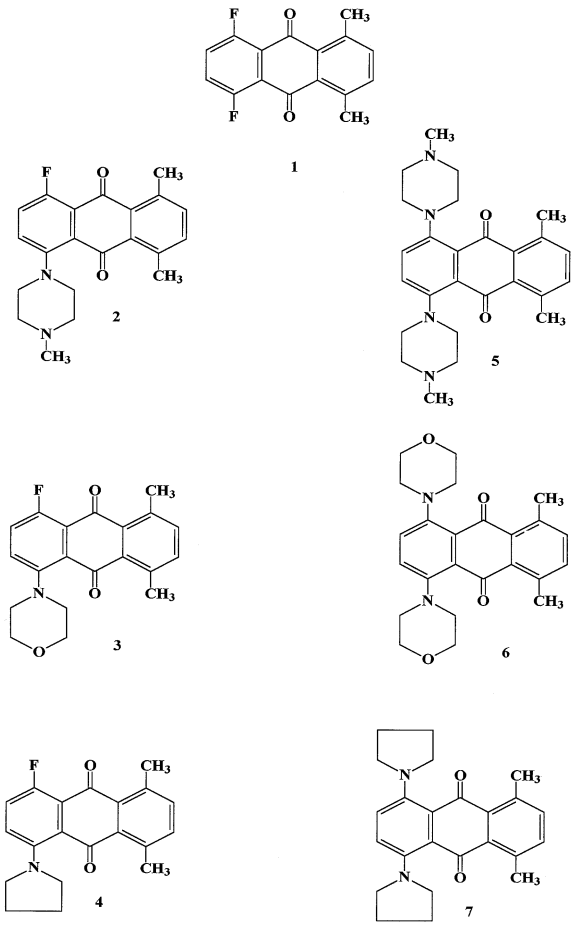
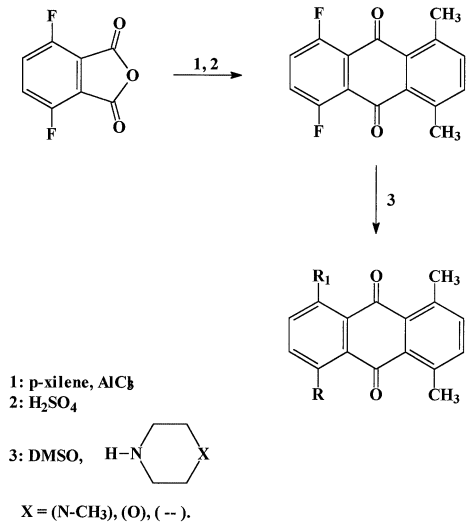


Fig. 1. Synthesised compounds.



Scheme 1.

The synthetic pathway to compounds **1**–**7** is reported in Scheme 1. All the synthesised compounds were fully characterised by means of analytical and spectral techniques. As an example, the ^{1}H NMR spectrum of compound **4** is reported in Fig. 2.

The anthraquinone skeleton was synthesised through a Friedel–Craft acylation of an appropriately substituted aromatic ring with difluorophthalic anhydride. The next step was the cyclisation of the acyl derivative in concentrated sulfuric acid.

On the 1,4-dimethyl-5,8-difluoro-9,10-anthracenedione obtained, the substitution of the fluorine atom

Table 1
MIC values of compounds **1**–**5** and **7** against different antimycobacterial species

Mycobacterium	MIC (μg/ml)					
	1	**2**	**3**	**4**	**5**	**7**
M. tubercolosis H37RV ATCC 25548	>100	25	>100	100	100	100
M. tubercolosis INH-R ATCC-35822	>100	25	>100	>100	100	25
M. tubercolosis SM-R ATCC 35820	>100	50	100	100	>100	25
M. tubercolosis RIF-R ATCC 35838	>100	50	>100	>100	100	25
M. tubercolisis PZA-R ATCC 35828	>100	50	>100	>100	100	50
M. avium NC 08559.06	>100	6.25	>100	>100	>100	>100
M. phlei NC 08151.07	>100	100	>100	>100	>100	>100
M. fortuitum NC 10394.02	>100	>100	>100	>100	>100	>100
M. scrofulaceum NC 10803.03	>100	100	>100	>100	>100	>100
M. kansasii NC 10268.07	>100	50	100	>100	100	100
M. intracellulare NC 10425.05	>100	100	>100	>100	>100	>100
M. szulgai NC 10831.03	>100	100	100	>100	>100	>100
M. gordonae NC 10267.05	>100	50	100	>100	>100	>100
M. chelonae subsp. *abscessus* NC 10882.02	>100	>100	>100	>100	>100	>100
M. bovis NC 10772.02	>100	25	50	100	12.5	25

