ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 55 (2000) 631–636

IL FARMACO

Synthesis and cytotoxic evaluation of N^1,N^m-bis[(tetrahydrobenzo[a]acridin-12-yl)phenyl]alkanediamides and N^1,N^m-bis[(tetrahydrobenzo[c]acridin-7-yl)phenyl]-alkanediamides

Roberto Martínez [a,*], Juan Antonio Cogordan [a], Claudia Mancera [b], Ma. Luisa Díaz [b]

[a] *Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, Mexico D.F., Mexico*

[b] *Departamento de Química Orgánica, DES de la Facultad de Ciencias Químicas Industriales, UAEM, Av. Universidad 1001, Cuernavaca, Mor., Mexico*

Received in revised form 16 August 2000

Abstract

The title compounds were synthesized in four steps from 5,5-dimethyl-1, 3-cyclohexanedione as starting material. These compounds were evaluated against 60 tumoral cell lines, whereas the N^1,N^m-bis[benzo[c]acridin-7-yl]phenyl]alkanediamides displaying significant cytotoxic activity, the corresponding N^1,N^m-bis[benzo[a]acridin-12-yl]phenyl]alkanediamides derivatives were found to be less cytotoxic.

Keywords: Synthesis; Benzacridines; Alkanediamides; Anticancer activity

1. Introduction

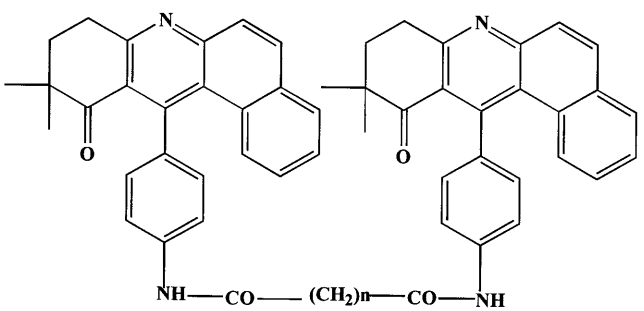
Cancer represents a serious human health problem despite much progress in understanding the biology and pharmacology. The main problem is that cancer is not one disease, but a group of diseases affecting different organs and systems of the body. It develops due to the abnormal and uncontrolled cell division, frequently at a rate greater than that of most normal body cells [1]. The traditional therapeutic strategies for the treatment of the cancer are surgery, radiotherapy, immunotherapy and chemotherapy. Today, 50% of the patients diagnosed with cancer are cured through one of these methods or by a combination of them. Chemotherapy is, at present, the only one effective therapy for some types of disseminated cancers because anticancer drugs travel through the circulatory system. Many compounds exert their action by interfering with the function of DNA. Their modes of interaction induce (1) non-covalent binding such as intercalation and groove binding, (2) covalent binding and (3) DNA backbone scission [2]. The essence of intercalation is the insertion of molecules which possess large planar ring systems (optimum at three to four rings, most often heteroaromatic) between adjacent base pairs of DNA, thereby extending and stabilizing the double helix; base-pair separation at an intercalation site thus increases from 3.7 to 8 Å [3]. The process of DNA transcription, translation and replication can all be affected by intercalation. Based on mechanistic studies, the intercalation into DNA seems to play the decisive role for their antitumor activity together with the ability to form stable hydrogen bonds with DNA [4]. In the last decade, anthracycline has become perhaps the best understood DNA intercalator [5]. However, there are other antitumor drugs (as 9-aminoacridine, whose intercalation has been experimentally verified [6]) which cannot build up hydrogen bonds with DNA-bases because of the lack of an appropriate side-chain with oxygen or nitrogen atoms. For this drug and other simple acridine derivatives, another stabilization factor must exist to build up an intercalation complex. One further important binding contribution of intercalators to DNA may be the formation of charge-transfer complexes with electron rich DNA-bases [7]. Bis-intercala-

* Corresponding author.
E-mail address: robmar@servidor.unam.mx (R. Martínez).

