Bioorganic Chemistry 36 (2008) 183–189

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

The usefulness of cyclic diamidines with different core-substituents as antitumor agents

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ARTICLE INFO

Article history:
Received 5 February 2008
Available online 20 June 2008

Keywords:
Antitumor
Carbazoles
Fluorenes
Furans
Imidazolines
Piperidines
Pyrenes
Tetrahydropyrimidines
Triazines
Xylenes

ABSTRACT

A series of related polycationic compounds has been screened for potential antitumor activity by the NCI's in vitro testing (one dose primary anticancer assay and the NCI-60 full panel screening). The GI_{50} values of triazines **3** and **4** are on average 1.9 μM and 2.4 μM, respectively. Furan **8** deserves mention too (1.9 μM). The biological test results showed that carbazole **10** possessed cytotoxic activity in the nanomolar range, much better than the other compounds tested, only against several cancer cell lines: CCRF-CEM, HL-60(TB), MOLT-4, NCI-H522, COLO 205, SF-268, but the average GI_{50} value was higher (15 μM). The activity appears closely dependent on the core-shape and length of the bisimidazoline molecules (important for both high cytotoxicity and DNA binding). The mechanism of DNA minor-groove binding of diamidines **1–12**, based on the anticancer parameters, is highly probable.

1. Introduction

Anticancer drugs have been classified as antitumor antibiotics, antimetabolites, antitubulin agents, topoisomerase inhibitors, and alkylating agents [1–5]. They often exhibit multiple mechanisms of action in tumor cells. During the past few decades, simple and cyclic amidines and their derivatives have been widely tested as antitumor agents in vitro and in vivo treatment [6–23]. Other exemplary compounds, containing the amidine N=C–N unit (e.g., pyrimidines, purines, triazines, guanidines, ureas), are usually considered separately.

From a general structure-activity standpoint, the above groups of amidines demonstrate that the structural variations can result in significant changes in specificity and potency with regard to anticancer activity. The active bisamidines increase the denaturation temperature of the calf thymus DNA (stimulate topoisomerase II—mediated DNA cleavage). A general strategy for the synthesis of drugs having a specific activity has been elaborated [20,24].

Hydrogen bond formation is involved in many biological processes. A model has been described for the biological activity against *Giardia lamblia* in which only one nitrogen atom of both amidine groups is in hydrogen bonding distance to the thymine O2 atom at AT rich sites of the minor-groove of DNA and the two cationic groups lie between phosphate groups on each strand of the duplex [24,25]. The complex results in the inhibition of the microbial topoisomerase II enzyme. Other findings of a molecular modeling study are in agreement with the crystal structure described [26].

Additional bifurcated hydrogen bonding interactions between the indole NH hydrogen and the thymine O2-carbonyl groups overstabilize the B-form helix and increase the ΔT_m values of the DAPI-DNA complex [27]. The complexation can occur in vivo and it causes inhibition of DNA synthesis at therapeutic levels in humans, in treatment of far advanced cancer cases.

The amidinium moiety is known to contribute to the stabilization of DNA recognition element through electrostatic and hydrogen bonding interactions [28–32]. Therefore, hydrogen bonds are frequently used as recognition elements due to their directionality. The strategy approach for the development of new drugs for the treatment of diseases, which are induced by RNA viruses, is based on the design of agents which bind to specific stem or loop units in the viral RNA genome [33,34].

The binding affinities and specificities observed suggest that the incorporation of a variety of moieties will lead to substances that interact with RNA targets. This approach greatly expands the utility of the amidines and related polycationic compounds for the construction of drugs in general. Hydrogen bonding is an attractive approach to the biological activity [35]. If there is the right size of a drug, the hydrogen bonds become stronger.

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0045-2068/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2008.05.002

It has been shown that various bisamidines can be effective for opportunistic infections and as antifungal, antileishmanial, antiplasmodial, antiprotozoal, and antitrypanosomal agents [36–43]. The structures, which possess adequate proportions of interactions among DNA [44] (e.g., Berenil, DAPI, Furamidine DB75, Hoechst 33258, Pentamidine), are mainly related to several antibiotics [45,46] (Amidinomycin, Anthelvencin A and B, Congocidin, Distamycin A, Kikumycin A and B, Noformycin) and their derivatives [32,47,48].

The radius of curvature of several defined groups in the mimic molecule shows that the shape of the latter affects the strength of nucleic acid binding. Therefore, many classes of drugs have substantial curvature, high DNA affinity, binding to the minor-groove, and interference with DNA-associated enzymes. Very often, the cytotoxicity remains at the micromolar level and confirms the results provided by the T_m studies [20].

This paper highlights usefulness in medicinal chemistry of the direct reaction of formation of cyclic amidines and secondary amides from nitriles. The practical value of this complex reaction has been demonstrated by preparation of some bisimidazolines [49] and tetrahydropyrimidines [50]. The reaction is an alternative method to the existing methodologies based on sulfur containing reagents [9,51–57].

In several papers, the formation of a range of cyclic amidines from thioamides or imidates has been described [58,59]. Both thioamides and nitriles were important synthetic intermediates for the preparation of simple amidines [60–62]. This paper describes the practical results obtained from anticancer screening of the products of the cited reactions. The presence of cationic groups and other core structural features make these compounds very suitable probes for nucleic acid binding modes. Therefore, it is desirable to design strong DNA binders by means of various anticancer parameters.

