Benzimidazo[1,2-*c*]quinazoline Dimers as Potential Antitumor Agents

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"In memoriam" of Professor F. Serratosa

The 6-substituted benzimidazo[1,2-*c*]quinazoline **1** is a lead structure from our DNA intercalator program and is cytotoxic to the human colon cancer tumor line HT-29 with an inhibitory concentration 50, IC_{50} of 4.00 μ*M*. In order to try and improve the limited cytotoxicity of this class of compound we prepared a series consisting of two benzimidazo[1,2-*c*]quinazoline moieties linked by a polyalkylamino bridge, of different length and substitution. The compound with the -NH-$(CH_2)_3$-N(CH_3)-$(CH_2)_3$-NH- bridge had an inhibitory concentration 50, IC_{50} of 0.5 μ*M*. When tested *in vivo*, however, no clear antitumor activity was produced in the human breast cancer tumor line MX-1 or the human melanoma tumor line LOX, human tumor xenografts models.

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Introduction.

In our search for compounds with anticancer properties we recently described [1] the design and synthesis of a new class of antitumor compounds derived from the benzimidazo[1,2-*c*]quinazoline system, being the most active of them the 6-(1-piperidyl)ethylamino derivative **1**. These molecules can bind to DNA by intercalation and are cytotoxic to tumor cells in tissue culture.

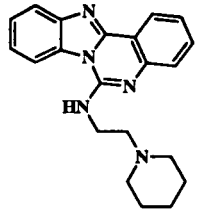

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Figure 1

Recently we have adopted a design strategy to develop [2] intercalating compounds with DNA affinities as high as possible. As a consequence, considerable effort has focused on the synthesis and characterization of agents capable of bis-intercalating into DNA. Because of the dual interaction in bis-intercalators the DNA binding is enhanced and the required disengagement of both chromophores for dissociation results at a slower dissociation rate [3]. However in addition to an intercalative capacity other attributes appear to be required for optimum antitumor activity. For example, our experience with the neutral chromophore naphthalimide, has indicated a cationic side chain can markedly improve antitumor activity of the monomeric forms [4,5]. Appropriate alkyl bridging of the ethylamino side chain in Amonafide and Mitonafide, results in a marked improvement in cellular cytotoxicity [4]. This is consistent with the marked increase in DNA binding and cellular cytotoxicity observed when acridine is dimerized using a positively charged polyamine chain [3,6,7]. In addition to dual intercalation with these dimerized molecules an interaction between the linking bridge and the sugar backbone of DNA, or nucleotides themselves may add to overall binding [8,9].

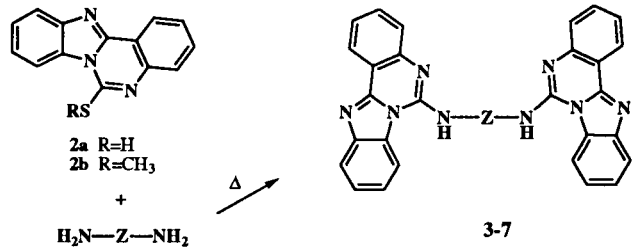
In our strategy of maximizing DNA interaction we have prepared a series of benzimidazo[1,2-*c*]quinazoline dimers bridged with a variety of polyalkylamine linkers, aimed at allowing bis-intercalation. We describe herein the synthesis, *in vitro* and *in vivo* activities of this new series of potential bis-intercalating agent.

Results.

Chemistry.

Bis-benzimidazo[1,2-*c*]quinazolines **3-7** were prepared as depicted in Scheme 1, from the previously described [1] 6-mercaptobenzimidazo[1,2-*c*]quinazoline **2a**, by a coupling reaction with the corresponding aliphatic polyamine in the absence of a solvent (Table I).