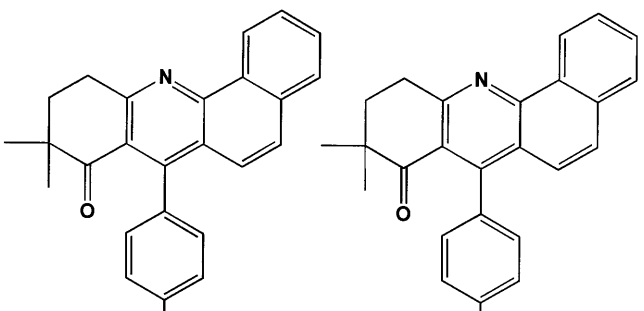
0014-827X/00/$ - see front matter
PII: S0014-827X(00)00073-2

tors consist of two separate chromophore squeleton joined by a spacer unit. The association constants of bis-intercalators with bDNA are considerably higher than mono-intercalators [8]; bis-daunorubicin is a structural example [9].

We are currently engaged in a program to devise the synthesis of heterocyclic compounds with possible pharmacological activity [10]. In the present work we report the synthesis of compounds **1**–**8** from 5,5-dimethyl-1,3-cyclohexanedione. Several desirable criteria were identified at the onset of this work. The first was that the products must have a planar moiety that could be intercalated into the DNA double strand. Second, the spacer unit must have a variable length that could be joined to the DNA strand by a hydrogen bond. Third, the products could be obtained in an easy way. The structure of these compounds was determined by IR, ^{1}H NMR and MS. The synthesized compounds were sent for pharmacological evaluation to the National Cancer Institute.