Table 1
Growth percentages of **1–13** (one dose primary anticancer assay)

Compound	NSC Number	NCI-H460 (Lung)	MCF7 (Breast)	SF-268 (CNS)
1	NSC 710604	12	10	8
2	NSC 710605	23	17	30
3	NSC 710607	−90	−79	−85
4	NSC 710608	−90	−91	−96
5	NSC 711986	20	14	31
6	NSC 711987	20	7	36
7	NSC 711989	−74	−74	−66
8	NSC 714616	8	−74	14
9	NSC 714620	29	49	57
10	NSC 715653	−80	3	−45
11	NSC 715654	−48	−33	−49
12	NSC 717052	9	16	72
13	NSC 717046	100	98	102

2. Materials and methods

2.1. Materials

Melting points were uncorrected and determined on a Boetius melting point apparatus. NMR spectra were recorded on a Varian Gemini (300 MHz) spectrometer with D_2O solutions using TMS as the internal standard. High-resolution mass spectra were obtained using an AMD 402 or 602 mass spectrometer in the EI or FAB mode, respectively. Thin layer chromatography was performed with Merck silica gel 60 F_{254} plates (0.25 mm thickness). The reagents were purchased from Aldrich. Compounds **1–11** are the author samples in the literature cited [9,24,42,49]. All final compounds were dried in an oven at 120 °C for several hours and then stored in a vacuum desiccator over P_2O_5.

2.2. Representative procedures for the preparation of amidines **12** and **13**

Polycationic compounds containing an α, α′-xylene linker have been found to exhibit various inhibitory effects [34,63]. Monocyclam and bicyclam have proven to be effective against HIV-1 and HIV-2 and have been tested in clinical trials [64–66]. They interfere with nucleic acid binding *via* non-covalent interactions. All xylene and toluene derivatives described in this paper were prepared following the synthetic way outlined in Fig. 2.

The syntheses of the cyclic amidines were previously disclosed by the author [49]. To illustrate the methodology, the synthetic scheme and experimental procedures for the representative compounds **12** and **13** are included. As starting compounds, the appropriate nitriles and diaminoalkanes, saturated with hydrogen sulfide, have been used for their preparation.

2.2.1. (±)-N,N′-Bis[4-(4,5-dihydro-4-methyl-1H-imidazol-2-yl) benzyl]-4,4′-propylenebispiperidine tetrahydrochloride (**12**)

Starting from 4,4′-propylenebispiperidine and α-bromo-*p*-tolunitrile, *N*,*N*′-bis(4-cyanobenzyl)-4,4′-propylenebispiperidine (mp 128 °C) was obtained by refluxing the reaction mixture in methanol in the presence of potassium carbonate [49]. The reaction of the bisnitrile with the (±)-1,2-diaminopropane-H_2S reagent in ethanol was carried out under literature conditions [49] and the title compound was obtained (90%): mp > 30 °C; ^{1}H NMR δ 1.24–1.48 (m, 14H), 1.50 (d, 6H, *J* = 6.3 Hz, CH_3), 1.63 (m, 2H), 2.19 (m, 4H), 3.08 (m, 3H), 3.55 (m, 3H), 3.76 (dd, 2H, *J* = 11.3, 8.0 Hz, imidazoline), 4.28 (t, 2H, *J* = 11.3 Hz, imidazoline), 4.45 (s, 4H, NCH_2),

Table 2
Overview of the results of the in vitro antitumor screening for compounds **1–12** scheduled automatically for evaluation against the full panel of about 60 human tumor cell lines

Compound	No. of the cell lines investigated	Number of the cell lines giving log GI_{50}, log TGI, and log $LC_{50} < -4$					
		−log GI_{50}		−log TGI		−log LC_{50}	
		No.	Range	No.	Range	No.	Range
1	58	43	4.06–4.75	15	4.01–4.41	2	4.06–4.07
2	58	45	4.04–4.70	11	4.00–4.30	0	<4.00
3	58	58	4.78–6.44	58	4.45–5.78	49	4.05–5.31
4	58	58	4.63–6.07	58	4.22–5.66	52	4.06–5.31
5	58	38	4.09–4.88	3	4.17–4.23	0	<4.00
6	58	48	4.00–5.24	12	4.01–4.41	2	4.01–4.05
7	58	57	4.12–4.86	49	4.02–4.53	23	4.01–4.26
8	58	58	4.55–5.92	54	4.10–5.60	49	4.24–5.30
9	58	43	4.01–5.65	2	4.02–4.87	0	<4.00
10	57	50	4.11–8.00	5	4.20–8.00	3	4.04–7.25
11	57	52	4.09–5.76	35	4.02–4.72	12	4.01–4.21
12	59	41	4.07–4.83	20	4.04–4.54	7	4.01–4.25

4.55–4.72 (m, 2H, imidazoline), 7.78 (d, 4H, J = 8.5 Hz), 7.95 (d, 4H, 8.3 Hz); ^{13}C NMR δ 22.7, 25.4, 32.0, 35.5, 37.7, 54.3, 55.9, 56.5, 62.6, 126.8, 131.7, 135.0, 138.1, 167.6; LRMS (EI): 554 (24), 382 (25), 381 (86), 174 (100),
158 (15); HRMS (EI): M^+, found 554.4067. $C_{35}H_{50}N_6$ requires 554.4097.

