Eur. J. Med. Chem. 36 (2001) 731–736

PII: S0223-5234(01)01260-0/SCO

Short communication

Cytotoxic activity and QSAR of *N*,*N*′-diarylalkanediamides

Luis Chacón-García, Roberto Martínez*

Instituto de Química, Universidad Nacional Autónoma de México, Circuito Interior, Ciudad Universitaria, Coyoacán, 04510 Mexico DF, Mexico

Received 28 February 2001; revised 10 July 2001; accepted 11 July 2001

Abstract – The one- and two-step syntheses of *N*,*N*′-(dinitrophenyl)alkanediamides and *N*,*N*′-(diaminophenyl)alkanediamides from 4-nitroaniline were carried out. These compounds were subjected to cytotoxic evaluation against different tumoural cell lines. A QSAR analysis for the aminated series, introducing a QSAR descriptor (D_{CL}) designed herein for groove binders, reveals that the activity in the K562 cell line is related to the charge, polarisability, volume and length of the molecules. © 2001 Éditions scientifiques et médicales Elsevier SAS

cytotoxic activity / QSAR / *N*,*N*′-diarylalkanediamides

1. Introduction

Among compounds with cytotoxic activity, the most important groups are those that interact reversibly with DNA without forming covalent bonds, such as DNA intercalators or groove binders. Some of the former are drugs used for cancer therapy, e.g. Daunorubicin, employed in leukaemia treatment. Amides have attracted attention in medicinal chemistry not only because of their antitumoural activity [1] but also in the treatment of other illnesses. In particular, some *N*,*N*′-diaryldiamides have been investigated as antibacterial agents [2] or HIV inhibitors [3] but not, to our knowledge, as cytotoxic agents. In a recent study we described the synthesis of bis-benzacridinones in which the linker portion was a diaryldiamide chain (*figure 1*) [4].

Taking into account the important role of the linker in the DNA bis-intercalators in recognising the double helix, and then increasing the affinity for this [5, 6], we decided to explore the cytotoxic activity of these portions against tumoural cell lines, using amino **1a**–**g** and nitro **2a**–**g** groups instead of the chromophore groups.

2. Chemistry

The *N*,*N*′-diarylalkanediamides were obtained from *p*-nitroaniline. In this method, 4-nitroaniline (2 equiv.) was condensed with the corresponding diacylchlorides (1 equiv.) in acetone while being cooled in an ice bath, leading to the formation of the respective *N*,*N*′-(4,4′-dinitrophenyl)alkanediamides. The products obtained were precipitated, filtered and washed with acetone. Chromatographic purification was not necessary. Yields varied from 50 to 65%, with the exception of the reaction with succinyl chloride, for which the yield was very low (18%). In neither case were the reactions carried out in the presence of an additional base.

The *N*,*N*′-(4,4′-diaminophenyl)alkanediamides were obtained by reduction of the nitrated compounds with hydrazine and palladium/carbon in ethanol at reflux temperature and recrystallised from methanol with yields over 80% in all the cases (*figure 2*).

* Correspondence and reprints
E-mail address: robmar@servidor.unam.mx (R. Martínez).

3. Pharmacological results and discussion

3.1. Cytotoxic activity

Compounds **1a**–**f** and **2a**–**f** were evaluated for cytotoxic activity in cultures of PC-3, U251 and K562 cells. The results are given in *table I*.

For the purpose of this study, and because of the structure of the diaryldiamides described herein, we consider the cytotoxic activity of these compounds to be due to an interaction with DNA as groove ligands [7].

In the group of nitrated compounds studied, the most active was **1f** in the PC3 and U251 cell lines followed by **1c**. The IC_{50} of **1f** was 34.78 (±5.9) μM in PC-3 and 3.84 (±0.65) μM in U251 and of **1c** 32 (±0.7) μM in U252.[1] Although this result is moderate, **1f** is considered to be active. The potency of this is high if compared with Distamicyn A, a known groove binder, whose IC_{50} in U251 is over 100 μM.