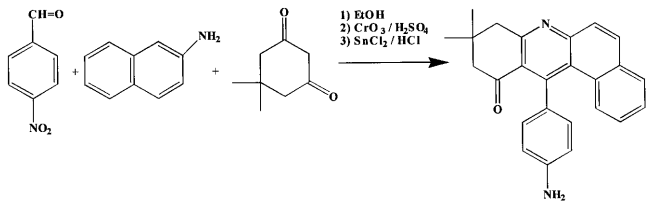
Compound	n
1	2
2	3
3	4
4	8

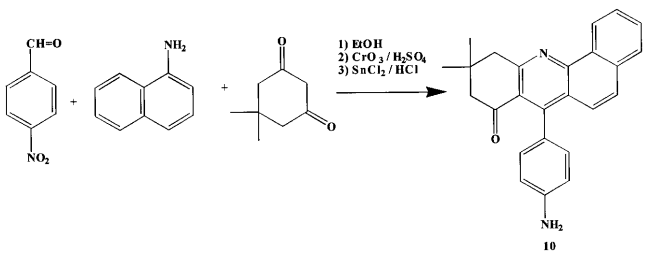


Compound	n
5	2
6	3
7	4
8	8

2. Chemistry

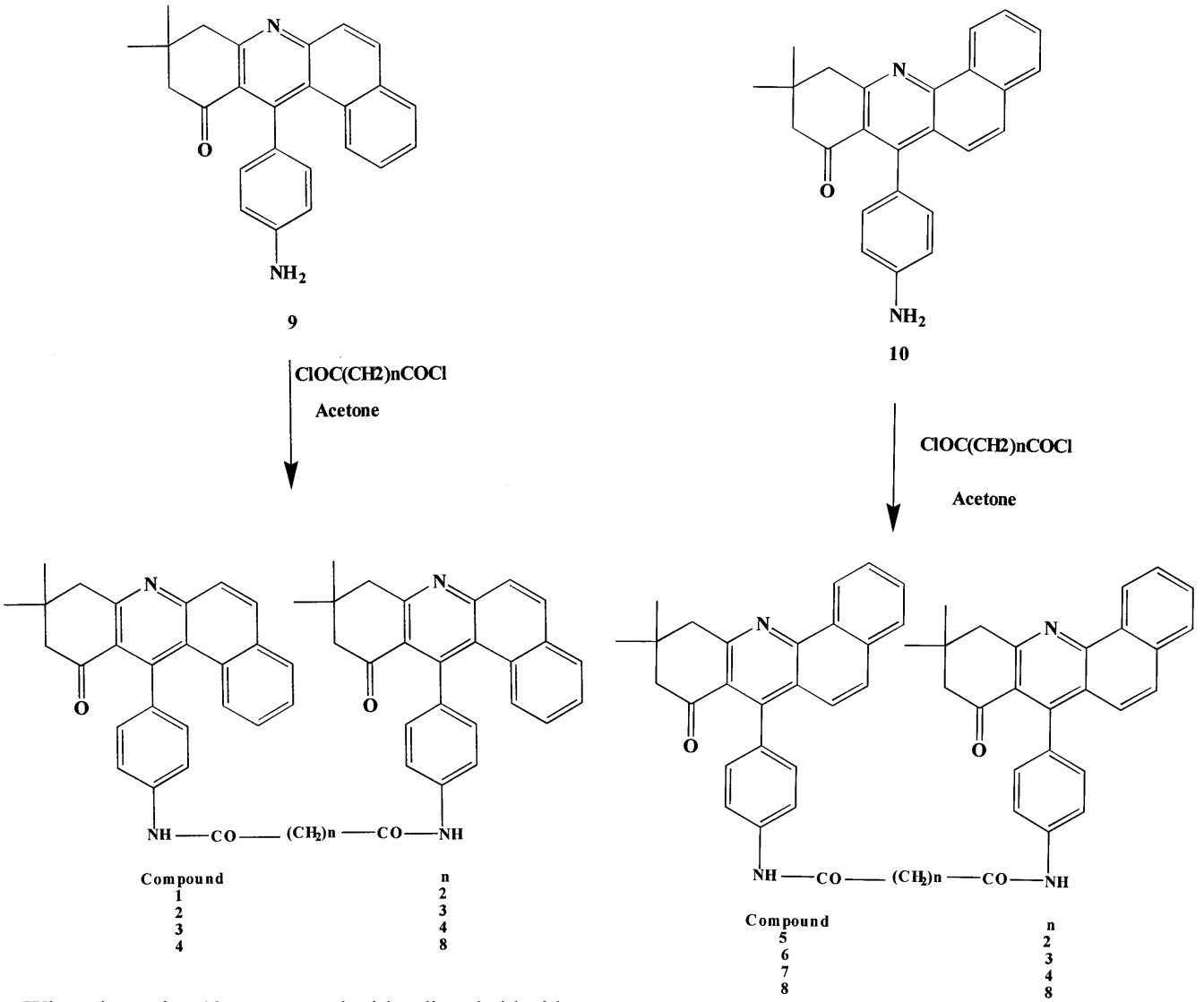
We prepared a series of N^1,N^m-bis[4-(9,9-dimethyl-11 - oxo - 8,9,10,11 - tetrahydrobenzo[a]acridin - 12 - yl)-phenyl]alkyldiamides (**1**–**4**) and N^1,N^m-bis[(10,10-dimethyl-8-oxo-8,9,10,11-tetrahydro-benzo[c]acridin-7-yl)phenyl]alkyldiamides (**5**–**8**) ($n = 2$, 3, 5, 8) in four steps. In the first step, compounds **9** and **10** were prepared following a reported procedure from 5,5-dimethyl-1,3-cyclohexanedione [11].





10

The acylation of **9** with adipoyl chloride in acetone furnishes the corresponding adipoyldiamide **3** in good yield. In agreement with the suggested structure, the IR spectra of compound **3** exhibited bands at 3329 cm^{-1} (–NH), 1666 cm^{-1} (amide carbonyl band) and 1695 cm^{-1} (ketone carbonyl band). Its ^{1}H NMR spectra showed a singlet at δ 1.45 for the methyl protons of C-9 and C-9′ as well as one broad singlet of the two methylene protons of aliphatic chain at δ 2.27. One eight-proton signal at δ 3.08 (broad signal) was given out to the four methylene protons joined to the amide carbonyl group and to the four methylene protons of C-10 and C-10′. A four-proton signal at δ 3.74 (singlet) was given out to the methylene protons joined to C-8 and C-8′. Two proton signal at δ 8.70 (doublet) was attributed to the C-1 and C-1′ protons. The remaining aromatic protons and –NH proton appeared at δ 7.50–8.27. The fast atom bombardment mass spectrum of these compounds showed their m/z ($M^+ + 1$) ions as their base peak. Compounds **1**, **2** and **4** were synthesized using a similar procedure and their spectral data are in agreement with their structure.



When the amine **10** was treated with adipoyl chloride in acetone, the adypoyl diamide **5** was produced**.** Structural assignment of the derivative **5** was made on spectroscopic grounds.