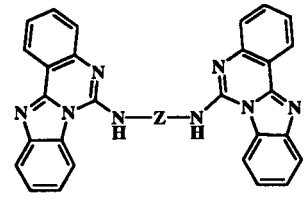
Scheme 1



3 Z = $(CH_2)_3$-N(CH_3)-$(CH_2)_3$
4 Z = $(CH_2)_3$-NH-$(CH_2)_4$
5 Z = $(CH_2)_3$-N(CH_3)-$(CH_2)_4$
6 Z = $(CH_2)_2$-N(CH_3)-$(CH_2)_2$-N(CH_3)-$(CH_2)_2$
7 Z = $(CH_2)_2$-N(CH_3)-$(CH_2)_3$-N(CH_3)-$(CH_2)_2$

Table I

Cytotoxic activities of compounds 3-7



Compound	Z	Formula	$IC_{50}(\mu M)$	Range	n
3	$(CH_2)_3$-N(CH_3)-$(CH_2)_3$	$C_{35}H_{33}N_9$	0.70	(0.5-0.8)	4
4	$(CH_2)_3$-NH-$(CH_2)_4$	$C_{35}H_{33}N_9$	0.45	(0.4-0.5)	2
5	$(CH_2)_3$-N(CH_3)-$(CH_2)_4$	$C_{36}H_{35}N_9$	0.70	(0.6-0.8)	2
6	$(CH_2)_2$-N(CH_3)-$(CH_2)_2$-N(CH_3)-$(CH_2)_2$	$C_{36}H_{36}N_{10}$	0.75	(0.7-0.8)	2
7	$(CH_2)_2$-N(CH_3)-$(CH_2)_3$-N(CH_3)-$(CH_2)_2$	$C_{41}H_{54}N_{10}O_{12}S_4$ [a]	1.50	(1.0-2.0)	2
1			3.50	(2.0-5.0)	2

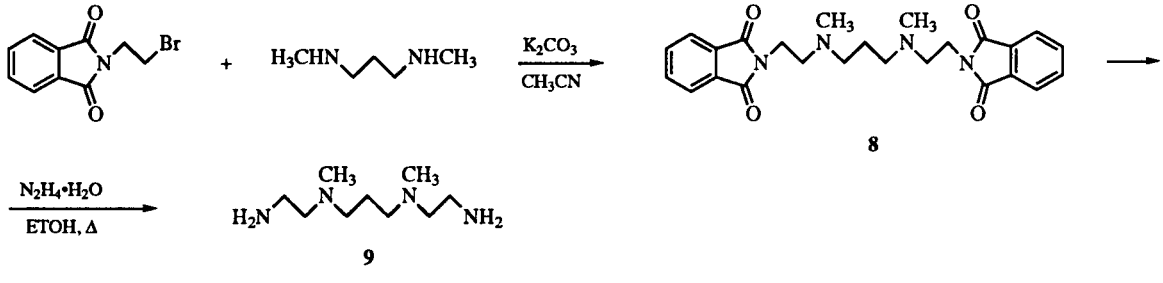
[a] Isolated as methanesulphonate.

The polyamines used for the synthesis of compounds **5** and **6** were known [10,11]. The *N,N'*-bis(2-aminoethyl)-*N,N'*-dimethyl-1,3-propanediamine **9**, necessary for the preparation of compound **7**, was prepared from *N*-(2-bromoethyl)phthalimide by reaction with *N,N'*-dimethyl-1,3-propanediamine and subsequent hydrazinolysis of the thus obtained bis-phthalimide, as under the conditions of a Gabriel synthesis (Scheme 2). This amine was used as a ligand by Lu *et al.* [12] in the preparation of an organocopper compound, but the authors did not provide any analytical data. The rest of the amines used were commercially available.

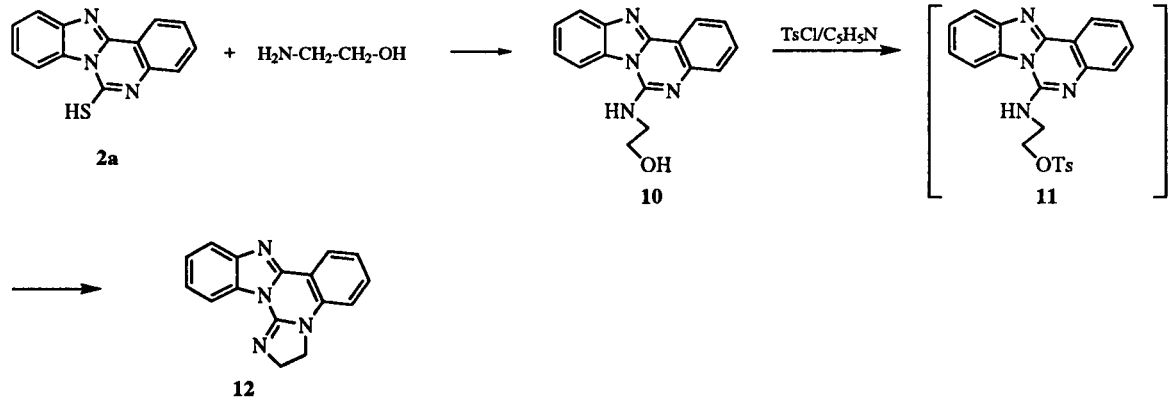
The preparation of **2b** was accomplished by treatment of **2a** with an excess of methyl iodide in the presence of sodium methoxide as a base. This method led to a better yield (80%) than the already described procedure (62%) [13].

