A Structure–Activity Relationship Study of Batracylin Analogues

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A number of isoindolo[1,2-*b*]quinazolines and some benzo[4,5]isoquinolino[1,2-*b*]quinazolines as structural modification analogues of the antitumor compound batracylin were synthesized and evaluated against HL-60 cell growth and in topoisomerase II-mediated DNA cleavage assays. Of the compounds studied, 10,12-dihydro-7,8-methylenedioxyisoindolo[1,2-*b*]quinazolin-12(10*H*)-one (**1d**), 2-amino-10,12-dihydroisoindolo[1,2-*b*]quinazolin-12(10*H*)-one (**1p**), and 2-amino-7,8-methylenedioxy-10,12-dihydroisoindolo[1,2-*b*]quinazolin-12(10*H*)-one (**1ab**) exhibited good inhibitory activities against HL-60 cell lines as well as induction of topo II-mediated DNA cleavage activities.

KEY WORDS: isoindolo[1,2-*b*]quinazolines; cytotoxicity; DNA topoisomerase; colon adenocarcinoma 38; structure–activity relationship.

INTRODUCTION

Compounds containing a condensed tetracyclic dihydroquinazoline ring system, isoindolo[1,2-*b*]quinazolin-12(10*H*)-one (**1a**), were originally reported by Gabriel in 1912 (1). Subsequently, the chemistry of compound **1a** and its 7,8-dimethoxy derivative **1b** were studied (2–5). In 1978, Kabbe (6) prepared the 8-amino derivative **1c** by the condensation of 2,5-diaminobenzylamine and phthalic anhydride. This compound, now called batracylin, was reported to inhibit completely early-stage colon adenocarcinoma 38 and to cause regression of advanced colon 38 tumor (7,8), a relatively refractory mouse solid tumor model. In addition, batracylin possesses collateral sensitivity against both adriamycin- and cisplatin-resistant stains of leukemia P-388 (7). This compound is active orally and intraperitoneally. However, because of unexpected toxicity of batracylin in the rat, the National Cancer Institute elected not to pursue further study of this compound (9). Colon cancer is one of the most common neoplastic diseases in developed countries and there is still no effective drug available for its treatment, therefore the inhibitory activity displayed by batracylin against colon 38 should not be overlooked. Since only limited compounds of this type were reported in the literature (1–6,10), we decided to synthesize and investigate the structure–activity relationships of batracylin analogues.

MATERIALS AND METHODS

For compounds substituted on ring A and/or ring D of the isoindolo[1,2-*b*]quinazolin-12(10*H*)-one ring system (**2**), the following derivatives were prepared and studied (Table I).

Formation of the isoindolo[1,2-*b*]quinazolin-12(10*H*)-one ring system (**2**) may be achieved by the following three approaches (see Fig. 1): *Method a*, condensation of a substituted nitrobenzene (**4**) with (*N*-hydroxymethyl)phthalimide (**5**) followed by reduction and cyclization; *Method b*, condensation of a substituted 2-nitrobenzyl halide (**6**) with the potassium salt of a substituted phthalimide (**7**) followed by reduction and cyclization; and *Method c*, condensation of a substituted 2-aminobenzylamine (**8**) with an appropriate phthalic anhydride (**9**) followed by cyclization of the resulting intermediate **3**. In most cases, cyclization of **3** to **2** occurred spontaneously. Many functional derivatives of **2** were then prepared from **2** by conventional methods.

Several compounds aiming on the structural modification of **2** at ring D (compounds **10–12**) and at rings C–D (compounds **13a, b**) were prepared by the condensation of the appropriate 2-aminobenzylamine with the corresponding anhydride followed by cyclization (see Fig. 2).

Batracylin (NSC-320846). This compound was obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, National Cancer Institute.

Enzymes and Nucleic Acids. DNA topoisomerase II was purified from calf thymus gland by a modification of published procedures (11). Plasmid YEPG, which is a derivative of YEP24, was purified by the alkali lysis method followed by phenol deproteination and by CsCl/ethidium isopycnic centrifugation (12).