Table 2
Analytical and spectral data for compounds **1**-**7**

Comp.	R	R_1	Formula	M^+	m.p. (°C)	Cryst. solvent	Yield%	C (%) [a]	H (%) [a]	N (%) [a]
1	F	F	$C_{16}H_{10}F_2O_2$	272	274–275	ethanol	91	70.59 (70.50)	3.70 (3.68)	
2	*c*-N$(CH_2CH_2)_2NCH_3$	F	$C_{21}H_{21}FN_2O_2$	352	150–151	water	34	71.57 (71.76)	6.01 (5.98)	7.95 (7.99)
3	*c*-N$(CH_2CH_2)_2O$	F	$C_{20}H_{18}FN0_3$	339	142–143	water	35	70.78 (70.96)	5.35 (5.32)	4.13 (4.15)
4	*c*-NC_4H_8	F	$C_{20}H_{18}FNO_2$	323	166–168	water	30	74.29 (74.37)	5.61 (5.60)	4.33 (4.35)
5	*c*-N$(CH_2CH_2)_2NCH_3$	*c*-N$(CH_2CH_2)_2NCH_3$	$C_{26}H_{32}N_4O_2$	432	230–231	water	62	72.19 (72.20)	7.46 (7.43)	12.95 (12.89)
6	*c*-N$(CH_2CH_2)_2O$	*c*-N$(CH_2CH_2)_2O$	$C_{24}H_{26}N_2O_4$	406	245–246	water	58	70.91 (71.00)	6.45 (6.43)	6.89 (6.86)
7	*c*-NC_4H_8	*c*-NC_4H_8	$C_{24}H_{26}N_2O_2$	374	250	water	67	76.96 (77.00)	7.00 (6.98)	7.48 (7.51)

[a] Found values in parentheses.

with secondary amines was carried out under different conditions in order to demonstrate the most convenient procedure.

Although it has been reported that halogen atoms on aromatic rings hardly undergo nucleophilic substitutions [10], in the case of our derivatives, the presence of the two carbonyl groups at positions 9 and 10 of the anthracene moiety strongly influences the reactivity of the fluorine atoms.

In fact, the substitution of the halogen atoms can be easily achieved at relatively low temperatures in the absence of catalysts.

Moreover, for all the newly synthesised products, the ratio between the monosubstituted and the disubstituted derivatives was 3:7, thus confirming not only the reactivity of the fluorine atoms towards the nucleophilic substitution, but also the lack of sterical hindrance when introducing the two basic substituents.

No changes in the products stoichiometry have been observed even when mixing the substrate and the nucleophile in a 1:1 ratio.

The biological activity was evaluated against a number of bacterial strains.

Unfortunately, no biological data were obtained for compound **6** due to its low solubility in the test medium.

Compounds **1**–**7** were almost ineffective in inhibiting the growth of the strains of both the Gram-negative and Gram-positive species tested. A poor activity was shown by compound **5** against *Staphylococcus epidermidis* (MIC 100 μg/ml). Likewise, compounds **1**–**7** were unable to inhibit the growth of *Candida albicans* even at the highest concentration tested. However, an interesting activity of compound **2** was detected against *Mycobacterium tubercolosis* II37Rv ATCC 25548 and *M. tubercolosis* INH-R ATCC 35822 (MIC 25 μg/ml) and *M. tubercolosis* SM-R ATCC 35820, *M. tubercolosis* RIF-R ATCC 35838 and *M. tubercolosis* PZA-R ATCC 35828 (MIC 50 μg/ml). Interestingly, only compound **2** was particularly effective against *M. avium* NC 08859.06 (MIC 6.25 μg/ml).