In addition, we planned the synthesis of bis-benzimidazo[1,2-*c*]quinazolines by substitution of the tosyl derivative **11** with the corresponding amine. Nevertheless,

Scheme 2



Scheme 3



preparation of tosylate **11** was followed by spontaneous cyclization to compound **12**.

The preparation of **10** was performed by heating **2a** in the presence of an excess of ethanolamine [13] (Scheme 3). Compound **12** had already been prepared by Leistner *et al* [13], by refluxing **10** in phosphorus oxychloride. The authors identified the product on the basis of mass and micro analytical data; no ^1H nmr data was supplied. An initial attempt, using FABms to identify the structure of the compound isolated from the tosylation reaction suggested a possible dimeric structure on the basis of a peak at m/z 521 $[2M+1]^+$. However comparison of this compound with a sample prepared by the procedure of Leistner *et al.* [13] pointed to the monomeric structure **12**. Performance of the FAB mass spectrum at different concentrations suggested the m/z 521 $[2M+1]^+$ peak may be an artefact of fragment/matrix interaction (relation between relative abundance of m/z 521 $[2M+1]^+$ peak and fragment 261 $[M+1]^+$ changes when the sample concentration is changed).

Biological Evaluation.

Cellular cytotoxicity of the compounds was assessed in the human colon cancer tumor line HT-29, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye assay [14]. This assay measures mitochondrial dehydrogenase activity that reduces 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to the colored formazan compound which absorbs at 550 nm. *In vivo* evaluation of antitumor efficacy utilized the human breast cancer tumor line MX-1 and the human LOX melanoma line implanted sub cutaneously in athymic mice. Activity was assessed on the basis of change in measured tumor size following drug treatment. We have previously utilized this combination of cellular and in vivo models to guide our strategy of optimizing the dimerization of DNA intercalators [2,5].

Discussion.

The preparation of bis-benzimidazo[1,2-*c*]quinazolines **3-7** outlined in Scheme 1, implies a nucleophilic substitution reaction of the mercapto group at position 6 of **2a** by the primary amino groups of the corresponding polyalkylamine. This is a similar method to the one used in the preparation of monobenzimidazo[1,2-*c*]quinazolines [1], but in our case the reaction is accomplished by heating the two components in the absence of a solvent and in a ratio of 2 equivalents of **2a** for each equivalent of polyamine. The reaction can be followed by evolution of hydrogen sulfide. Compounds **3-7** were obtained in moderate to good yields, but recovering of unaltered starting material in all cases has to be considered. Several attempts to improve the reaction yields, by increasing the reaction time, using an excess of polyamine, using solvents such as dimethylformamide or even the presence of bases like dimethylaminopyridine or triethylamine, were unsuccessful.

An assay to improve the reaction yield by transformation of the mercapto group of compound **2a** in a better leaving group like the methylmercapto functionality, compound **2b**, which will favour the nucleophilic substitution reaction was also tried without success (no better yields were obtained).