Preparation of End-Labeled DNA Fragments. The procedure used for end-labeling of plasmid DNA was followed by the method described previously (13).

Topoisomerase II-Mediated DNA Cleavage Assay. Reactions were carried out as described previously (14).

Cytotoxic Assay. The synthesized compounds were evaluated for their cytotoxic effects of HL-60 (human promyelocytic leukemia) cells. The cytotoxicity of the compounds was determined by XTT-microculture tetrazolium assay (15). The medium-effect inhibitory concentration (ID_{50}) was determined by the medium-effect plot (16) using computer software (17).

Synthesis

Melting points were determined on a Thomas-Hoover melting point apparatus. The ultraviolet spectra were measured on a Varian Superscan 3 ultraviolet–visible spectrophotometer. The mass spectra were determined with an Atlas CH-5 mass spectrophotometer and the infrared spectra were recorded on a Perkin–Elmer 337 Grating infrared spectrometer.

For the methods of synthesis of dihydroisoindolo[1,2-

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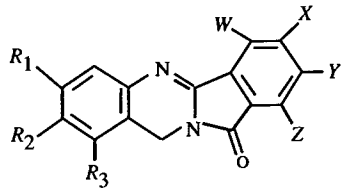
Table I. Substituted Isoindolo[1,2-*b*]quinazolin-12(10*H*)-ones (1)



Compound	R_1	R_2	R_3	W	X	Y	Z
1a[a]	H	H	H	H	H	H	H
1b	CH_3O	CH_3O	H	H	H	H	H
1c (batracylin)	H	NH_2	H	H	H	H	H
1d	OCH_2O		H	H	H	H	H
1e	H	H	CO_2H	H	H	H	H
1f	H	H	CO_2CH_3	H	H	H	H
1g	H	H	$CONHNH_2$	H	H	H	H
1h	H	H	$CONH(CH_2)_2N(CH_3)_2$	H	H	H	H
1i	H	H	$CO_2(CH_2)_2N(CH_3)_2$	H	H	H	H
1j	H	CH_3	H	H	H	H	H
1k	H	H	H	H	CH_3	H	H
1l	H	H	H	H		OCH_2O	H
1m	H	H	H	NO_2	H	H	H
1n	H	H	H	NH_2	H	H	H
1o	H	H	H	H	H	NO_2	H
1p	H	H	H	H	H	NH_2	H
1q	H	H	H	H	H	CO_2H	H
1r	H	H	H	H	H	CO_2CH_3	H
1s	H	H	H	H	H	$CONH(CH_2)_2N(CH_3)_2$	H
1t	H	H	H	Br	Br	Br	Br
1u	H	H	H	Cl	Cl	Cl	Cl
1v	CH_3O	CH_3O	H	H	H	CO_2H	H
1w	CH_3O	CH_3O	H	H	H	NO_2	H
1x	H	NO_2	H	H	CH_3 (H)	H (CH_3)	H
1y	H	NO_2	H	H		OCH_2O	H
1z	H	NH_2	H	H	CH_3 (H)	H (CH_3)	H
1aa	OCH_2O		H	H	H	NO_2	H
1ab	OCH_2O		H	H	H	NH_2	H

[a] Compounds synthesized previously (1–6, 10), reprepared for biological activity comparison.

b]quinazolin-12(10*H*)-ones, Method a was initially reported by Downs and Lions (2) and compound **1b** was synthesized by this route. Method b is illustrated by the following examples.