Compound **1a** exhibited no activity in any line, while compounds **1b**, **1d**, and **1e** showed almost negligible activity in all entries.

With respect to the group of the aminated compounds, **2a** ($n = 2$), **2b** ($n = 3$), **2c** ($n = 4$), **2e** ($n = 6$) and **2f** ($n = 7$) showed medium activity in K562 (**2d** was not probed in this line) and very little cytotoxicity in all the others. This set of compounds, in contrast with the preceding, could have additional intermolecular interactions with DNA due to hydrogen bonds between the amine group at the end of the molecule and base pairs, either directly or through water molecules. It is probably due to these increased interactions that almost all compounds in this group present cytotoxicity. One obvious hypothesis for these systems would be that the inhibition of the growth is proportional to the length of the chain. However, it is easily seen from the results (K562) that in the first three compounds (**2a**–**c**) there is a decrease in the percentage of inhibition followed by an increase in compounds **2e**–**f** (see *figure 3*).

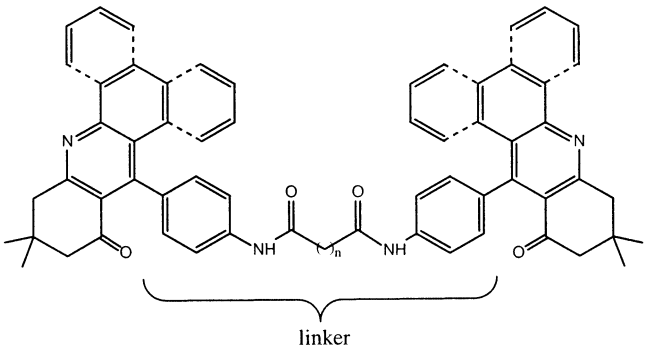

Figure 1. Structure of bis-benzacridines with the linker portion shown.

3.2. QSAR

The above results indicate that the activity cannot be explained only in terms of liposolubility, chain length or molecular weight. To address this point, a QSAR analysis of these compounds was carried out.[2] Since the number of compounds was small, the study was made for the purpose of interpolation, not for extrapolation. Due to the high correlation between almost all 16 of the descriptors used by the program, it was not possible to carry out the QSAR analysis with the remaining descriptors, and it was necessary to incorporate a new descriptor (D_{CL}) related to the topological recognition of DNA. This descriptor was obtained from the length of the molecule geometrically optimised using PM3, assuming that a specific length is required for an optimal recognition of the double helix, as shown in *figure 4*. The distance within the molecule between the extreme groups (NH_2–NH_2), divided by 4.202 or 3.4/cos 36, should in principle be the number of gaps (or base pairs plus one) that the cross-linker will recognise. Therefore, if the closest whole number to this value is subtracted from its value, the resulting difference will be the separation of the optimal (zero) or the worst (0.5) relative recognition. This can be appreciated in *figure 4* and Eq. (1).

$$D_{CL} = \left|(NH_2\text{–}NH_2 \text{ distance}/4.202) - N\right| \qquad (1)$$

where N is the closest whole number.

Considering this descriptor it was possible to formulate a mathematical expression. Eq. (2) was found to be the best expression, containing the topological (D_{CL}), polarisability/volume (Sp.Pol) and charges (ABSQ) descriptors.[3]

[1] IC_{50} of this compound was evaluated by duplicate in three different experiments using concentrations of 3.1, 10, 31 and 100 μM.

[2] QSAR analysis was made by multiple linear regressions. All descriptors considered (with exception of D_{CL}, see below) were taken from SCIQSAR, version 3.0, SciVision, Inc., 1998. Statistical criteria for avoidance chance were carefully taken into account.

[3] Linear estimation and Student's t-distribution ($\alpha = 0.05$) between these descriptors discarded H_0.