Superposition of 6-dimethylaminoethylbenzimidazo-[1,2-*c*]quinazoline with the naphthalimide, Mitonafide, has previously been demonstrated to position the dimethylaminoethyl side chain of each molecule in a corresponding position [1]. Our previous experience with the dimerization of the naphthalimides has indicated maximum activity with the $-(CH_2)_2$-NH-$(CH_2)_3$-NH-$(CH_2)_2$- bridge between both chromophores [5]. It was anticipated a similar bridge in the benzimidazo[1,2-*c*]quinazoline series would also result in good activity. However a similar bridge between the chromophores would lead to an unstable aminal derivative, therefore we elected to synthesize the compound with the bridge -HN-$(CH_2)_2$-NH-$(CH_2)_3$-NH-$(CH_2)_2$-NH-. Therefore we decided to synthesize the compound with the bridge -HN-$(CH_2)_2$-NH-$(CH_2)_3$-NH-$(CH_2)_2$-NH-. However, several attempts at reaction **2a** with the *N,N'*-bis(2-aminoethyl)-1,3-propanediamine using different reaction conditions were unsuccessful, possibly by a polyreaction between the different amino groups of the row polyamine. To circumvent this problem, we have decided two strategies. First at all, the preparation of derivative **10** by treatment of **2a** with 2-ethanolamine with the intention of preparing the desired compound by reaction of the tosyl derivative of **10** with *N,N'*-dimethyl-1,3-propanediamine. However, tosylation of **10** in the presence of tosyl chloride in pyridine did not lead to the expected derivative **11**, but the intramolecular cyclization product **12** in an excellent yield. On the other hand, to avoid the presence of secondary amino groups, we assayed to bridging the benzimidazo[1,2-*c*]quinazoline moiety with the $-(CH_2)_2$-N(CH_3)-$(CH_2)_3$-N(CH_3)-$(CH_2)_2$- linker **7**, which resulted in little improvement in cellular cytotoxicity over the monomer **1**. In contrast, the cellular cytotoxicity was increased approximately 5 fold when the shorter $-(CH_2)_2$-N(CH_3)-$(CH_2)_2$-N(CH_3)-$(CH_2)_2$- bridge **6** was used. A 5-10 fold increase in cellular cytotoxicity was also produced over the monomer **1** when benzimidazo[1,2-*c*]-quinazoline moieties were bridged with a $-(CH_2)_x$-N(A)-$(CH_2)_y$- linker, were x and y were 3 or 4 and A was either H or CH_3 (compounds **3**, **4** and **5**, Table I). The cellular cytotoxicity of optimally substituted benzimidazo[1,2-*c*]-quinazoline monomers is in the same range as naphthalimide monomers such as Amonafide and Mitonafide [1,4]. However, the potency of these optimized benzimidazo-[1,2-*c*]quinazoline dimers appears less than the bis-naphthalimides [5]. The monomer **1** was examined in the human breast cancer MX-1 tumor xenograft model. No meaningful

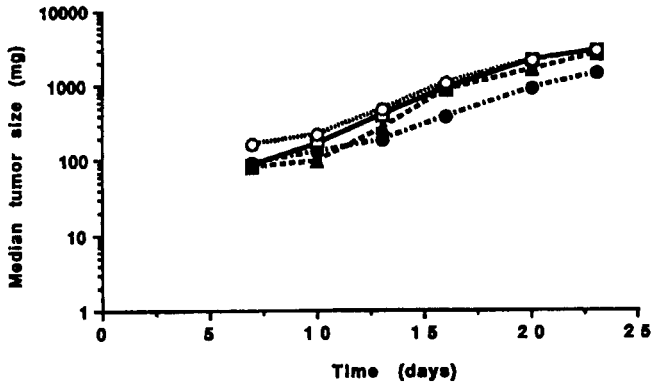


Figure 2. Effect of compound **1** on MX-1 tumor growth. Groups of 6 athymic mice implanted s.c. with MX-1 tumor were treated i.v. on days 7-10 and 20-24 with either control (□—□), or 20 mg/kg (●—●), 30 mg/kg (▲—▲), or 40 mg/kg (○—○) of compound **1**.

weight loss, see Figure 2). Dimer **3**, which was nearly 10 fold more potent than the monomer **1**, was also tested in the human breast cancer tumor model MX-1. Tumors on animals dosed intra venously on days 6-10 or 6, 10 and 14 at dose levels up to 30 mg/kg showed no meaningful delay in growth compared to control (see Figure 3). Similarly no antitumor activity was observed for **3** against the human LOX melanoma tumor line when given intra venously on days 3-7 at 33 mg/kg and drug related animal deaths were produced with 40 mg/kg (see Figure 4). The lack of activity of compound **3** contrast with the good activity of the bis-naphthalimide, LU 79553 seen in these models [5]. These initial studies suggest polyamine bridging of benzimidazo-[1,2-*c*]quinazoline moieties may not lead to effective anti-tumor agents and further studies have been abandoned.