2.2.2. 1,3,6,8-Tetrakis(1,4,5,6-tetrahydro-2-pyrimidinyl)pyrene tetrahydrochloride (**13**)

This compound was prepared by analogy to the 2-imidazoline homologues [49] from 1,3,6,8-tetracyanopyrene and 1,3-diaminopropane (61%): mp > 300 °C; ^{1}H NMR δ 2.53 (br quintet, 8H, J = 5.1 Hz, CH_2), 4.00 (br t, 16H, J = 5.2 Hz, NCH_2), 8.75 (s, 2H, C2H, C7H), 8.82 (s, 4H, CH); ^{13}C NMR δ 20.6, 42.6, 126.7, 128.6, 129.8, 129.9, 133.6, 162.3; HRMS (FAB, NBA): MH^+, found 531.2961. $C_{32}H_{35}N_8$ requires 531.2985.

2.3. Determination of growth percentages in the 3-cell line, one dose primary anticancer assay

As a primary screening, compounds **1–13** were submitted to the National Cancer Institute (NCI) cell line screen for evaluation of

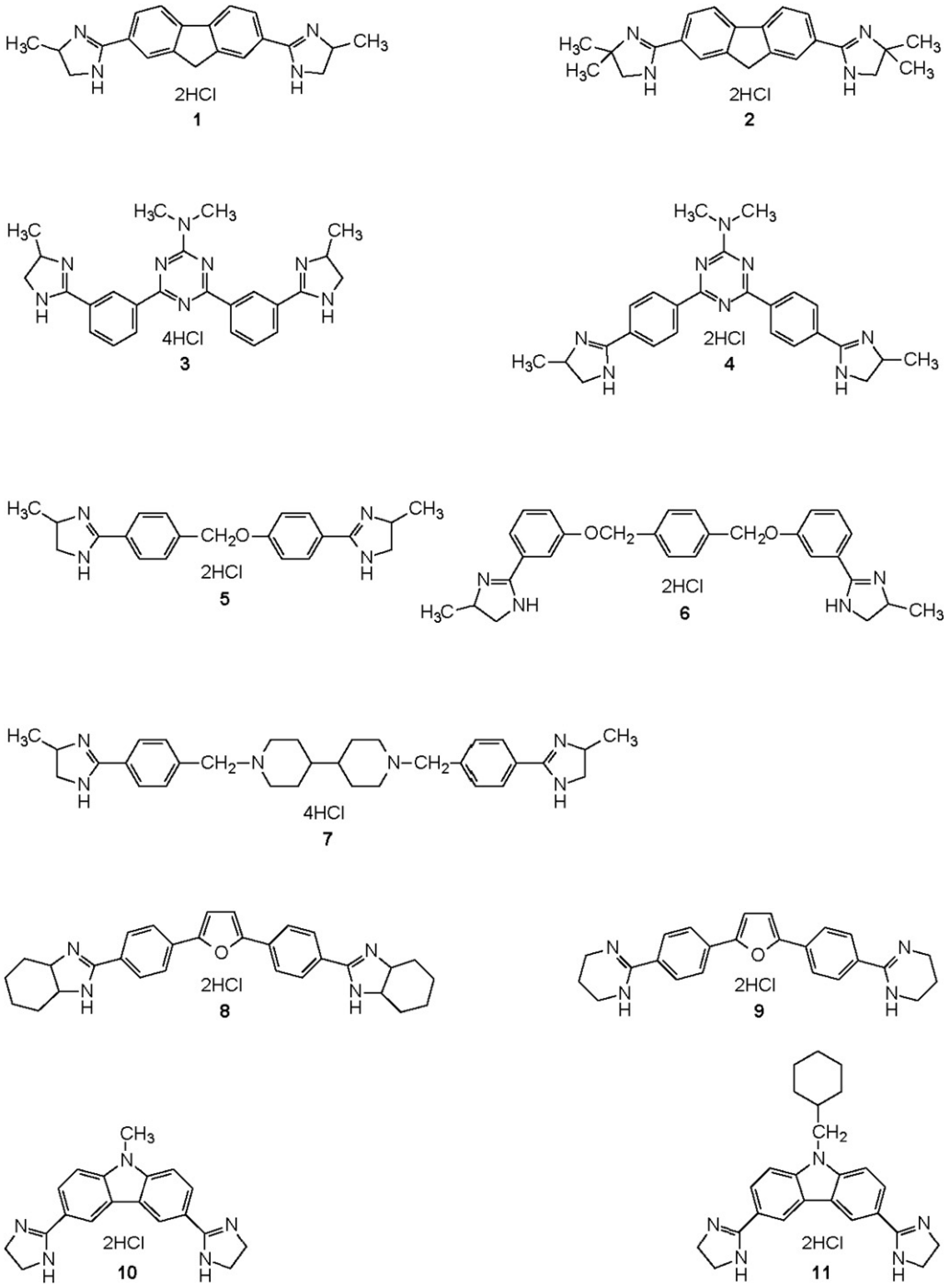

Fig. 1. Compounds **1–11**, active against most tumor cell lines, may serve as useful lead compounds for the search of more powerful anticancer agents. The presence of opposite cationic groups in the molecule is essential for cytotoxicity.

their anticancer activity [67]. From the data analysis [68] it follows that approximately 95% of the actives from the 60 cell line screen can be identified using only three cell lines. Thus a 48 h continuous drug exposure protocol and a protein-binding dye sulforhodamine B (SRB) were used to estimate cell growth. In a preliminary test at a single concentration (100 μM) against three human cell lines (NCI-H460 lung cancer, MCF7 breast cancer, and SF-268 glioma), a compound is considered active when it reduces the growth of any of the cell lines to 32% or less.