10,12-Dihydro-7,8-methylenedioxyisoindolo[1,2-b]quinazolin-12(10H)-one (1d). Ten grams (0.05 mol) of 6-nitropiperonyl alcohol (mp 122–125°C) was refluxed with 6 mL of thionyl chloride in 100 mL of chloroform for 30 min. The reaction mixture was concentrated under reduced pressure, and the residue was diluted with 50 mL of dimethylformamide. To the solution was added, with stirring, 9.5 g (0.05 mol) of potassium phthalimide. A percipitate formed immediately. The mixture was stirred for 30 min and filtered to give 9 g of *N*-(2-nitro-4,5-methylenedioxybenzyl)phthalimide. Recrystallization from 90 mL of dimethyformamide yielded 4.0 g of the condensation intermediate as brown crystals, mp 221–223°C. This intermediate was hydrogenated with 0.5 g of 5% Pd–C at 5 kg/cm^2. The isolated product (3.3 g) was fused at 220–230°C for 30 min to give 2.4 g of a greenish yellow solid, mp 250–251°C. Recrystallization from dimethylformamide raised the mp to 253–255.5°C; the yield was 76%. *Anal.* Calcd. for $C_{16}H_{10}N_2O_3$ (278.3): C, 69.06; H, 3.62; N, 10.07. Found: C, 69.26; H, 3.80; N, 10.22.

9-Carbomethoxy-10,12-dihydroisoindolo[1,2-b]quinazolin-12(10H)-one (1f). This was prepared in a similar manner by the reaction of 2-carbomethoxy-6-nitrobenzyl bromide (prepared by the *N*-bromosuccimide bromination of methyl 2-methyl-3-nitrobenzoate with potassium phthalimide (90–100°C for 5 hr in dimethylformamide) followed by catalytic hydrogenation of the resulting *N*-(2-carbomethoxy-6-nitrobenzyl)phthalimide (mp 167–168°C) to give the desired product **1f** in an overall yield of 30%, mp 231–232°C. *Anal.* Calcd. for $C_{17}H_{12}N_2O_3$ (292.3): C, 69.86; H, 4.14; N, 9.59. Found: C, 69.88; H, 4.23; N, 9.47.

10,12-Dihydro-8-methylisoindolo[1,2-b]quinazolin-12(10H)-one (1j). A mixture of 10 g (0.054 mol) of 5-methyl-2-nitrobenzyl chloride, 0.2 g of potassium iodide, 11 g (0.05 mol) of potassium phthalimide, and 50 mL of dimethylformamide was stirred at 80°C overnight. The solid was collected after cooling. It was washed successively with water,

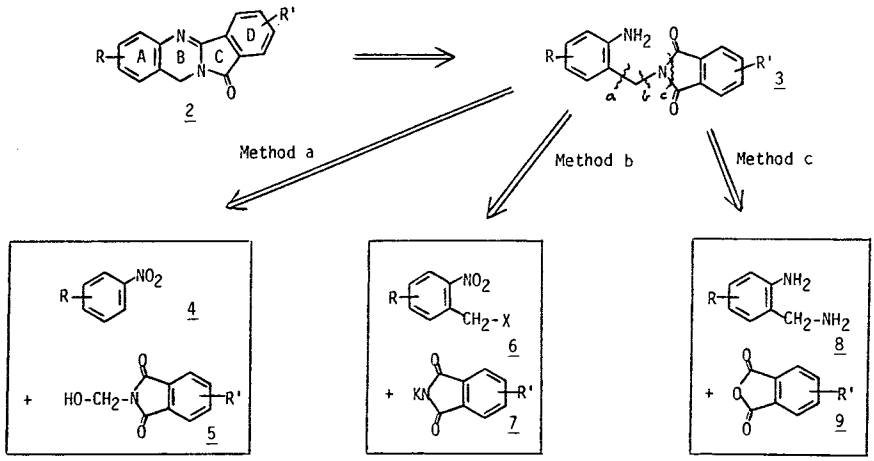


Fig. 1. Retrospective synthetic approaches to isoindolo[1,2-*b*]quinazolin-12(10*H*)-ones.