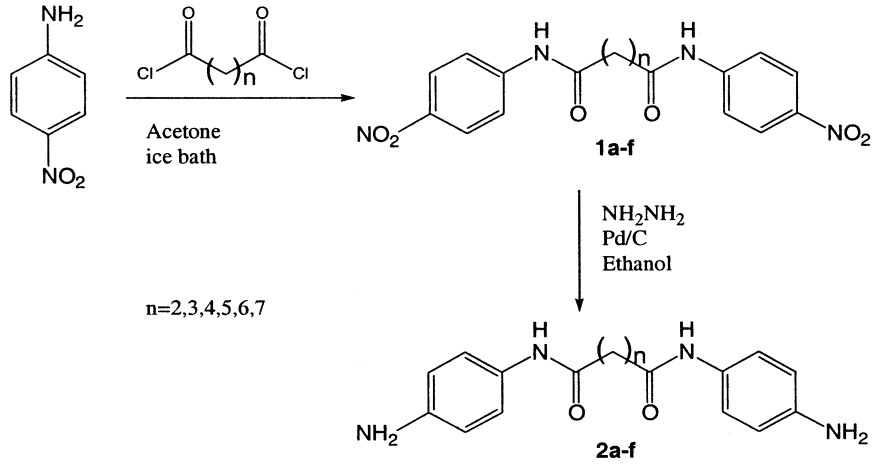

Figure 2. Synthesis of the *N*,*N*′-diarylalkanediamides **1a**–**f** and **2a**–**f**.

Table I. Percentage of inhibition of the growth (at 31 μM concentration). [a]

Comp.	*n*	M.p.	PC-3 (prostate)	U251 (CNS)	K562 (leukaemia)
1a	2	286–288	0	0	[b]
1b	3	278–280	0	50	50.7
1c	4	280–285	0	>50	[b]
1d	5	206–208	5	23	[b]
1e	6	220–222	2	20	[b]
1f	7	155–158	>50	82	43.8
2a	2	232–234	16.8	15.4	67.4
2b	3	228–230	14.8	23.9	59.3
2c	4	238–240	4.7	23.9	51.6
2d	5	203–204	0	0	[b]
2e	6	211–212	21.3	20.5	54.2
2f	7	195–197	0	18.2	54.9

[a] A concentration of 31 μM was chosen because it represents the medium point between log −4 and log −5, which is the range where inhibition of the growth was found.
[b] Activity was not measured.

$$\log_{(\%\ \text{growth})} = -20.55\times10^{-2}D_{CL} + 70.92\times10^{-2}\text{ABSQ} + 29.617\text{Sp.Pol} - 2.107,\ n=5,\ r^2=0.99,\ F=31\ 782_{(1,3)},\alpha=0.05,\ s=0.045 \qquad (2)$$

where D_{CL} (see Eq. (1)); $\text{ABSQ} = \Sigma_i\,|Q_i|$, where Q_i is the charge on the ith atom; $\text{Sp.Pol} = \Sigma_A|\alpha_A|/\text{Vol}$, where α_A is the average atomic hybrid polarisability for atom A and Vol is the molecular volume of the molecule.

The good correlation between Eq. (2) and the experimental data can be seen in *table II*.

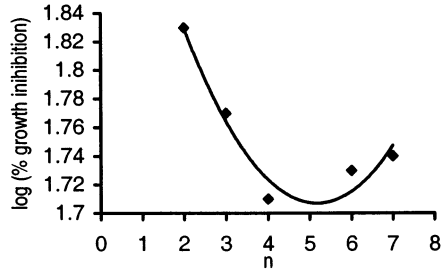

Figure 3. Behaviour of the inhibition of the growth in K562 cell vs. the number of methylenes (*n*) in the alkyl chain of the amino series.

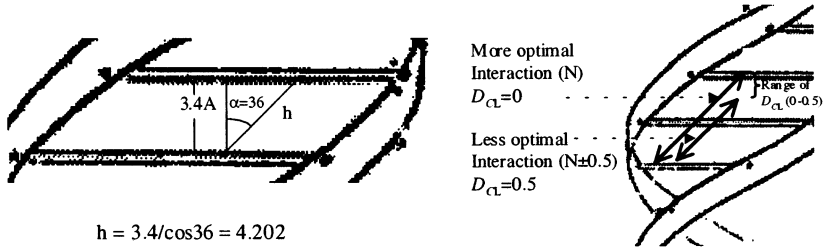

Figure 4. Schematic representation of D_{CL}.