In the IR spectra of **5** the appearance of absorption bands at 3288 and 1652 cm^{-1} were consistent with the presence of an amide carbonyl band group and also showed the ketone carbonyl band at 1693 cm^{-1}. In the ^1H NMR spectra of derivative **5** the presence of a 12-proton singlet at δ 1.3 confirmed the presence of the four-methyl groups joined to C-10 and C-10′; two four-protons singlets at δ 3.30 and 3.70 were assigned to the methylene protons joined to C-9, C-9′ and C-11 and C-11′. The four-proton multiplet at δ 2.85 was assigned to the methylene protons of the aliphatic chain and the downfield two-proton doublet at 8.90 signal to H1 and H1′. The remaining aromatic protons and the –NH proton appeared at δ 7.26–8.17. Further evidence of the structure of **5** is derived from it mass spectral data that showed the $(M^+ + 1)$ ion.

Table 1
Physicochemical properties of the investigated compounds **1–8**

Comp.	*n*	Yield (%)	Analysis
1 [a]	2	96	$C_{54}H_{46}O_4N_4$ (814.97)
2	3	93	$C_{55}H_{48}O_4N_4$ (829.02)
3	4	97	$C_{56}H_{50}O_4N_4$ (843.05)
4	8	98	$C_{60}H_{58}O_4N_4$ (899.15)
5	2	94	$C_{54}H_{46}O_4N_4$ (814.97)
6	3	98	$C_{55}H_{48}O_4N_4$ (829.02)
7	4	95	$C_{56}H_{50}O_4N_4$ (843.05)
8	8	96	$C_{60}H_{58}O_4N_4$ (899.15)

[a] The melting point of each compound is >350°C.

3. Experimental

3.1. Procedures

All melting points are uncorrected. The IR spectra were recorded on a Nicolet FT-55X spectrophotometer.

Table 2
Spectroscopic data for the alkanediamides **1**–**8**

Comp.	IR (nujol)	^{1}H NMR (CF_3CO_2D)
1	3248, 1693,1658	1.45 (s, 12H, 2×CH_3–C9), 3.08 (bs, 8H, H10+CH_2–CO), 3.52 (s, 4H, H8), 7.48–8.10 (m, 20H, Ar–H+NH), 8.68 (d, 2H, J = 7.8 Hz, H1)
2	3290, 1692, 1656	1.43 (s, 12H, 2×CH_3–C9), 2.27 (m, 2H, –CH_2–), 3.06 (bs, 8H, H10+CH_2–CO), 3.64 (s, 4H, H8), 7.50–8.17 (m, 20H, Ar–H+NH), 8.69 (d, 1H, J = 7.8 Hz, H1)
3	3329, 1695, 1666	1.45 (s, 12H, 2×CH_3–C9), 2.27 (bs, 4H, –CH_2CH_2–), 3.08 (bs, 8H, H10+CH_2–CO), 3.74 (s, 4H, H8), 7.50–8.27 (m, 20H, Ar–H+NH), 8.70(d, 2H, J = 7.8 Hz, H1)
4	3326, 1696, 1658	1.42 (s, 12H, 2×CH_3–C9), 2.27 (m, 12H, –CH_2–), 3.05 (bs, 8H, H10+CH_2–CO), 3.68 (s, 4H, H8), 7.50–8.17 (m, 20H, Ar–H+NH), 8.71 (d, 1H, J = 7.8 Hz, H1)
5	3258, 1693, 1652	1.30 (s, 12H, 2×CH_3–C10), 2.85 (m, 4H, –CH_2–), 3.30 (bs, 4H, H9), 3.70 (s, 4H, H11), 7.26–8.17 (m, 20H, Ar–H+NH), 8.90 (d, 2H, J = 8.0 Hz, H1)
6	3232, 1702, 1681	1.48 (s, 12H, 2×CH_3–C10), 2.28 (m, 2H, –CH_2–), 2.90 (bs, 8H, H9+CH_2–CO), 3.69 (s, 4H, H11), 7.30–8.18 (m, 20H, Ar–H+NH), 8.87 (d, 1H, J = 8.0 Hz, H1)
7	3306, 1695, 1652	1.30 (s, 12H, 2×CH_3–C10), 2.10 (m, 4H, –CH_2–), 2.90 (bs, 8H, H9+CH_2–CO), 3.75 (s, 4H, H11), 7.25–8.20 (m, 20H, Ar–H+NH), 8.90 (d,1H, J = 8.0 Hz, H1)
8	3325, 1694, 1670	1.40 (s, 12H, 2×CH_3–C10), 1.50 (s, 8H, –CH_2–) 2.0 (bs, 4H, –CH_2–), 2.81 (bs, 4H, –CH_2–CO), 2.98 (s, 4H, H9), 3.86 (s, 4H, H11), 7.43–8.4 (m, 20H, Ar–H+NH), 9.10 (d, 2H, J = 8.0 Hz, H1)