ethanol, and ether and dried to give 13 g of crude *N*-(5-methyl-2-nitrobenzyl)phthalimide, mp 205–209°C. Recrystallized from dimethylformamide gave 9 g of the pure intermediate, mp 213–214°C. This was hydrogenated with 5% Pd/C in dimethylformamide to give *N*-(2-amino-5-methylbenzyl)phthalimide (3; *R* = 4-methyl, *R'* = *H*), mp 140–160°C. This intermediate resolidified on continued heating and remelted at 218–219°C. It was thus fused at 180°C and the fused solid recrystallized from a mixture of methanol and dimethylformamide to give the desired compound **1j**, mp 218–219°C. The overall yield was 74.6%. *Anal.* Calcd. for $C_{16}H_{12}N_2O$ (248.2): C, 77.40; H, 4.87; N, 11.28. Found: C, 77.58; H, 5.00; N, 11.31.

Method c is illustrated by the following examples.

10,12-Dihydro-2-nitroisoindolo[1,2-b]quinazolin-12(10H)-one (1o). To a solution of 12.2 g (0.1 mol) of 2-aminobenzylamine in 100 mL of dry pyridine was added 21 g (0.108 mol) of 4-nitrophthalic anhydride in 100 mL of pyridine. The mixture was refluxed for 22 hr and cooled. The resulting solid was collected by filtration to give 19 g (68.1% yield) of **1o**, mp 258–261°C (dec). Recrystallization from pyridine, mp 258–261°C (dec). UV: λ_{max}, 378 nm (ε 11,600). IR: ν_{max}, 1715, 1625, and 1510 cm^{-1}. Mass spec: *m/e* 279 (M^+).

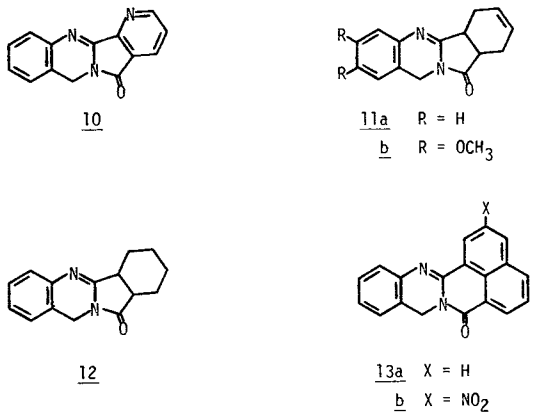


Fig. 2. Batracylin analogues: structural modification at rings A, C, and/or D.

Anal. Calcd. for $C_{15}H_9N_3O_3$ (279.3): C, 64.50; H, 3.25; N, 15.05. Found: C, 64.62; H, 3.35; N, 15.09.

Other compounds prepared in a similar manner by this method include compounds **1k, 1l, 1m, 1w, 1y, 1aa,** and **11b.**

2-Amino-10,12-dihydroisoindolo[1,2-b]quinazolin-12(10H)-one (1p). A solution of 0.9 g of **1o** in 150 mL of dimethylformamide was hydrogenated with 100 mg of 5% Pd/C under 5 kg/cm^2 of H_2 for 2 hr. The reaction mixture was filtered and the filter cake was washed with dimethylformamide (3 × 10 mL). The combined filtrate and washings were evaporated under reduced pressure to dryness to give 760 mg (95% yield) of **1p**. The residue was recrystallized from 1-butanol, mp 282–284.5°C. UV: λ_{max}, 378 nm (ε 14,400), 312 nm (ε 18,900). IR: ν_{max}, 3440, 3310, 3200, 1715, 1620, and 1590 cm^{-1}. Mass spec: *m/e* 249 (M^+). *Anal.* Calcd. for $C_{15}H_{11}N_3O$ (249.3): C, 72.27; H, 4,45; N, 16.86. Found: C, 72.05; H, 4.57; N, 16.88.

Compounds **1n, 1z,** and **1ab** were prepared by catalytic reduction from compounds **1m, 1x,** and **1aa,** respectively, in a similar manner (for physical constants, see Table II). Elemental analyses of both compounds were within ±0.3% of the theoretical values for C, H, and N.