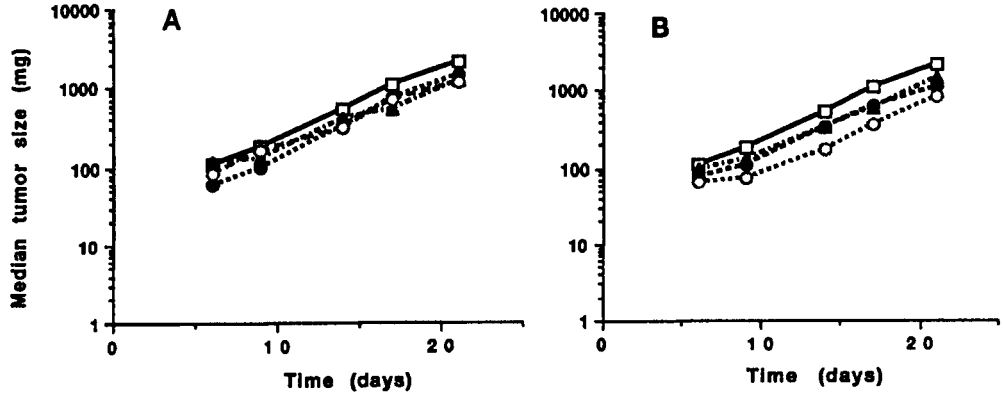


Figure 3. Effect of compound **3** on MX-1 tumor growth. Groups of 6 athymic mice implanted s.c. with MX-1 tumor were treated i.v. on (A) days 6-10 or (B) days 6,10 and 14 with either control (□—□), or 30 mg/kg (●—●), 20 mg/kg (▲—▲), or 13 mg/kg (○—○) of compound **3**.

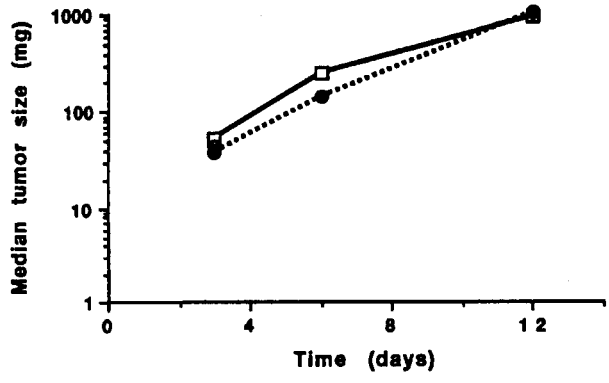


Figure 4. Effect of compound **3** on LOX melanoma growth. Groups of 6 athymic mice implanted s.c. with LOX melanoma were treated i.v. on days 3-7 with either control (□—□), or 33 mg/kg (●—●) or 40 mg/kg (○) of compound **3** (40 mg/kg produced drug related deaths shortly after starting treatment.

tumor growth delay was produced when animals were treated intra venously on days 7-10 and 20-24 up to 40 mg/kg (40 mg/kg produced seizures and marked body

EXPERIMENTAL

Melting points were determined using a Büchi 510 and an Electrotermal capillary melting apparatus, and are uncorrected. The ^{1}H nmr spectra at 200 MHz were recorded on Bruker AC-200 spectrometer, with tetramethylsilane as internal standard. High-resolution FAB mass spectra were measured in a VG Auto Spec mass spectrometer. The ir spectra were obtained (potassium bromide discs) on a Perkin Elmer 1310 spectrophotometer. Analyses indicated by the symbols of the elements or functions were within ± 0.4% of theoretical values. The reactions were checked on hptlc fertigplatten silica gel 60 F_{254} plats (Merck).

2-Methylmercaptobenzimidazo[1,2-*c*]quinazoline (**2b**).