The ^{1}H NMR spectra were determined on a Varian FT-200 and Varian FT-300 instrument. All NMR spectra were obtained with the pulse sequence as part of the spectrometer's software and was determined in deuterotrifluoroacetic acid solution containing tetramethylsilane as the internal standard with chemical shifts (δ) expressed downfield from tetramethylsilane. Mass spectra were recorded using a JEOL SX-102 mass spectrometer using the direct inlet system with an ionization energy of 70 eV, an emission current of 100 μA and ion source temperature of 150°C. Column chromatography was carried out on Merck Kieselgel 60 F_{254}. Thin layer chromatography was carried out on Merck Kieselgel 60 PF_{254}. All the used solvents were dried over appropriate drying agent.

The starting 12-(*p*-aminophenyl)-9,9-dimethyl-,8,9,10,11-tetrahydrobenz[a]acridin-11-one (**9**) and 7-(*p*-aminophenyl) - 10,10 - dimethyl - 8,9,10,11 - tetrahydrobenz[c]acridin-8-ones (**10**) were prepared following our reported procedure from dimedone, para-nitrobenzaldehyde and 2-naphthylamine or 1-naphtylamine, respectively. The structure of compounds **9** and **10** was supported by IR, ^{1}H NMR and mass spectral data, which are identical to that reported [11].

*3.2. Synthesis of N^1,N^m-bis[4-(9,9-dimethyl-11-oxo-8,9,10,11-tetrahydrobenzo[a]acridin-12-yl) phenyl]-alkanediamides (**1–4**) and N^1,N^m-bis[4-(10,10-dimethyl-8-oxo-8,9,10,11-tetrahydrobenzo [c]acridin-7-yl) phenyl]alkyldiamides (**5–8**)*

*3.2.1. N^1,N^6-bis[4-(9,9-dimethyl-11-oxo-8,9,10,11-tetrahydrobenzo[a]acridin-12-yl)phenyl]hexane diamide (**3**) (n = 4)*

3.2.1.1. General procedure. Adipoyl chloride (0.025 g, 0.13 mmol) was added to a solution of 0.1 g of **9** (0.27 mmol) in 15 ml of acetone at 5°C. After 15 min stirring, the mixture was poured into a 10% potassium carbonate solution. The resulting amorphous solid **3** was crystallized from acetone. Analytical and spectroscopic data of the diamides **1**–**8** are shown in Tables 1 and 2, respectively.

3.3. Biological activity

The biological studies were carried out by the National Cancer Institute in Betseda, USA. The NCI screening procedures are described [12] as the origins and processing of the cell lines. Briefly, cell suspensions that were diluted according to the particular cell type and the expected target cell density (5000–40 000 cells per well based on cell growth characteristics), were added by pipet (100 μl) into 96-well microtiter plates. Inoculates were allowed a preincubation period of 24 h at 37°C for stabilization. Dilutions at twice the intended test concentration were added at time zero in 100-μl aliquots to the microtiter plate wells. Usually, test compounds were evaluated at five tenfold dilutions. In routine testing, the highest well concentration is 10E − 4 M, but for the standard agents the highest well concentration used depended on the agent. Incubations lasted for 48 h in a 5% CO_2 and 95% air atmosphere and 100% humidity. The cells were assayed by using the sulforhodamine B assay [13,14]. A plate reader was used to read the optical densities, and a microcomputer processed the optical densities into the special concentration parameters defined later.