By these criteria, 12 compounds reported were active (six compounds: **3**, **4**, **7**, **8**, **10**, and **11** with negative numbers indicate cell kills) and passed on for evaluation in the full panel of 57–59 human tumor cell lines. Pyrene **13**, which bears four tetrahydropyrimidine groups on the pyrene residue, did not inhibit growth of the tumor cells. The results of **1–13** are summarized in Table 1.

2.4. Methodology of the NCI-60 in vitro cancer screening

The panel is organized into nine subpanels representing diverse histologies: leukemia, melanoma, and cancers of lung, colon, kidney, ovary, breast, prostate, and central nervous system [68]. The test compounds **1–12** were dissolved in DMSO and evaluated using five concentrations at 10-fold dilutions, the highest being 10^{-4} M and the others being 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M. Table 2 reports the results obtained with this test expressed as the −log of the molar concentration that inhibited the cell growth by 50% (pGI_{50}), that caused total cytostasis (pTGI, total growth inhibition), or that killed half of the cells (pLC_{50}) when compared with values of untreated control cells. For the calculation of the MG_MID values, insensitive cell lines are included with the highest test concentration.

3. Results and discussion

A current trend in chemical modifications has appending the examples that have already been tested. Efforts were directed towards extending the approach to the synthesis of other biologically interesting antibiotic analogues. Molecules used in the present assay and their numbering are given in Figs. 1–2. Since it has been well known that amidines in general exhibit biological and pharmacological activities, it was interesting to investigate the anticancer properties of some polycationic molecules and to compare their activities with the relevant literature data.

Consequently, the parent structures are altered by varying the core-substituents (shape of the molecules). On the basis of the growth inhibition parameters, a structure-activity relationship was obtained. Overall, the results show that the presence of amidinium groups is not the unique requirement for the compounds to induce activity. Inactive tetraamidine **13** led to consider the shape of molecules responsible for the activity exerted by the compounds.

Most cell lines were sensitive to the agents. It appeared, as indicated in Table 2, that weak structural modifications were responsible for the activity variation. The range between the least sensitive and most sensitive cell lines was about 1–2 log units for **3**, **4**, **6**, **8**, **9**, **10**, and **11**. The range was smaller, less than 1 log unit, for the other compounds. Thus, the growth inhibition results allow classification of the compounds according to their activity profiles.

Averaged values, designated as mean graph midpoints (MG_MID), are calculated for each of the three parameters mentioned by averaging the log parameters of all cell lines. All anticancer agents possessed three different average potencies at the parameters pGI_{50}, pTGI, and pLC_{50}, respectively: **1** (4.33, 4.06, 4.00), **2** (4.34, 4.03, 4.00), **3** (5.73, 5.17, 4.54), **4** (5.62, 5.13, 4.59), **5** (4.30, 4.01, 4.00), **6** (4.41, 4.04, 4.00), **7** (4.66, 4.29, 4.05), **8** (5.72, 5.31, 4.73), **9** (4.28, 4.02, 4.00), **10** (4.83, 4.15, 4.11), **11** (4.77, 4.21, 4.02), **12** (4.37, 4.09, 4.01).

Among all compounds, the data for selected members **1**, **2**, **3**, **4**, **8**, and **10** are summarized in Table 3. The assays of **3** and **4** were repeated once; other compounds were assayed once. At the pGI_{50} endpoint isomers **3** and **4** were of approximately equal overall potency; the ranges of the −log molar concentrations for all cell lines were from 4.78 to 6.44 for **3** (average 5.73) and 4.63 to 6.07 for **4** (average 5.62), compared to the average potency of **8**, which was 5.72, ranging from 4.55 to 5.92.

For the most active compounds, there were considerable differences between log GI_{50} and log LC_{50} values (MG_MID parameters). At the pTGI endpoint the average log molar potencies were very close to the pLC_{50} in most cases. The ranges at the pTGI between the least sensitive and the most sensitive cell lines were tighter than the ranges seen at the pGI_{50}, though the overall patterns showed less difference among the cell lines of the same tumor, except for **10** (data shown in Tables 2–3).

The average TGI values for triazines **3** and **4** were 6.8 μM and 7.4 μM, respectively. The hexahydrobenzimidazolyl to tetrahydropyrimidinyl change in **8** (4.9 μM) to **9** (95.5 μM) causes such a large increase in the TGI. The ΔT_m data from biophysical studies with DNA and **8** (ΔT_m 24.5 °C) or **9** (ΔT_m > 28 °C) [24] show that these compounds exhibit dissimilar binding affinities. It is reflected in their different dose response curves (Fig. 3). These results support