An MIC value of 25 μg/ml was shown by compound **7** against *M. tubercolosis* INH-R ATCC 35820, *M. tubercolosis* SM-R ATCC 35820 and *M. tubercolosis* RIF-R ATCC 35838.

Furthermore, *M. bovis* NC 10772.02 was susceptible to compounds **2**, **3**, **5** and **7** (MIC 25, 50, 12.5 and 25 μg/ml, respectively.)

According to these data, the methylpiperazine group seems to be the choice for both antibacterial and antimycobacterial activity. In particular, considering the latter activity, the concomitant presence of a fluorine atom leads to an increase in potency. This can be explained by a modification of both the partition coefficient and the molecule steric hindrance.

Also, the introduction of two pyrrolidine molecules in positions 5 and 8 leads to increasing antimycobacterial activity.

In conclusion, an easy synthesis to obtain fluorinated and nitrogen-substituted anthracenediones has been set up. The information stemming from the structure–activity relationships will constitute the basis for the design of future compounds that are hopefully more active.

3. Experimental

3.1. Materials and methods

Melting points are uncorrected and were determined on a Reichert Kofler thermopan apparatus. IR spectra were recorded on a Perkin–Elmer 1640 FT spectrometer (KBr discs, in cm^{-1}). ^1H NMR spectra were recorded on a Bruker AMX (300 MHz) using tetramethylsilane as internal standard (chemical shifts in δ values). Electron ionisation mass spectra were obtained by a Fisons QMD 1000 mass spectrometer (70 eV, 200 μA, ion source temperature 200°C) The samples were introduced directly into the ion source. Elemental analyses were obtained on a Perkin–Elmer 240 B microanalyser.

3.2. Chemistry

3.2.1. Synthesis of 1,4-dimethyl-5,8-difluoro-9,10-anthracendione (*1*)

In a three-necked nitrogen flushed flask 1 g of 3,6-difluorophthalic anhydride and 50 ml of *p*-xylene were stirred. Aluminium trichloride (2 g) was added in three instalments and the mixture was then refluxed for 12 h. A yellow residue was obtained and poured on to a mixture of 50 g of crushed ice and 2.5 ml of hydrochloric acid.

The mixture was stirred for 1 h and then washed with acidic water.

A white solid was obtained and crystallised from isopropyl ether.

Yield 72.8%, m.p. 145°C.

The carboxylic acid obtained (1 g) was heated at 60°C for 1 h in 15 ml of concentrated sulfuric acid.

The mixture was then allowed to cool down, poured on to 150 g of crushed ice and stirred for 1 h. After filtration the white solid obtained was crystallised from ethanol to yield 1,4-dimethyl-5,8-difluoro-9,10-anthracendione (**1**).

Yield 91%, m.p. 274–275°C.

^1H NMR (DMSO): δ 2.62 (s, 6H, $ArCH_3$), 7.06–7.47 (m, 4H, Ar).

3.2.2. Synthesis of 1,4-dimethyl-5-methylpiperazino-8-fluoro-9,10-anthracendione (*2*) (general procedure)

In a three-necked flask, under nitrogen atmosphere, 1 g (37 mmol) of compound **1** was allowed to react with 3.7 g (37 mmol) of methylpiperazine in 20 ml of dimethylsulfoxide. The reaction mixture was heated at 70°C for 1 h and then allowed to cool down at room temperature. Water (20 ml) was then added and a red solid precipitated. The solid was filtered, washed three times with water and finally exsiccated. Thin-layer chromatography revealed the presence of two compounds which were separated by column chromatogra-