Table II. Comparison of the calculated data from Eq. (2) with experimental.

Comp.	log (%inhib)			Dist. N–N [a]	D_{CL}	ABSQ	Sp.Pol
	Exp.	Calc.	Δ ($\times 10^{-4}$)				
2a	1.8286	1.8287	1	16.85	0.00939	4.496668	0.122193
2b	1.7730	1.7728	2	17.56	0.17833	4.560416	0.121323
2c	1.7126	1.7127	1	19.32	0.40287	4.663736	0.120606
2e	1.7339	1.7340	1	21.8	0.18722	4.875879	0.11932
2f	1.7395	1.7396	1	22.3	0.30619	5.268802	0.119391

[a] Distance between amines groups, taken from the geometry optimised using PM3. ALCHEMY 2000, version 2.05, Tripos, Inc., 1998.

4. Conclusions

The molecules described in this study are easily obtained and the significant activity and high selectivity displayed by some of them is noteworthy.

The results from Eq. (1) are in agreement with the molecular mechanism of action proposed for these compounds. Firstly, the relative length between the cross-linker and the number of DNA base pairs that it will recognise through H-bonds, directly or by water molecules, is given by D_{CL}. It is noteworthy that the length in DNA groove ligands is still continuing to be a topic in the design of this kind of molecules [8–10]. Secondly, as is expected for cross-linker agents, charges (ABSQ) and polarisability (Sp.Pol) are directly related to repulsive and/or attractive interactions with the DNA grooves. Finally, volume contributes to the correct docking of the molecule.

Unfortunately, the presence of more than seven methylenes in the alkyl chain could present additional factors that affect the activity, such as additional degrees of freedom, many more than one low energy conformation, the marked effect of the partition coefficient, etc. In these cases another mathematical expression should be sought.

However, the descriptor D_{CL} can be used in this and any other structurally different cross-linker and could be very useful in the design of DNA bis-intercalating compounds in the spacer region.

5. Experimental protocols

5.1. Chemistry

All melting points are uncorrected. The IR spectra were recorded in a Nicolet FT-55X spectrophotometer. The ^{1}H-NMR spectra were determined in a Varian FT-200 instrument. All NMR spectra were obtained with the pulse sequence as part of the spectrometer's software and were determined in DMSO-d_6. Chemical shifts are expressed in δ (ppm) relative to TMS as internal standard and coupling constants J in Hz.

5.1.1. General procedure for the preparation of the *N,N′-(4,4′-dinitrophenyl)alkanediamides*

Diacyl chloride (0.72 mmol) was added to a solution of 4-nitroaniline (1.44 mmol) in 15 mL of acetone at 5 °C. After stirring for 2 h, the mixture was filtered and washed with acetone to afford **1a**–**c**.

*5.1.1.1. N,N′-Bis(4-nitrophenyl)ethanediamide (**1a**)*

M.p. 286–288 °C, 18% yield, ^{1}H-NMR: δ 2.75 (s, 2H), 2.32 (s, 2H), 7.8 (d, $J = 9$ Hz, 4H), 8.19 (d, $J = 9$ Hz, 4H), 10.65 (s, 2H).

*5.1.1.2. N,N′-Bis(4-nitrophenyl)propanediamide (**1b**)*

M.p. 278–280 °C, 59% yield, ^{1}H-NMR: δ 1.92 (q, $J = 7$ Hz, 2H), 2.45 (t, $J = 7$ Hz, 4H), 7.8 (d, $J = 9$ Hz, 4H), 8.18 (d, $J = 9$ Hz, 4H), 10.52 (s, 2H).

*5.1.1.3. N,N′-Bis(4-nitrophenyl)butanediamide (**1c**)*

M.p. 280–285 °C, 63% yield, ^{1}H-NMR: δ 1.64 (q, $J = 6$ Hz, 4H), 2.42 (s, $J = 6$ Hz, 4H), 7.82 (d, $J = 9$ Hz, 4H), 8.19 (d, $J = 9$ Hz, 4H), 10.53 (s, 2H).