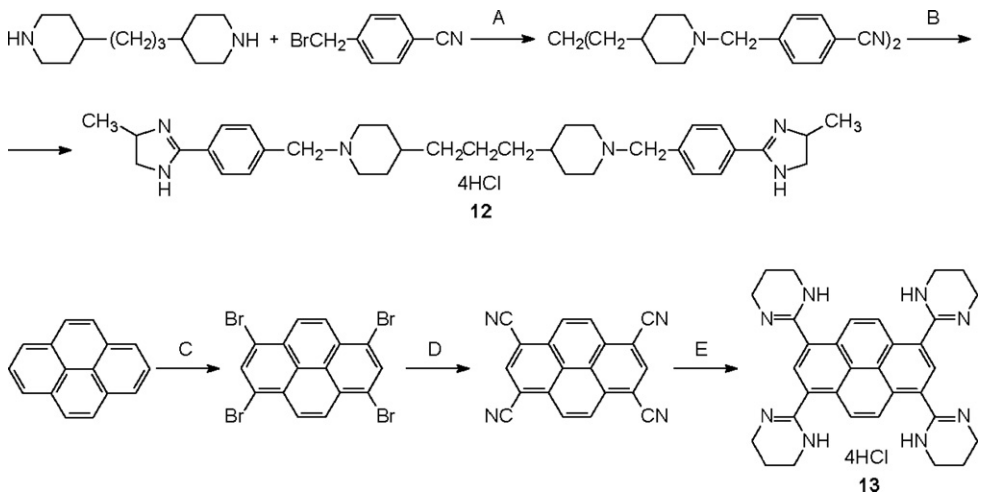

Fig. 2. Representative syntheses of **12** and **13**. Reagents: (A) K_2CO_3, MeOH; (B) $H_2NCH_2CH(CH_3)NH_2$-H_2S, EtOH; (C) Br_2, CCl_4; (D) $Cu_2(CN)_2$, quinoline; (E) $H_2N(CH_2)_3NH_2$-H_2S.

Table 3
Inhibition of in vitro cancer lines by active compounds **1**, **2**, **3**, **4**, **8**, and **10** ($-\log GI_{50} > 4.00$ for active compounds)