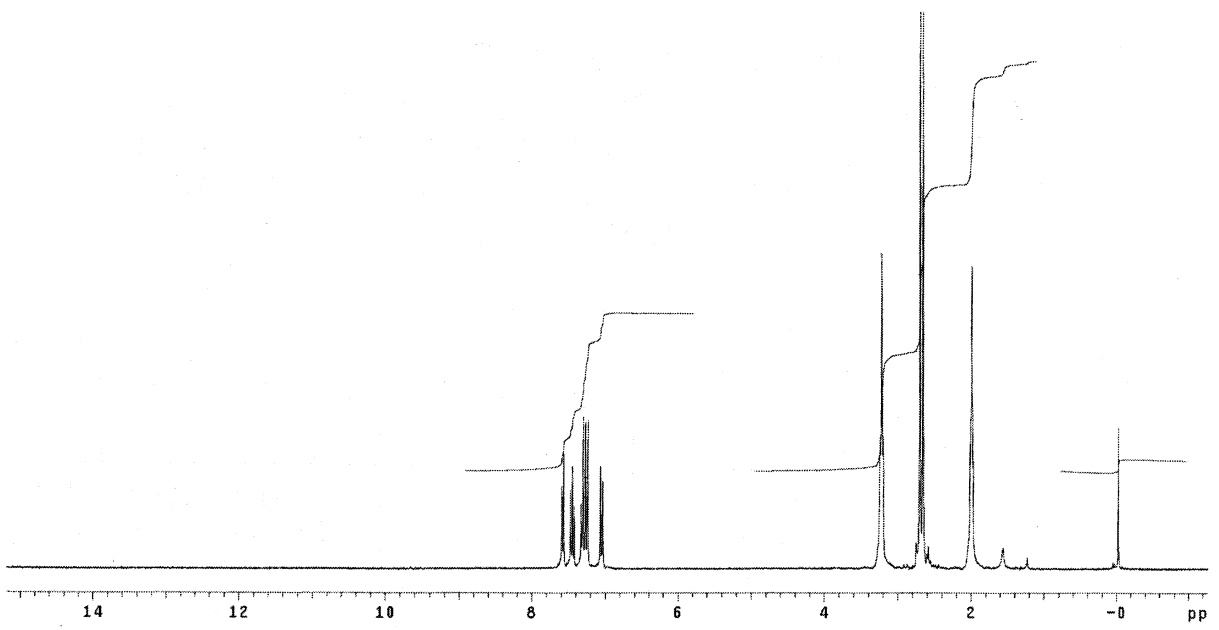


Fig. 2. ^1H NMR spectrum of compound **4**.

phy performed on silica gel eluent hexane/methylene chloride gradient.

In the first fractions of the column the monosubstituted product (**2**) was isolated, which was further purified by crystallisation from water.

Red crystalline solid, yield 34%, m.p. 150–151°C.

^{1}H NMR (DMSO): δ 1.20 (s, 3H, NCH_3), 2.66 (s, 3H, $ArCH_3$), 2.71 (s, 3H, $ArCH_3$), 3.67 (t, 4H, CH_2NMe), 3.85 (t, 4H, CH_2NAr), 7.03–7.59 (m, 4H, Ar).

3.2.3. Synthesis of 1,4-dimethyl-5,8-di-(methylpiperazino)-9,10-anthracendione (5)

Compound **5** was isolated from the last fractions of the column and crystallised from water. Dark red crystalline solid, yield 62%, m.p. 230–231°C.

^{1}H NMR (DMSO): δ 2.32 (s, 6H, NCH_3), 2.59 (t, 8H, CH_2NMe), 2.75 (s, 6H, $ArCH_3$), 3.33 (t, 8H, CH_2NAr), 7.23–7.74 (m, 4H, Ar).

Using the same synthetic and purification pathway, the following compounds were obtained:

1,4-dimethyl-5-morpholino-8-fluoro-9,10-anthracenedione (3)

Orange crystalline solid, yield 35%, m.p. 142–143°C.

^{1}H NMR (DMSO): δ 2.70 (s, 3H, $ArCH_3$), 2.73 (s, 3H, $ArCH_3$), 3.33 (t, 4H, NCH_2), 3.95 (t, 4H, OCH_2), 7.20–7.83 (m, 4H, Ar).

1,4-dimethyl-5,8-dimorpholino-9,10-anthracenedione (6)

Red crystalline solid, yield 58%, m.p. 245–246°C.

^{1}H NMR (DMSO): δ 2.80 (s, 6H, $ArCH_3$), 3.28 (t, 8H, NCH_2), 3.92 (t, 8H, OCH_2), 7.24–7.75 (m, 4H, Ar).