7,9-Dihydrobenz[4,5]isoquinolino[1,2-b]quinazolin-7(9H)-one (13a). A mixture of 4.4 g (36 mmol) of 2-aminobenzylamine and 7.5 g (38 mmol) of 1,8-naphthalic anhydride in 100 mL of dimethylformamide was refluxed for 17.5 hr. After cooling in a refrigerator overnight, the crude product was collected, washed with ethanol, and dried to give 6.13 g, mp 191–193°C. Recrystallization from dimethylformamide gave 5.46 g of **13a**, mp 209–210.5°C. UV: λ_{max}, 394 nm (ε 14,400). This compound was found to be identical to that prepared by other procedures [lit (19,20): mp 211–212°C (19), mp 211°C (20)]. Other compounds prepared in a similar manner by this method include **1q, 1t, 1u, 1v, 1x** (refluxed in dimethylacetamide), **10, 11a, 12,** and **13b** (see Table II). Elemental analyses of these compounds were all within ±0.3% of the theoretical values for C, H, and N.

9-Carboxy-10,12-dihydroisoindolo[1,2-b]quinazolin-12(10H)-one (1e). A mixture of 1.5 of **1f**, 2 g of potassium carbonate, 5 mL of water, and 30 mL of dimethylformamide was refluxed for 90 min. All solids dissolved initially during heating then gradually precipitated out. The solvent was re-

Table II. Characterization and Biological Activity of Isoindolo[1,2-*b*]quinazolines and Benz[4,5]isoquinolino[1,2-*b*]quinazolines

Compound[a]	Method of preparation	Yield (%)	mp (°C)	HL-60 ID_{50} (μM)[b]	Topo II-mediated DNA cleavage activity[c]
1a	b, c	70	184–185.5	54	100
1b	a, c	48	245–246	>1000	—[d]
1c (batracylin)	c	56	287–288	32	20
1d	b	76	253–255.5	8	200
1e	(from **1f**)	63	338	373	5
1f	b	30	231–232	>1000	—
1g	(from **1f**)	84	338–340	>100	—
1h	(from **1f**)	57	213–214	13	10
1i	(from **1f**)	67	169–170	13	10
1j	b	75	218–219	>1000	—
1k	c	27	208–209	219	100
1l	c	76	253.5–255	253	—
1m	c	69	272–273	>100	0
1n	(from **1m**)	56	222–224	90	—
1o	c	68	258–261	117	5
1p	(from **1o**)	95	282–284.5	12	2000
1q	c	69	300	>100	1
1r	(from **1q**)	70	247–248	>1000	—
1s	(from **1q**)	64	263–265	11	—
1t	c	44	288 (dec)	14	0
1u	c	30	287 (dec)	30	0
1v	c	65	341 (dec)	>100	0
1w	c	42	258–260 (dec)	277	—
1x	c	62	253–256	>1000	—
1y	c	51	295 (dec)	85	—
1z	(from **1x**)	56	253–258	4	—
1aa	c	47	265 (dec)	>100	0
1ab	(from **1aa**)	63	322 (dec)	4	100
10	c	27	242–244	>100	100
11a	c	47	175–176.5	>100	—
11b	c	30	188–190	>100	200
12	c	21	122–123.5	761	0
13a	c	54	209.5–210.5	>125	0
13b	c	27	281–283	>100	0

[a] Each compound was correctly analyzed for C, H, and N.
[b] ID_{50} values of 20 μM or less are considered active against HL-60 cell growth.
[c] Relative activity to induce Topo II-mediated DNA cleavage is based on the effective doses which caused 90% of Topo II-mediated DNA fragmentation of linear 8.4-kb YEPG DNA. The activity of VM-26 to stimulate Topo II-mediated DNA cleavage is taken as 100,000.
[d] Not tested.

moved under reduced pressure and the residue dissolved in water. A small amount of impurity was filtered, then the filtrate acidified to give 1.1 g of **1e**, mp 321–324°C. Recrystallization from dimethylformamide gave 0.9 g (63% yield) of pure **1e**, mp 338°C. *Anal.* Calcd. for $C_{16}H_{10}N_2O_3$ (278.3): C, 69.06; H, 3.62; N, 10.07. Found: C, 69.02; H, 3.70; N, 10.16.