A solution of sodium methoxide was prepared by adding sodium (0.18 g, 7.9 mmoles) to absolute methanol (50 ml). To this solution 2-mercaptobenzimidazo[1,2-*c*]quinazoline (**2a**) (2 g, 7.9 mmoles) and methyl iodide (6.7 g, 47.4 mmoles) were added and the mixture was refluxed for 15 minutes. After this time the solution was allowed to cool and the precipitated solid was filtered, washed and crystallized from methanol to give **2b** (1.7 g, 80 %), mp 159-160°, lit [5], 158-160°; ^{1}H nmr (deuteriochloroform): δ 2.90 (s, 3H, SCH_3), 7.41-7.60 (m, 3H), 7.71 (td, 1H, J = 7.5, 1.5

Hz), 7.81 (dd, 1H, J = 8.1, 1.2 Hz), 7.99 (dd, 1H, 7.4, 1.1 Hz), 8.46 (d, 1H, J = 7.7 Hz), 8.63 (dd, 1H, J = 7.9, 1.5 Hz).

Anal. Calcd. for $C_{15}H_{11}N_3S$: C, 67.89; H, 4.18; N, 15.84. Found: C, 67.57; H, 4.22; N, 15.70.

General Procedure for the Preparation of Compounds **3-7**.

6-Mercaptobenzimidazo[1,2-*c*]quinazoline (**2a**) (1 g, 0.004 mole) and the corresponding amine (0.0019 mole) were heated for 20 hours at 120-130°. After this time the reaction mixture was allowed to cool to room temperature and triturated with ice water. The resulting precipitate was filtered, dried, chromatographed and recrystallized from the appropriate solvent to provide the title compounds.

Bis[*N*-(benzimidazo[1,2-*c*]quinazolyl)-3-aminopropyl]methylamine (**3**).

This compound was obtained in 50% overall yield from 0.3 g of recovered starting material, as white powder (metanol), mp 201-202°; ^{1}H-nmr (deuteriotrifluoroacetic acid): δ 2.65 (m, 4H, 2 CCH_2C), 3.07 (s, 3H, CH_3), 3.48-3.62 (m, 4H, 2 CH_2NCH_3), 4.26 (m, 4H, 2 CH_2NH), 7.80-8.20 (m, 12H, H arom), 8.39 (d, 2H, J = 8.1 Hz, H arom), 8.60 (d, 2H, J = 8.1 Hz, H arom).

Anal. Calcd. for $C_{35}H_{33}N_9$: C, 72.51; H, 5.74; N, 21.74. Found: C, 72.37; H, 5.96; N, 21.94.

[*N*-(Benzimidazo[1,2-*c*]quinazolyl)-3-aminopropyl]-[*N*-(benzimidazo[1,2-*c*]quinazolyl)-2-aminoethyl]amine (**4**).

This compound was obtained in yield 50% as white powder (acetonitrile), mp 150-151°; ^{1}H nmr (deuteriotrifluoroacetic acid): δ 2.00 (s broad, 4H, 2 CCH_2C), 2.59 (s broad, 2H, 1 CCH_2C), 3.33 (s broad, 2H, 1 CH_2NHCC), 3.48 (m, 2H, 1 CH_2NHCC), 4.15 (m, 2H, 1 CH_2NHCN), 4.28 (t, 2H, J = 7.0 Hz, 1 CH_2NHCN), 7.78-8.19 (m, 12H, H arom), 8.36 (t, 2H, J = 7.6 Hz, H arom), 8.61 (dd, 2H, J = 7.8, 2.4 Hz, H arom).

Anal. Calcd. for $C_{35}H_{33}N_9 \cdot 2.5\ H_2O$: C, 67.28; H, 6.13; N, 20.17. Found: C, 67.59; H, 6.01; N, 19.78.

[*N*-(Benzimidazo[1,2-*c*]quinazolyl)-3-aminopropyl]-[*N*-(benzimidazo[1,2-*c*]quinazolyl)-2-aminoethyl]methylamine (**5**).

This compound was obtained in 42% yield as a white powder (methanol), mp 145-146°; ^{1}H nmr (deuteriochloroform): δ 1.78 (s, 2H, 1 CCH_2C), 1.99-2.06 (m, 4H, 2 CCH_2C), 2.51 (s, 3H, CH_3), 2.75 (t, 4H, 2 CH_2NCH_3, J = 6.3 Hz), 3.74-3.83 (m, 4H, 2 CH_2NH), 6.52 (t, 2H, J = 4.6 Hz, 2 NH), 7.24-7.57 (m, 10H, H arom), 7.80-7.88 (m, 4H, H arom), 8.44 (dd, 2H, J = 7.7, 1.0 Hz, H arom).

