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Investigación

Cytotoxic Evaluation of a Series of Bisalkanoic Anilides and Bisbenzoyl Diamines

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Dedicated to Profesor Alfonso Romo de Vivar

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Abstract. A series of bisalkanoic anilides and bisbenzoyl diamines were synthesized with the aim of elucidating the relationship between molecular structure and cytotoxic activity. Twenty-one derivatives were synthesized and tested on three tumoral cell lines. No apparent relationship was observed between electronic effects and cytotoxic activity, but it was found that compounds in which the 4'-phenyl substituent is fluoride or bromide gave the best inhibition of tumoral cell growth.
Keywords: Diamides, alkanediamides, cytotoxic activity.

Resumen. El objetivo del presente trabajo fue encontrar la relación entre la estructura molecular y la actividad citotóxica de una serie de anilidas de diácidos y diamidas bisbenzoiladas, para lo cual se sintetizaron veintiuno de los compuestos mencionados. Los resultados de la evaluación citotóxica de estos derivados, en tres líneas celulares, no indicaron ninguna relación con respecto a efectos electrónicos de los substituyentes, si bien los derivados 4-bromofenil y 4-fluorofenil son los más activos.
Palabras clave: Diamidas, alcano diamidas, actividad citotóxica.

Introduction

DNA recognizing molecules such as DNA-intercalators and groove binders have been the subject of increasing interest due to the ongoing search for more active antitumoral compounds. DNA-groove binders have been widely studied as anticancer compounds. In addition, they have been studied as anti-HIV agents and have been incorporated as a linker in DNA bis-intercalators [1-4]. The most typical DNA-groove binders are the antibiotics Distamycine A (**1**) and Netropsin (**2**), which are characterized by polyamide and polyaromatic functional groups along the DNA recognizing chain [5]. The aromatic portion of these compounds is the pyrrolo system; however, recent studies have investigated compounds incorporating thiazolyl (**3**) or phenyl (**4**) (Fig. 1) instead of pyrrolyl, and groove binders that contain the benzimidazolyl moiety have been described in earlier reports [6-8]. Recently, we reported a series of *N,N*'-(diaminophenyl)alkanediamides 5 which differ in the length of the aliphatic portion. These compounds were shown to inhibit the growth of tumoral cell lines, indicating that this topographical factor has an important influence on DNA recognition [9]. However, the cytotoxic activity of the *N,N*'-(diaminophenyl)alkanediamides was low. The present investigation was undertaken to study the influence of aryl substituents in these compounds and to find compounds of this type with improved cytotoxic activity. To achieve this, we synthesized a series of bisalkanoic anilides and bisbenzoyl diammines (**6-27**) and their activities as cytotoxic agents were evaluated.

Results and discussion

The *N,N*'-diarylalkanediamides (**6-20**) (Fig. 2) were synthesized by condensation of the respective 4-substitued aniline (2 equiv.) with succinyl, glutaryl or adipoyl chloride (1 equiv.) in acetone while being stirred and cooled in an iced bath. The products were precipitated, filtered, and washed with acetone. Yields varied from 65 to 96 %.

Compounds **21-23** and **25-27** were obtained as described for **6-20** but from condensation of the respective benzoyl chloride and ethylenediamine, 1,2-propanediamine, or piperazine as shown in Figure 2. The compounds were obtained in yields of 75 to 95 %. Compound **24** was obtained by reduction of the nitro derivative **23**, using Pd/C and hydrazine in ethanol at reflux for 1 h. Recrystallization from methanol afforded the amine derivative. The yields and spectroscopic data of compounds **6-27** are summarized in Table 1.

The percentage of inhibition of the growth of the three tumoral cell lines after treatment with each compound at a concentration of 31 μM is given in Table 1. The groups bonded at the 4' position were selected on the basis of their electron withdrawing or donating properties, and their hydrogen bonding capabilities.

The first series of compounds comprises *N,N*'-diarylalkanediamides with different numbers of methylenes in the aliphatic chain. The first compounds synthesized and probed were **6** to **10** ($n = 2$). These compounds displayed little activity in the three cell lines. The compound which inhibits cell growth to the greatest extent (57 % in K562) is **6** (R = F), followed by **7** (R = Br) in the same cell line.

Table 1. Physical properties, spectroscopic data and inhibition of the growth of compounds **6-27** at concentration 31 μM.