3.4. Special concentration parameters GI_{50}, TGI, and LC_{50}

The NCI renamed the IC_{50} value, the concentration that causes 50% growth inhibition, the GI_{50} value to emphasize the correction for the cell count at time zero; thus, GI_{50} is the concentration of test drug where $100 \times (T - T_0)/(C - T_0) = 50$ [3,9]. The optical density of the test well after a 48-h period of exposure to test drug is T, the optical density at time zero is T_0, and the control optical density is C. The '50' is called the GI50PRCNT, a T/C-like parameter that can have values from $+100$ to -100. The GI_{50} measures the growth inhibitory power of the test agent. The TGI is the concentration of test drug where $100 \times (T - T_0)/(C - T_0) = 0$. Thus, the TGI signifies a cytostatic effect. The LC_{50}, which signifies a cytotoxic effect, is the concentration of drug where $100 \times (T - T_0)/T_0 = -50$. The control optical density is not used in the calculation of LC_{50}.

Table 3
Selected data of anticancer activity in vitro of compounds **1–4**

Cell line	GI_{50}	TGI	LC_{50}
Compound **1** ($n = 2$)			
EKVX (lung)	9.53×10^{-5}	$>1.00 \times 10^{-4}$	$>1.00 \times 10^{-4}$
SNB-75 (CNS)	2.5×10^{-5}	$>1.00 \times 10^{-4}$	$>1.00 \times 10^{-4}$
Compound **2** (n = 3)			
HL-60(TB) (leukemia)	5.94×10^{-6}	$>1.00 \times 10^{-4}$	$>1.00 \times 10^{-4}$
SNB-75 (CNS)	4.16×10^{-5}	$>1.00 \times 10^{-4}$	$>1.00 \times 10^{-4}$
HB578T (breast)	6.03×10^{-5}	$>1.00 \times 10^{-4}$	$>1.00 \times 10^{-4}$
Compound **3** ($n = 4$)	all	values	$>1.00 \times 10^{-4}$
Compound **4** ($n = 8$)	all	values	$>1.00 \times 10^{-4}$

Table 4
Selected data of anticancer activity in vitro of compounds **5–8**

Cell line	GI_{50}	TGI	LC_{50}
Compound **5** ($n = 2$)			
HCT-15 (colon)	4.65×10^{-5}	$>1.00 \times 10^{-4}$	$>1.00 \times 10^{-4}$
INGROVI (ovarian)	5.85×10^{-5}	$>1.00 \times 10^{-4}$	$>1.00 \times 10^{-4}$
Compound **6** ($n = 3$)			
HCT-15 (colon)	2.78×10^{-5}	5.09×10^{-5}	9.32×10^{-5}
SF-295 (CNS)	1.96×10^{-5}	4.16×10^{-5}	8.84×10^{-5}
LOX IMVI (melano.)	1.76×10^{-5}	3.33×10^{-5}	6.32×10^{-5}
Compound **7** ($n = 4$)			
INGROVI (ovarian)	1.70×10^{-8}	$>1.00 \times 10^{-4}$	$>1.00 \times 10^{-4}$
Compound **8** ($n = 8$)			
SNB-75 (CNS)	2.82×10^{-5}	$>1.00 \times 10^{-4}$	$>1.00 \times 10^{-4}$

4. Results and discussion

The results of the in vitro anticancer studies of the compounds **1–4** and **5–8** are summarized in Tables 3 and 4. A study of the results shows that the type of fusion of benzene ring to the acridine moiety together with the length of the aliphatic chain might play some role in the selectivity of the anticancer activity. One of the facts observed in Table 1 is that the benz(a) compounds **1** ($n = 2$) and **2** ($n = 3$) display moderate cytostatic activity against EKVX (lung), HL-60(TB) (leukemia) and HB578T (breast) cell lines, respectively. Also seen in Table 1 is the marked loss of the growth inhibitory power when the aliphatic chain is increased as in compounds **3** ($n = 4$) and **4** ($n = 8$). As seen in Table 2 the benz(c) compounds **5** ($n = 2$) and **6** ($n = 3$) display moderate activity against HCT-15 (colon) cell lines; compound **5** ($n = 2$) and and **7** ($n = 4$) contra INGROVI (ovarian) cell line.