Panel	Cell lines (cytotoxicity: $-\log GI_{50} > 4.00$)
*2,7-Bis(4,5-dihydro-4-methyl-1H-imidazol-2-yl)fluorene dihydrochloride (**1**)*	
Leukemia	CCRF-CEM (<4.00), K-562 (4.46), MOLT-4 (4.42), RPMI-8226 (4.44), SR (4.37)
Non-small cell lung cancer	A549/ATCC (<4.00), EKVX (<4.00), HOP-62 (4.39), HOP-92 (<4.00), NCI-H226 (4.25), NCI-H23 (4.43), NCI-H322M (4.63), NCI-H460 (4.58), NCI-H522 (4.65)
Colon cancer	COLO 205 (4.27), HCC-2998 (4.74), HCT-116 (4.50), HCT-15 (<4.00), HT29 (4.21), KM12 (4.69), SW-620 (4.25)
CNS cancer	SF-268 (4.58), SF-295 (4.14), SNB-19 (4.44), SNB-75 (4.27), U251 (4.71)
Melanoma	LOX IMVI (4.33), MALME-3M (4.72), M14 (4.40), SK-MEL-2 (4.63), SK-MEL-28 (4.06), SK-MEL-5 (4.14), UACC-257 (4.49), UACC-62 (4.54)
Ovarin cancer	IGROV1 (<4.00), OVCAR-3 (4.68), OVCAR-4 (4.72), OVCAR-5 (<4.00), OVCAR-8 (4.43), SK-OV-3 (4.42)
Renal cancer	786-0 (4.26), A498 (<4.00), ACHN (<4.00), CAKI-1 (<4.00), RXF 393 (<4.00), SN12C (4.32), TK-10 (<4.00), UO-31 (<4.00)
Prostate cancer	PC-3 (4.36), DU-145 (4.18)
Breast cancer	MCF7 (4.75), NCI/ADR-RES (<4.00), MDA-MB-231/ATCC (4.47), HS 578T (4.42), MDA-MB-435 (4.59), MDA-N (4.46), BT-549 (<4.00), T-47D (4.45)
*2,7-Bis(4,5-dihydro-4,4-dimethyl-1H-imidazol-2-yl)fluorene dihydrochloride (**2**)*	
Leukemia	CCRF-CEM (4.32), K-562 (4.61), MOLT-4 (4.50), RPMI-8226 (4.60), SR (4.49)
Non-small cell lung cancer	A549/ATCC (<4.00), EKVX (4.22), HOP-62 (4.47), HOP-92 (4.04), NCI-H226 (4.31), NCI-H23 (4.45), NCI-H322M (4.60), NCI-H460 (4.51), NCI-H522 (4.52)
Colon cancer	COLO 205 (4.55), HCC-2998 (4.58), HCT-116 (4.48), HCT-15 (<4.00), HT29 (4.49), KM12 (4.55), SW-620 (4.46)
CNS cancer	SF-268 (4.46), SF-295 (<4.00), SNB-19 (4.45), SNB-75 (4.04), U251 (4.66)
Melanoma	LOX IMVI (4.43), MALME-3M (4.61), M14 (4.35), SK-MEL-2 (4.70), SK-MEL-28 (4.41), SK-MEL-5 (4.22), UACC-257 (4.51), UACC-62 (4.60)
Ovarin cancer	IGROV1 (<4.00), OVCAR-3 (4.45), OVCAR-4 (4.48), OVCAR-5 (<4.00), OVCAR-8 (4.43), SK-OV-3 (4.35)
Renal cancer	786-0 (4.30), A498 (4.11), ACHN (<4.00), CAKI-1 (<4.00), RXF 393 (<4.00), SN12C (4.44), TK-10 (<4.00), UO-31 (<4.00)
Prostate cancer	PC-3 (4.40), DU-145 (<4.00)
Breast cancer	MCF7 (4.63), NCI/ADR-RES (<4.00), MDA-MB-231/ATCC (4.41), HS 578T (4.19), MDA-MB-435 (4.48), MDA-N (4.40), BT-549 (<4.00), T-47D (4.21)
*4,6-Bis [3-(4,5-dihydro-4-methyl-1H-imidazol-2-yl)phenyl]-2-dimethylamino-1,3,5-triazine tetrahydrochloride (**3**)*	
Leukemia	CCRF-CEM (6.33), HL-60(TB) (5.69), K-562 (6.21), MOLT-4 (6.44), RPMI-8226 (5.55)
Non-small cell lung cancer	A549/ATCC (5.53), EKVX (5.56), HOP-62 (5.92), HOP-92 (5.63), NCI-H23 (5.60), NCI-H322M (5.98), NCI-H460 (5.69), NCI-H522 (5.97)
Colon cancer	COLO 205 (5.90), HCC-2998 (5.94), HCT-116 (6.26), HCT-15 (5.01), HT29 (5.88), KM12 (5.81), SW-620 (6.12)
CNS cancer	SF-268 (5.56), SF-295 (5.36), SF-539 (6.34), SNB-19 (6.07), SNB-75 (5.76), U251 (6.15)
Melanoma	LOX IMVI (5.96), MALME-3M (5.29), M14 (5.45), SK-MEL-2 (5.66), SK-MEL-28 (5.44), SK-MEL-5 (5.69), UACC-257 (5.65), UACC-62 (5.73)
Ovarin cancer	IGROV1 (5.53), OVCAR-3 (5.67), OVCAR-4 (5.91), OVCAR-5 (5.55), OVCAR-8 (5.69), SK-OV-3 (5.69)
Renal cancer	786-0 (5.67), A498 (5.63), ACHN (5.47), CAKI-1 (4.78), RXF 393 (5.57), SN12C (5.91), TK-10 (5.63), UO-31 (5.48)
Prostate cancer	PC-3 (6.13), DU-145 (5.66)
Breast cancer	MCF7 (6.06), NCI/ADR-RES (4.78), MDA-MB-231/ATCC (5.86), HS 578T (5.55), MDA-MB-435 (5.85), MDA-N (5.82), BT-549 (5.43), T-47D (5.67)
*4,6-Bis[4-(4,5-dihydro-4-methyl-1H-imidazol-2-yl)phenyl]-2-dimethylamino-1,3,5-triazine dihydrochloride (**4**)*	
Leukemia	CCRF-CEM (6.06), HL-60(TB) (5.58), K-562 (5.79), MOLT-4 (6.02), RPMI-8226 (5.37)
Non-small cell lung cancer	A549/ATCC (5.47), EKVX (5.46), HOP-62 (5.82), HOP-92 (5.70), NCI-H23 (5.54), NCI-H322M (5.79), NCI-H460 (5.45), NCI-H522 (5.82)
Colon cancer	COLO 205 (5.93), HCC-2998 (5.70), HCT-116 (5.81), HCT-15 (4.77), HT29 (5.64), KM12 (5.75), SW-620 (5.82)
CNS cancer	SF-268 (5.64), SF-295 (5.42), SF-539 (6.07), SNB-19 (5.72), SNB75 (5.78), U251 (5.94)
Melanoma	LOX IMVI (5.83), MALME-3M (5.42), M14 (5.46), SK-MEL-2 (5.69), SK-MEL-28 (5.43), SK-MEL-5 (5.50), UACC-257 (5.69), UACC-62 (5.75)
Ovarin cancer	IGROV1 (5.51), OVCAR-3 (5.68), OVCAR-4 (5.79), OVCAR-5 (5.41), OVCAR-8 (5.63), SK-OV-3 (5.71)
Renal cancer	786-0 (5.62), A498 (5.60), ACHN (5.48), CAKI-1 (4.79), RXF 393 (5.59), SN12C (5.69), TK-10 (5.57), UO-31 (5.31)
Prostate cancer	PC-3 (5.74), DU-145 (5.76)
Breast cancer	MCF7 (5.75), NCI/ADR-RES (4.63), MDA-MB-231/ATCC (5.84), HS 578T (5.54), MDA-MB-435 (5.81), MDA-N (5.67), BT-549 (5.40), T-47D (5.65)
*2,5-Bis[4-(4,5,6,7,8,9-hexahydro-1H-benzimidazol-2-yl)phenyl]furan dihydrochloride (**8**)*	
Leukemia	CCRF-CEM (5.75), HL-60(TB) (5.64), K-562 (5.85), MOLT-4 (5.74), RPMI-8226 (5.75)
Non-small cell lung cancer	A549/ATCC (5.77), EKVX (5.77), HOP-62 (5.82), HOP-92 (5.84), NCI-H226 (5.73), NCI-H23 (5.80), NCI-H322M (5.87), NCI-H460 (5.74), NCI-H522 (5.79)
Colon cancer	COLO 205 (5.82), HCC-2998 (5.89), HCT-116 (5.82), HCT-15 (5.28), HT29 (5.86), KM12 (5.84), SW-620 (5.59)
CNS cancer	SF-268 (5.81), SF-295 (5.74), SF-539 (5.76), SNB-19 (5.83), SNB-75 (5.77), U251 (5.84)
Melanoma	LOX IMVI (5.81), MALME-3M (5.76), M14 (5.78), SK-MEL-2 (5.74), SK-MEL-28 (5.74), SK-MEL-5 (5.80), UACC-257 (5.76), UACC-62 (5.77)
Ovarin cancer	OVCAR-3 (5.74), OVCAR-4 (5.81), OVCAR-5 (5.68), OVCAR-8 (5.75), SK-OV-3 (5.79)
Renal cancer	786-0 (5.83), A498 (5.54), ACHN (5.63), CAKI-1 (5.23), RXF 393 (5.71), SN12C (5.83), TK-10 (5.29), UO-31 (5.53)
Prostate cancer	PC-3 (5.84), DU-145 (5.80)
Breast cancer	MCF7 (5.92), NCI/ADR-RES (4.55), MDA-MB-231/ATCC (5.82), HS 578T (5.81), MDA-MB-435 (5.73), MDA-N (5.78), BT-549 (5.67), T-47D (5.70)
*3,6-Bis(4,5-dihydro-1H-imidazol-2-yl)-9-methylcarbazole dihydrochloride (**10**)*	
Leukemia	CCRF-CEM (>8.00), HL-60(TB) (>8.00), K-562 (4.58), MOLT-4 (6.20), RPMI-8226 (4.61), SR (4.74)
Non-small cell lung cancer	A549/ATCC (4.34), EKVX (4.77), HOP-62 (4.82), HOP-92 (4.87), NCI-H226 (4.52), NCI-H23 (5.38), NCI-H322M (4.90), NCI-H460 (5.27), NCI-H522 (7.11)
Colon cancer	COLO 205 (6.14), HCT-116 (4.66), HCT-15 (<4.00), HT29 (5.11), KM12 (5.81), SW-620 (4.75)
CNS cancer	SF-268 (7.33), SF-295 (4.37), SF-539 (4.53), SNB-19 (4.37), SNB-75 (4.11), U251 (4.83)
Melanoma	LOX IMVI (4.31), MALME-3M (4.74), M14 (4.31), SK-MEL-2 (4.47), SK-MEL-28 (4.62), SK-MEL-5 (4.50), UACC-257 (4.36)
Ovarin cancer	IGROV1 (4.61), OVCAR-3 (4.38), OVCAR-4 (4.87), OVCAR-5 (4.42), OVCAR-8 (4.54), SK-OV-3 (4.36)
Renal cancer	786-0 (4.41), A498 (<4.00), ACHN (4.42), CAKI-1 (<4.00), RXF 393 (4.46), SN12C (5.20), TK-10 (4.22), UO-31 (4.25)
Prostate cancer	PC-3 (4.46), DU-145 (4.58)
Breast cancer	NCI/ADR-RES (<4.00), MDA-MB-231/ATCC (4.39), HS 578T (4.97), MDA-MB-435 (4.21), MDA-N (4.28), BT-549 (<4.00), T-47D (5.10)