*5.1.1.4. N,N′-Bis(4-nitrophenyl)pentanediamide (**1d**)*

M.p. 206–208 °C, 57% yield, ^{1}H-NMR: δ 1.34 (m, 2H), 1.62 (q, $J = 7$ Hz, 4H), 2.38 (t, $J = 7$ Hz, 4H), 7.79 (d, $J = 8$ Hz, 4H), 8.16 (d, $J = 8$ Hz, 4H), 10.46 (s, 2H).

*5.1.1.5. N,N′-Bis(4-nitrophenyl)hexanediamide (**1e**)*

M.p. 220–222 °C, 49% yield, ^{1}H-NMR: δ 1.26–1.38 (m, 4H), 1.60 (q, $J = 7$ Hz, 4H), 2.37 (t, $J = 7$ Hz, 4H), 7.8 (d, $J = 9$ Hz, 4H), 8.17 (d, $J = 9$ Hz, 4H), 10.46 (s, 2H).

*5.1.1.6. N,N′-Bis(4-nitrophenyl)heptanediamide (**1f**)*

M.p. 155–158 °C, 52% yield, ^{1}H-NMR: δ 1.24–1.36 (q, $J = 7$ Hz, 6H), 1.58 (q, $J = 7$ Hz, 4H), 2.36 (t, $J = 7$ Hz, 4H), 7.81 (d, $J = 9$ Hz, 4H), 8.18 (d, $J = 9$ Hz, 4H), 10.46 (s, 2H).

5.1.2. General procedure for the preparation of the N,N′-(4,4′-diaminophenyl)alkanodiamides

Ethanol (10 mL), Pd/C 5% (0.046 g), hydrazine (0.818 mL, 25.9 mmol), water (0.93 mL) and **1a**–**c** (2.59 mmol) were mixed in a round bottom flask. The mixture was refluxed for 2 h. The resulting solid was dissolved in methanol with heat and filtered at vacuum. Methanol was eliminated upon precipitation of a solid that was filtered and crystallised from methanol to afford **2a**–**c**.

*5.1.2.1. N,N′-Bis(4-aminophenyl)ethanediamide (**2a**)*

M.p. 232–234 °C, 92% yield, ^{1}H-NMR: δ 2.52 (s, 4H), 4.78 (s, 4H), 6.47 (d, $J = 9$ Hz, 4H), 7.20 (d, $J = 9$ Hz, 4H), 9.51 (s, 2H).

*5.1.2.2. N,N′-Bis(4-aminophenyl)propanediamide (**2b**)*

M.p. 228–230 °C, 94% yield, ^{1}H-NMR: δ 1.83 (q, $J = 7$ Hz, 2H), 2.25 (t, $J = 7$ Hz, 4H), 4.79 (s, 4H), 6.47 (d, $J = 10$ Hz, 4H), 7.20 (d, $J = 10$ Hz, 4H), 9.44 (s, 2H).

*5.1.2.3. N,N′-Bis(4-aminophenyl)butanediamide (**2c**)*

M.p. 238–240 °C, 93% yield, ^{1}H-NMR: δ 1.56 (t, $J = 7$ Hz, 4H), 2.21 (t, $J = 7$ Hz, 4H), 4.81 (s, 4H), 6.46 (d, $J = 9$ Hz, 4H), 7.19 (d, $J = 9$ Hz, 4H), 9.43 (s, 2H).

*5.1.2.4. N,N′-Bis(4-aminophenyl)pentanediamide (**2d**)*

M.p. 203–204 °C, 83% yield, ^{1}H-NMR: δ 1.26–1.31 (m, 2H), 1.55 (q, $J = 7$ Hz, 4H), 2.19 (t, $J = 7$ Hz, 4H), 4.79 (s, 4H), 6.46 (d, $J = 9$ Hz, 4H), 7.17 (d, $J = 9$ Hz, 4H), 9.40 (s, 2H).