The benz(a) compounds **1** ($n = 2$) and **2** ($n = 3$) showed inhibitory potency equivalent to that of the benz(c) compound **8** ($n = 8$) towards the SNB-75 (CNS) cell line. This result suggests that in the SNB-75 cell line there are not restrictions on the type of fusion between the benzene ring and the acridine moiety or the length of the alkane chain.

Acknowledgements

We are grateful to C. Contreras, H. Ríos, B. Quiroz, R. Gabiño, L. Velasco and F.J. Pérez for their assistance in obtaining the IR, ^{1}H NMR and MS spectral data. We also thank the Consejo Nacional de Ciencia y Tecnología (CONACYT) Proy-1427P-N9507 for financial support. Contribution No.1713 from Instituto de Química, UNAM.

References

[1] S.P. Gupta, Quantitative structure–activity relationship studies on anticancer drugs, Chem. Rev. 94 (1994) 1507–1551.
[2] S.R Rajski, R.M. Williams, DNA cross-linking agents as antitumor drugs, Chem. Rev. 98 (1998) 2723–2795.
[3] L.S. Lerman, Structural considerations in the interaction of deoxyribonucleic acid and acridines, J. Mol. Biol. 3 (1961) 18–30.
[4] K. Reska, P. Kolodziejczyk, J.A. Hartley, W.D. Wilson, J.W. Lown, Molecular pharmacology of anthracenedione-based anti-

cancer drugs, in: J.W Lown (Ed.), Bioactive Molecules, Anthracycline and Anthracenedione-based Anticancer Agents, vol. 6, Elsevier, Amsterdam, 1998, p. 401.

[5] J.W. Lown, Discovery and development of anthracycline antitumor antibiotics, Chem. Soc. Rev. 22 (1993) 165–176.

[6] T.D. Sakore, B.S Reddy, H.M. Sobell, Visualization of drug-nucleic acid interactions at atomic resolution. IV. Structure of an aminoacridine/dinucleoside monophosphate, J. Mol. Biol. 135 (1979) 763–785.

[7] C. Rehn, U. Pindur, Molecular modeling of intercalation complexes of antitumor active 9-aminoacridine and a [d,e]-anellated isoquinoline derivative with base paired deoxytetranucleotides, Monatsh. Chem. 127 (1996) 645–658.

[8] U. Pindur, M. Haber, K. Sattler, Antitumor active drugs as intercalators of deoxyribonucleic acid, J. Chem. Educ. 70 (1993) 263–272.

[9] F. Leng, W. Priebe, J.B. Chaires, Ultratight DNA binding of a new bisintercalating anthracycline antibiotic, Biochemistry 37 (1998) 1743–1753.

[10] V.O. Nava-Salgado, R. Martínez, M.F. Rubio Arroyo, G. Ramírez Galicia, Molecular association for the design of anti-HIV-1 agents. Conformational study of {3′-azido-3′-deoxythymidine}-{4,5,6,7-tetrahydro-5-methylimidazo-[4,5,1-jk]-[1,4]benzodiazepin-2(1H)-one} derivatives, Teochem. J. Mol. Struct. 504 (2000) 69–75.

[11] R. Martinez, R. Gaviño, J.G. Avila, Synthesis, reactivity and spectral properties of some 12-(*o*- and *p*-*R*-phenyl)-9,9-dimethyl-8,9,10,11-tetrahydrobenz[a]acridin-11-ones and 7-(*o*- and *p*-*R*-phenyl)-10,10-dimethyl-8,9,10,11-tetrahydrobenz[c]acridin-8-ones, Trends Heterocyc. Chem. 5 (1997) 75–81.

[12] A. Monks, D.A Scudiero, P. Skehan, R.H. Shoemaker, K.D. Paull, D.T Vistica, C. Hose, J. Langley, P. Cronise, M. Vaigro-Wolff, Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines, J. Natl. Cancer Inst. 83 (1991) 757–766.

[13] L.V. Rubinstein, R.H. Shoemaker, K.D. Paull, R.M. Simon, S. Tosini, P. Skehan, D.A. Scudiero, A. Monks, M.R. Boyd, Comparison of in vitro anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines, J. Natl. Cancer Inst. 82 (1990) 1113–1118.

[14] P. Skehan, R. Storeng, D.A. Scudiero, A. Monks, J. Mcmahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drug screening, J. Natl. Cancer Inst. 82 (1990) 1107–1112.