the putative mechanism that minor-groove binding to DNA (topoisomerase inhibition) is a component of the anticancer activity.

The highest sensitivity of **10** described here was found for leukemia (CCRF-CEM, $GI_{50} < 0.01$ μM; HL-60 (TB), $GI_{50} < 0.01$ μM; MOLT-4, GI_{50} 0.6 μM) (Fig. 4), non-small cell lung cancer (NCI-H522, GI_{50} 0.08 μM), colon cancer (COLO 205, GI_{50} 0.7 μM), and CNS cancer (SF-268, GI_{50} 0.05 μM), but the average GI_{50} value was 15 μM. Only three cancer cell lines possessed values repre-

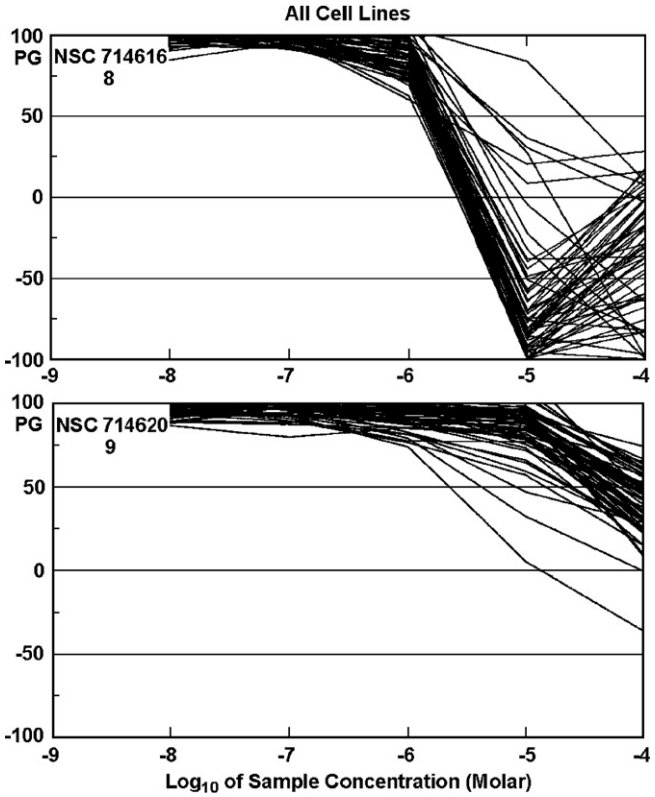

Fig. 3. Dose–response curves of **8** (top) and **9** (bottom) for all nine subpanels of cell lines. Horizontal lines are provided at the PG (Percentage Growth) values of +50, 0, and −50. The concentrations corresponding to points where the curves cross these lines are the GI_{50}, TGI, and LC_{50}, respectively.

senting concentrations at which the PG is −50: CCRF-CEM, 0.2 μM; HL-60(TB), 0.06 μM; NCI-H460, 90 μM. None of the three response parameters can be obtained by interpolation in several cases (the PGs in a given row exceed +50).