*5.1.2.5. N,N′-Bis(4-aminophenyl)hexanediamide (**2e**)*

M.p. 211–212 °C, 87% yield, ^{1}H-NMR: δ 1.25–1.35 (m, 4H), 1.56 (q, $J = 7$ Hz, 4H), 2.2 (t, $J = 7$ Hz, 4H), 4.74 (s, 4H), 6.47 (d, $J = 9$ Hz, 4H), 7.19 (d, $J = 9$ Hz, 4H), 9.39 (s, 2H).

*5.1.2.6. N,N′-Bis(4-aminophenyl)heptanediamide (**2f**)*

M.p. 195–197°C, 88% yield, ^{1}H-NMR: δ 1.25–1.3 (m, 6H), 1.54 (q, $J = 7$ Hz, 4H), 2.19 (t, $J = 7$ Hz, 4H), 4.79 (s, 4H), 6.46 (d, $J = 9$ Hz, 4H), 7.18 (d, $J = 9$ Hz, 4H), 9.39 (s, 2H).

5.2. Cytotoxic activity

The tumoural cell lines were supplied by the National Cancer Institute. The cytotoxicity assays were carried out at 5000–7500 cells mL^{-1} as reported by Skehan et al. [11] and Monks et al. [12] using the sulforhodamine B (SRB) protein assay to estimate cell growth. The percentage growth was evaluated spectrophotometrically in a Bio kinetics reader spectrophotometer.

Acknowledgements

We thank CONACYT (32633-E) and DGAPA-UNAM (IN206598) for financial support. L.C.G. thanks CONACYT for a doctoral fellowship support. We also thank M.T. Ramírez Apan for obtaining the biological data. Contribution No. 1737 from Instituto de Química, UNAM.

References

[1] Lown J.W., Drug Dev. Res. 34 (1995) 145–183.

[2] Kubicova L., Waisser K., Kunes J., Kralova K., Odlerova Z., Slosarek M., Janota J., Svoboda Z., Molecules 5 (2000) 714–726.

[3] Serradji N., Bensaid O., Martin M., Kan E., Dereuddre-Bosquet N., Redeuilh C., Huet J., Heymans F., Lamouri A., Clayette P., Dong C.Z., Dormont D., Godfroid J.-J., J. Med. Chem. 43 (2000) 2149–2154.

[4] Martínez R., Cogordan J.A., Mancera C., Diaz M.L., Il Farmaco 55 (2000) 631–636.

[5] Canellakis E.S., Shaw H.Y., Hanners W.E., Schwartz R.A., Biochim. Biophys. Acta 418 (1976) 277–289.

[6] Wakelin L.P.G., Romanos M., Chan T.K., Glaubiger D., Canellakis E.S., Waring M.J., Biochemistry 17 (1978) 5057–5063.

[7] The structural analogy with some groove binders is seen for example when compared with compounds cited in Ref. [8] and Cozzi, P., Farmaco 56 (2001) 57–65.

[8] Wemmer D.E., Dervan P.B., Curr. Opin. Struct. Biol. 7 (1997) 355–361.

[9] Bhattacharya S., Thomas M., Tetrahedron Lett. 41 (2000) 5571–5575.

[10] Ryabinin V.A., Sinyakov A.N., Soultrait V.R., Caumont A., Parissi V., Zakharova O.D., Vasyutina E.L., Yurchenko E., Bayandin R., Litvak S., Tarrago-Litvak L., Nevinsky G.A., Eur. J. Med. Chem. 35 (2000) 989–1000.

[11] Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., Vistica D., Warren J.T., Bokesch H., Kenney S., Boyd M.R., J. Nat. Cancer Inst. 82 (1990) 1107–1112.

[12] Monks A., Scudiero D., Skehan P., Shoemaker R., Paull K., Vistica D., Hose C., Langley J., Cronise P., Vaigro-Wolff A., Gray-Goodrich M., Campbell H., Mayo J., Boyd M., J. Nat. Cancer Inst. 83 (1991) 757–765.