Anal. Calcd. for $C_{36}H_{35}N_9 \cdot 0.25\ H_2O$: C, 72.27; H, 5.98; N, 21.07. Found: C, 72.04; H, 5.72; N, 21.41.

N,N'-Bis[*N*-(benzimidazo[1,2-*c*]quinazolyl)-2-aminoethyl]-*N,N'*-dimethyl-1,2-ethanediamine (**6**).

This compound was obtained in 29% yield as yellow prisms (dimethylformamide), mp 215-217°; ^{1}H nmr (deuteriotrifluoroacetic acid): δ 3.34 (s, 6H, 2 CH_3N), 3.70-3.80 (m, 2H, 1 CH_2), 3.90-4.20 (m, 6H, 2 CH_2), 4.30-4.60 (m, 4H, 2 CH_2), 7.60-8.03 (m, 12H, H arom), 8.30 (d, 2H, J = 8.4 Hz, H arom), 8.40 (d, 2H, J = 8.1 Hz, H arom).

Anal. Calcd. for $C_{36}H_{36}N_{10} \cdot 0.25\ H_2O$: C, 70.50; H, 5.99; N, 22.84. Found: C, 70.21; H, 6.09; N, 23.09.

N,N'-Bis[*N*-(benzimidazo[1,2-*c*]quinazolyl)-2-aminoethyl]-*N,N'*-dimethyl-1,3-propanediamine (**7**).

This compound was isolated as its methanesulfonate salt, by treatment of the crude chromatographed product (0.3 g, 0.48 mmole, 24%) with four equivalents of methanesulfonic acid (0.185 g, 1.92 mmoles) in dichloromethane (50 ml). The mixture was stirred overnight at room temperature under nitrogen atmosphere. Then, the solvent was evaporated to dryness and the residue crystallized from methanol/ether to give the tetramethanesulfonate of **7** in 25% as a white powder, mp 125-127°; ^{1}H nmr (deuteriotrifluoroacetic acid): δ 2.63 (s broad, 2H, CCH_2C), 2.97 (s, 12H, 4 CH_3S), 3.31 (s, 6H, 2 CH_3N), 3.60-3.78 (m, 4H, 2 CH_2), 3.81-4.12 (m, 4H, 2 CH_2), 4.70 (s broad, 4H, 2 CH_2NH), 7.83-8.16 (m, 12 H, H arom), 8.47 (d, 2H, J = 8.1 Hz, H arom), 8.60 (d, 2H, J = 8.1 Hz, H arom).

Anal. Calcd. for $C_{41}H_{54}N_{10}O_{12}S_4 \cdot 4H_2O$: C, 45.63; N, 5.79; H, 12.98. Found: C, 45.33; H, 5.64; N, 12.62.

N,N'-Bis[2-(*N*-phthalimido)ethyl]-*N,N'*-dimethyl-1,3-propane diamine (**8**).

N,N'-Dimethyl-1,3-propanediamine (2.5 g; 0.025 mole) was added to a suspension of *N*-(2-bromoethyl)phthalimide (12.70 g; 0.050 mole) and potassium carbonate (13.8 g, 0.100 mole) in dry acetone (500 ml).The reaction mixture was refluxed under nitrogen for 24 hours. After concentration to half the original volume, the precipitated solid was removed by filtration and washed with dichloromethane. The filtrate was evaporated to dryness and then extracted with dichloromethane and washed with water. The organic layer was evaporated to dryness to give crude **8** as a yellow oil (12.7 g, 74%) which was used without further purification. ^{1}H nmr (deuteriochloroform): δ 1.47 (m, 2H, CCH_2C), 2.19 (s, 6H, 2 CH_3N), 2.30 (t, 4H, J = 7.3 Hz, 2 CH_2N), 2.55 (t, 4H, J = 6.7 Hz, 2 CH_2N), 3.74 (t, 4H, J = 6.7 Hz, 2 CH_2NCO), 7.66-7.73 (m, 4H, H arom), 7.79-7.85 (m, 4H, H arom).

N,N'-Bis(3-aminoethyl)-*N,N'*-dimethyl-1,3-propanediamine (**9**).