In the current article, the following has to be noted regarding the tumor cell growth inhibition data with two carbazoles. The substitution of the carbazole unit at the position 9 by the cyclohexylmethyl group led to less interesting compound **11** (the lowest GI_{50} is 1.7 μM for COLO-205), nevertheless their average GI_{50} values were very close (17 μM for **11**). Both carbazoles **10** (ΔT_m 24.0 °C [42]) and **11** (ΔT_m 16.8 °C [42]) possess the MG_MID parameters similar to **9**. The presence of two amidine groups is important concerning the activity of this class of derivatives, but care should be also taken to steric differences between the active compounds and to the distance of the cationic groups.

The activity may be due to a sufficient structural similarity to the amidine antibiotics (the pyrene series is not comparable in terms of structure-activity relationships). The average cytotoxic effects of **3**, **4**, and **8** against all cell lines investigated were comparable to those of *N*-alkylated furamidine derivatives [20]. They remain at the low-micromolar level and confirm the results provided by the previous ΔT_m studies [24,34,42,53]. The high values indicate strong DNA affinities for these molecules.

It appeared that the most active compounds are DNA associated [69]. The poorer binding to DNA may be due to the presence of the out-of-shape less active molecules which prevent a suitable fit into the electronegative minor-groove of the DNA helix. In addition, some agents show significant affinity for RNA and have weaker affinity for DNA (nonspecific interaction mode) [34,53]. The discovery of antibiotic analogs with new selectivity patterns sets the stage for further effort to explore therapeutic potential, especially for AIDS-defining cancers [70].

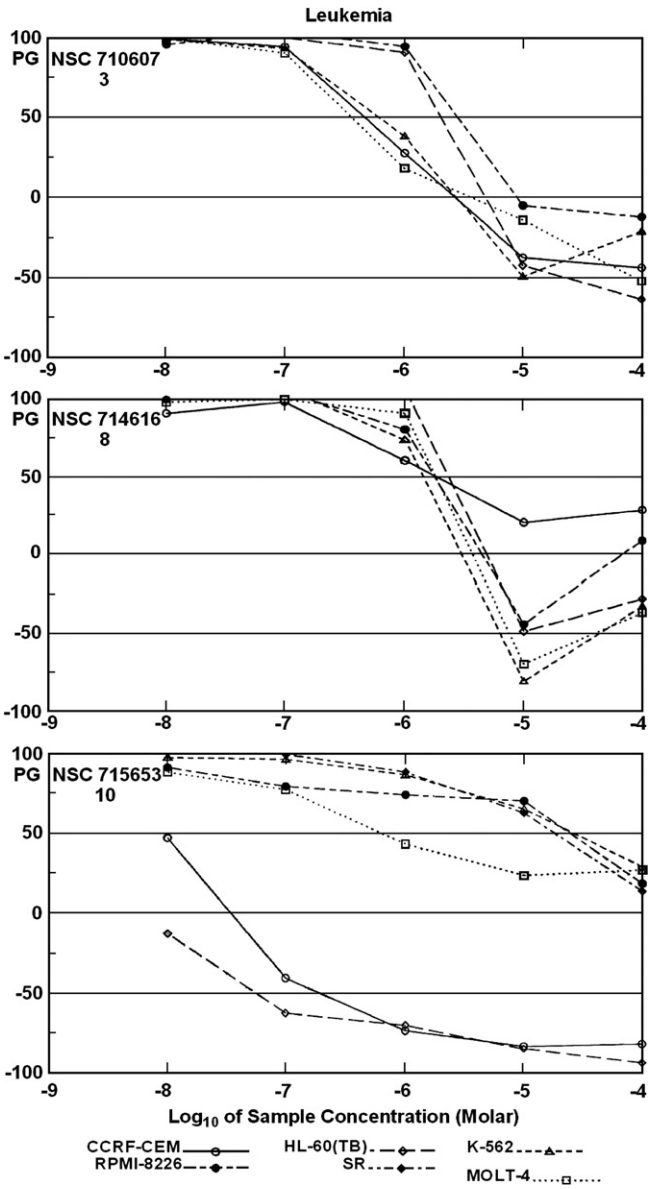

Fig. 4. In vitro activity on leukemia cell lines. Triazine **3** (top), selected for further studies with the hollow fiber assay, in comparison with furan **8** (middle) and carbazole **10** (bottom).

4. Conclusion

In summary, the group of active compounds comprises 12 cyclic diamidines. Compounds **1–12** exhibited activity against most of cancer cell lines and, in particular, the in vitro anticancer data in the low-micromolar range prove usefulness of the cationic system in the design of active anticancer agents **3**, **4**, and **8**. Triazines **3** and **4** were the most promising agents (point of view of the NCI's Biological Evaluation Committee for Cancer Drugs), and carbazole **10** was of special interest because of its high activity in the nanomolar range against some cell lines: leukemia, non-small cell lung, colon, and CNS cell lines. Compounds **3**, **4**, and furan **8** exhibited activities against all tumor cell lines investigated.

The anticancer screening data presented are well correlated with mode of binding to DNA. They can be used to explore the strength of the DNA interactions by comparison of the NCI's activity profiles (all dose response curves). The strength of these interactions is likely a sum of both hydrogen bonding capabilities and

the linker length (shape). Strong likeness among the cell line responses (MG_MID values) suggests that the compounds may be acting similarly through the same mechanism of action.

Acknowledgments

I am particularly indebted to Dr. E. Sausville and Dr. V.L. Narayanan (Department of Health & Human Services, NIH, NCI, Bethesda, Maryland 20892, USA) for supporting the evaluation against the full panel of 60 human tumor cell lines.

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