Comp. No.	R	n	M.W.	Yield (%)	m.p.	Ref. (a)	K562 (b)	PC-3 (c)	U251 (d)
6	F	2	304	65	243-245	[10]	57	25	0
7	Br	2	426	69	281-282	[10,11, 12]	40	6	0
8	OMe	2	328	80	255-256	[10]	22	13	10
9	OH	2	300	92	273-274	[13]	0	13	0
10	H	2	268	83	231-232	[10, 12]	0	16	0
11	Br	3	318	70	254-256	[10]	55	0	4
12	F	4	332	65	230-233	—	96	10	39
13	Cl	4	365	75	255-256	[10]	13	0	0
14	Br	4	454	75	287-288	[12]	5	0	3
15	I	4	548	77		—	0	0	0
16	OMe	4	356	89	233-235	[10, 14]	74	18	17
17	OH	4	328	96		[13, 15]	80	34	4
18	H	4	296	95	244-245	[10, 12]	24	18	0
19	$HNCOCH_3$	4	410	80	> 350	—	0	0	0
20	CN	4	346	85	272-233	[14]	9	13	42
21	Br	—	440	75	268-270	—	83	72	100
22	Br	—	426	80	281-283	—	0	0	5
23	NO_2	—	358	80	253-254	—	0	8	0
24	NH_2	—	298	85	284-285	—	0	3	0
25	NO_2	—	372	75	235-237	—	0	26	0
26	Br	—	452	89	270-273	—	0	0	6
27	NO_2	—	384	95	318-320	[16]	0	4	0

(a) References of the synthesis for previously reported compounds. (b) Leukemia (c) Prostate (d) CNS.

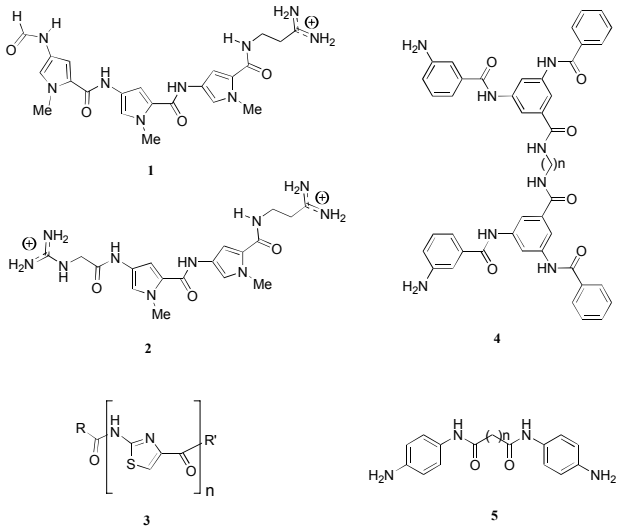

Fig. 1. Examples of compounds containing polyamide and polyaromatic functional groups along the DNA recognizing chain.

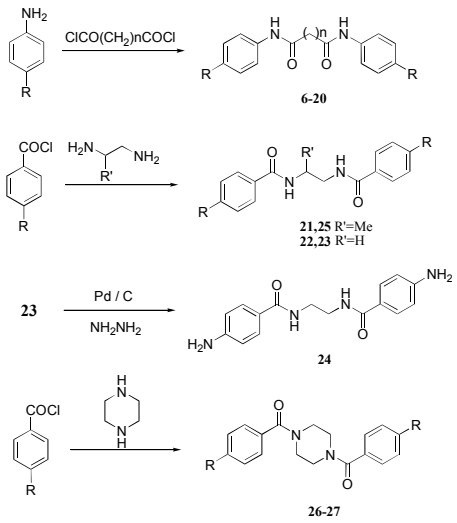

Fig. 2. Synthesis of compounds **6-27**. (R values are reported on Table 1.)

To study the influence of the length of the aliphatic chain on cytotoxic activity, we prepared compounds with a four methylene chain (**12**, **14**, **16-18**). It should be pointed out that compounds **13** (R = Cl) and **15** (R = I) were included due to the apparent tendency of halogens to present activity. In addition, compounds **19** (R = $NHCOCH_3$) and **20** (R = CN) were included in the study to investigate the effects of the $NHCOCH_3$ and CN functional groups. In contrast to the almost complete lack of activity shown by the first series (*n* = 2), compound **12** (R = F) induced almost 100 % inhibition of growth in K562 cell line and the functional groups OMe (**16**) and OH (**17**) were found to enhance cytotoxicity. The rest of the compounds showed no activity.

Compounds **21-25** were examined to analyze the importance of the relative position of the amide group and the presence of branching in the aliphatic chain. Surprisingly, compound **21** was the most active in the bromide series, displaying relatively good inhibition in the three cell lines. Given the activity of **21**, it is surprising that **22** was inactive. To complete the series of bromide compounds, **11** (n = 3, R = Br) was obtained; it showed greater activity than **7** (R = Br, n = 2) but less than **14** (R = Br, n = 4) in K562 cell line.

The inhibition resulted by **21**, lead to the resentment that conformation could be implicated in the cytotoxic activity. To test this idea, compounds **26** and **27** were synthesized; however, both of these compounds were inactive. Although these molecules are structurally similar to **21-25**, the formers (**26** and **27**) are not very capable of interacting by hydrogen bonding. This is a very important factor affecting cytotoxicity in DNA groove binders due to the stability of the DNA-ligand complex.