A mixture of **8** (8.7 g, 0.019 mole),, hydrazine hydrate (3.8 g, 0.076 mole), in ethanol was refluxed for 3 hours. After removal of the solvent, excess hydrazine hydrate was eliminated by addition of ethanol and evaporation (5 x 10 ml). The residue was suspended in ethanol (200 ml), and the mixture was adjusted to *p*H 1 with hydrochloric acid, then refluxed for 0.5 hour. After cooling the white precipitate was collected by filtration and washed with water. The water was adjusted to *p*H 12-13 with sodium hydroxide and then evaporated to dryness. The residue was dried over potassium hydroxide pellets, then extracted with toluene. Evaporation of the solvent and distillation in vacuum (bp 32-33°, 0.5 mm Hg) gave **8** as a colourless oil (0.75 g, 20%). ^{1}H nmr (deuteriochloroform): δ 1.63 (m, 2H, CCH_2C), 1.93 (s broad, 4H, 2 NH_2), 2.21 (s, 6H, 2 CH_3N), 2.34-2.43 (m, 8H, 4 CH_2), 2.77 (t, 4H, J = 6.0 Hz, 2 CH_2).

Benzimidazo[1,2-*c*]-1,2-dihydroimidazo[1,2-*a*]quinazoline (**12**).

Tosyl chloride (0.376 g, 1.97 mmoles) was added to a solution of **10** (0.5 g, 1.79 mmoles) in dry pyridine (10 ml) at 0°, and stirred for 2 hours. After additional stirring overnight at room temperature, the mixture was poured into ice water (50 ml), and the precipitated solid was filtered, dried and crystallized from ethanol to afford **12** (0.42 g, 90%) as a white powder: mp 176-177°, lit [13] 174-176°; ^{1}H nmr (deuteriodimethyl sulfoxide): δ 4.11 (s, 8H, 4 CH_2), 7.06 (d, 2H, J = 8.1 Hz, H arom), 7.18 (t, 2H, J = 7.5 Hz, H arom), 7.36-7.47 (m, 4H, H arom), 7.59 (m, 2H, H arom), 7.79-7.83 (m, 2H, H arom), 8.19-8.31 (m, 4H, H arom);

ms: $^+$FABms (3-$NO_2C_6H_4CH_2OH$) m/z (%) 261 ([M+1]$^+$, 100).

Anal. Calcd. for $C_{32}H_{24}N_8$: C, 73.83; H, 4.64; N, 21.52. Found: C, 73.55; H, 4.80; N, 21.38.

In Vitro Assay.

The cytotoxicity of the benzimidazo[1,2-*c*]quinazolines was measured using a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay [14]. Human colon carcinoma cell line HT-29 was obtained from American Type Culture Collection and cultured in the recommended media. Exponentially growing cells were plated at 3000/well into 96 well plates in 150 µl complete Dulbecco's minimum essential media containing 10% fetal bovine serum. Cells were allowed to attach for 24 hours before the addition of a serial (1:4) dilution of drug in 50 µl fresh media. After 72 hours of incubation at 37°, 5% carbon dioxide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (50 µl, 3 mg/ml in fetal bovine serum) was added to each well and the plates incubated for 4 hours. Formazan crystals formed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide metabolism were solubilized by the addition of 50 µl of 25% sodium dodecil sulfate *p*H 2 to each well and incubated overnight. The cellular metabolism of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was then quantified by reading the absorbance of the solubilized product at 550 nm with a 96 well plate reader. Inhibitory concentration 50 values were calculated as the concentration of drug to inhibit cell growth to 50% of controls.

In Vivo Evaluation.

MX-1 and LOX tumors were obtained from the tumor repository of the National Cancer Institute (Bethesda, MD) and grown in female athymic Ncr-nude mice (Taconic Farms, Germantown, NY). MX-1 tumor was grown sub cutaneously and passaged as tumor fragments (50 mg) dissected from non-necrotic portion of a donor tumor. LOX was grown intra peritoneally as an ascites and 10^6 cells implanted sub cutaneusly for experimental studies. Tumor size was determined by caliper measurement and weight estimated according to a published method [15]. Effects of drug treatment on tumor growth were assessed on the basis of comparison of median time to reach a 1000 mg tumor size between treated (T) and control (C) groups. A T-C value of less than 6 days was not considered meaningful.

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