1,4-dimethyl-5-pyrrolidino-8-fluoro-9,10-anthracenedione (4)

Red crystalline solid, yield 30%, m.p. 166–168°C.

^{1}H NMR (DMSO): δ 2.00 (m, 4H, CH_2), 2.65 (s, 3H, $ArCH_3$), 2.70 (s, 3H, $ArCH_3$), 3.22 (t, 4H, NCH_2), 7.03–7.59 (m, 4H, Ar).

1,4-dimethyl-5,8-dipyrrolidino-9,10-anthracenedione (7)

Red crystalline solid, yield 67%, m.p. 250°C.

^{1}H NMR (DMSO): δ 2.02 (m, 8H, CH_2), 2.69 (s, 6H, $ArCH_3$), 3.25 (t, 8H, NCH_2), 7.19–7.85 (m, 4H, Ar).

3.3. Microbiology

Compounds. For antimicrobial studies the compounds were dissolved in dimethylsulfoxide at 10 mg/ml and stored at -20°C.

The working solutions were prepared in the same medium employed for the tests. To avoid interference from the solvent [11], the highest DMSO concentration was 1%.

Bacteria. The antimicrobial activity of compounds **1**–**7** was evaluated against five Gram-positive species (*Staphylococcus aureus*, *S. epidermidis*, *Streptococcus agalactiae*, *S. faecalis* and *Bacillus subtilis*) and five Gram-negative species (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus mirabilis*, *Klebsiella pneumoniae*) isolated from clinical specimens. For the evaluation of the antifungal activity *C. albicans* ATCC E10931 was employed. The effect on the growth of mycobacteria was investigated against *M. tubercolosis* H37Rv ATCC 25548, *M. tubercolosis* resistant to isoniazid (INH-R) ATCC 35822, *M. tubercolosis* resistant to streptomycin (SM-R) ATCC 35820, *M. tubercolosis* resistant to rifampicin (RIF-R) ATCC 35838 and *M. tubercolosis* resistant to pyrazinamide (PZA-R) ATCC 35828, *M. avium* NC 08559.06, *M. phley* NC 08151.07, *M. fortuitum* NC 10394.02, *M. scrofulaceum* NC 10803.03, *M. kansasii* NC 10268.07, *M. intracellulare* NC 10425.05, *M. szulgai* NC 10831.03, *M. gordonae* NC 10267.05, *M. chelonae* subspecies *abscessus* NC 10882.02 and *M. bovis* NC 10772.02.

Determination of MICs. The MICs of the compounds against Gram-positive, Gram-negative and *C. albicans* were determined by a standard broth macro-dilution method [12,13]. Tests with Gram-positive and Gram-negative bacteria were carried out in Mueller Hinton broth (Difco Laboratories, Detroit, MI). Antifungal activity, against *C. albicans* ATCC E10231, was evaluated in yeast extract peptone dextrose medium (Difco) [14]. The compounds were diluted in the test medium to obtain final concentrations ranging between 100 and 0.19 μg/ml. Tubes containing 1 ml of the diluted compounds were inoculated with 1×10^5 bacteria and incubated at 37°C for 18 or 24 h. The determination of MIC against mycobacteria was carried out by the two-fold agar-dilution method [15] in 24-multiwell plates (Nunc, Naperville, IL) using 7H11 agar (Difco) containing compounds **1**–**7** at concentrations that ranged between 100 and 0.19 μg/ml on which 100 μl of the test bacterial suspension was spotted.

Suspensions to be used for drug susceptibility testing were prepared from 7H9 broth cultures containing 0.05% Tween 80, washed, suspended in 0.1% Tween 80-saline to yield a turbidity no. 1 McFarland and then diluted in saline to obtain inocula of 3×10^5–1.5×10^4 cells/100 μl of bacterial suspension. After a 21 day (slow growers) or 7 day (rapid growers) cultivation in a CO_2 (5% CO_2–95% humidified air) incubator at 37°C (33°C for *M. chelonae*) the growth of organisms was scored. The MIC was defined as the minimum concentration causing complete growth inhibition of organisms or allowing no more than five colonies to grow.

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