Conclusions

The data presented here are inconclusive regarding the relationship between electronic factors or hydrogen bonding capability and inhibition of the growth in tumor cell lines. The present results also show no clear link between the presence of halogens or the length of the aliphatic chain and the cytotoxicity of a compound. However, this study did reveal the interesting finding that the compounds which presented cytotoxic activities were primarily those containing fluoride or bromide.

Experimental

Chemistry

General procedure for the preparation of **6-20**. Diacyl chloride (0.72 mmol) was added to a solution of 4-*R*-aniline (1.44 mmol) in 15 mL of acetone at 5 °C. After 2 h stirring, the mixture was filtered and washed with acetone to afford **6-20**.
12: ^{1}H NMR (δ, J(Hz)): 1.60 (s, 4H), 2.30 (s, 4H), 7.10 (m, 4H), 7.57 (m, 4H), 9.94 (s, 2H); IR ν (cm^{-1}) 1652, 3305. **15:** ^{1}H NMR (δ, J(Hz)): 1.59 (s, 4H), 2.31 (s, 4H), 7.41 (d, J = 8.8, 4H), 7.60 (d, J 8.7, 4H), 9.97 (s, 2H); IR ν (cm^{-1}) 1657, 3292. **19:** ^{1}H NMR (δ, J(Hz)) 1.59 (m, 4H), 1.99 (s, 6H), 2.28 (s, 4H), 7.46 (s, 8H), 9.80 (s, 2H), 9.83 (s, 2H); IR ν (cm^{-1}) 1659, 3298.

General procedure for the preparation of **21-23** and **25-27**. 4-bromobenzoyl chloride (1 mmol) was added to a solution of diamine (0.7 mmol) in 15 mL of acetone at 5 °C. After 2 h stirring, water was added and the precipitated filtered and washed with water and acetone to afford **21-23** or **25**.
21: ^{1}H NMR (δ, J(Hz)) 1.15 (d, J 6.64, 3H), 3.35 (t, J 9, 2H), 4.23 (m, 1H), 7.64 (d, J 8.6, 4H), 7.76 (d, J 8.5, 2H), 8.34 (d, J 8.2, 1H) 8.63 (t, J 5.6, 1H); IR ν (cm^{-1}) 1637, 3301.
22: ^{1}H NMR (δ, J(Hz)): 1.33 (s, 4H), 7.65 (d, J 8.85, 4H), 7.77 (d, J 8.5, 4H), 8.67 (s, 2H); IR ν (cm^{-1}) 1633, 3287. **23:** ^{1}H NMR (δ, J(Hz) 3.47 (d, J=2.7, 4H), 8.07 (d, J = 8.8, 4H), 8.30 (d, J 8.9, 4H), 9.00 (s, 2H); IR ν (cm^{-1}) 1640-3319. **25:** ^{1}H NMR (δ, J(Hz)): 1.2 (d, J 6.7, 3H), 3.45 (t, J 6.3, 2H), 4.3 (m, 1H), 8.03 (d, J 8.96, 2H), 8.05 (d, J 9, 2H), 8.63 (d, J 8.14, 1H), 8.92 (t, J 5.6, 1H); IR ν (cm^{-1}) 1661, 3318. **26:** ^{1}H NMR (δ, J (Hz)) 3.54 (m, 8H), 7.37 (d, J 8.4, 4H), 7.64 (d, J 8, 4H); IR ν (cm^{-1}). 1635.

Preparation of **24**.
Ethanol (10 ml), Pd/C 5% (0.046 g), Hidrazine (0.818 ml, 25.9 mmol), water (0.93 ml) and **23** (756 mg, 2.59 mmol) were mixed in a bottom flask. The mixture was refluxed for 2h. The resulting solid was dissolved in methanol with heat and filtered at vacuum. Methanol was eliminated up precipitation of a solid that was filtered and crystallized from methanol to afford **24**. ^{1}H NMR (δ, J(Hz)): 3.33 (d, J 7.2, 4H), 5.58 (d, J 3.18, 4H), 6.51 (d, J 8.5, 4H), 7.54 (d, J 8.5, 4H), 8.12 (s, 2H); IR ν (cm^{-1}) 1600, 3333, 3437.

Cytotoxic Activity

Tumoral cell lines were supplied by the National Cancer Institute. The cytotoxicity assays were carried out at 5000 to 7500 cells / mL as reported by Skehan *et al.* and Monks et al using the sulforhodamine **B** (SRB) protein assay to estimate cell growth [17, 18]. Compounds were dissolved in DMSO which has not effect on the inhibition has shown by the control. The percentage of inhibition of the growth described for all compounds were obtained from three different experiments. The percentage growth was evaluated spectrophotometrically in a Bio kinetics reader spectrophotometer. Daunomicyne and 5-fluorouracyl were used as references. These compounds under the described conditions gave 100 % of inhibition. Each experiment was made two times by gave triplicate.

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