10,12-Dihydro-9-[2-(dimethylamino)ethylamino]carbonylisoindolo[1,2-b]quinazolin-12(10H)-one (1h). A mixture of 3.7 g of **1e** and 35 mL of thionyl chloride was refluxed for 4 hr. To the reaction mixture was added another 40 mL of thionyl chloride and reflux was continued for another 2 hr, whereupon a complete solution was attained. Excess thionyl chloride was removed under reduced pressure. To the residue was added 50 mL of chloroform, then the mixture was evaporated again to remove the last traces of thionyl chloride. The impure acid chloride was used for the preparation of the following two compounds (**1h** and **1i**) without further purification. To 0.7 g of the acid chloride was added 3 g of 2-(dimethylamino)ethylamine. The mixture was refluxed until a solution resulted. A solid separated on cooling. This was collected by filtration and recrystallized from diglyme to give 0.5 g (57.1% overall) of **1h**, mp 213–214°C. *Anal.* Calcd. for $C_{20}H_{20}N_4O_2$ (378.4): C, 68.95; H, 5.79; N, 16.08. Found: C, 69.08; H, 5.84; N, 15.98.

10,12-Dihydro-9-[2-(dimethylamino)ethylaminocarbonyl]isoindolo[1,2-b]quinazolin-12(10H)-one (1i). A mixture of 0.9 g of the acid chloride and 2.7 g of 2-(dimethylamino)-ethanol was heated until a complete solution was achieved (30 min). On cooling, solid separated, which was collected and recrystallized from diglyme to give 0.75 g (66.8% yield) of **1i**, mp 169–170°C. *Anal.* Calcd. for $C_{20}H_{19}N_3O_3$ (349.4): C, 68.75; H, 5.48; N, 12.03. Found: C, 68.83; H, 5.66; N, 11.92.

RESULTS AND DISCUSSION

Structural Assignment for Compounds 1k, 1x, and 1r (Prepared from 1q)

When unsymmetrically substituted anhydrides are used in Method c, there is the possibility of isomeric product formulation. As expected, electronic effects of substituents would exert different influences on the two carbonyl groups during cyclization (see Fig. 1). It was found that the refluxing temperature during condensation (which depends on the type of solvents used) is also an important factor. NMR analyses indicated that compound **1k**, which was prepared in refluxing pyridine, is a single compound and the structural assignment for **1k** is correct, since its methyl group absorptions of ^{1}H and ^{13}C are single peaks at δ 2.517 and δ 21.928, respectively. This infers that at a relatively low reaction temperature, even a weak electron-releasing property exerted by the methyl group (hyperconjugation) plays a role in determining the orientation of the condensation product. In the case of compound **1x**, it is a mixture of two (2- and 3-methyl) isomers at a ratio of 1:1 (methyl absorptions for ^{1}H and ^{13}C are δ 2.549 and 2.542 and δ 22.04 and 22.01, respectively), since the reaction was conducted at a much higher temperature (refluxed in dimethylacetamide), and at that temperature the carbonyl condensation took place regardless of the weak electronic effect rather than being determined by the weak electron-releasing effect of the methyl group. In contrast, a stronger electron-withdrawing carboxyl group predominates the selection of the proper carbonyl group for condensation even at refluxing temperature (in dimethylformamide), so it is concluded that **1q** was formed as a single compound. This is indicated by the NMR analysis of its derivative **1r**, the HMBC of which showed that H-4 is at δ 8.1 with a small additional splitting doublet. The structural assignments for compounds **1q** and **1r** are therefore correct.

DNA Topoisomerase II-Mediated DNA Cleavage Assay

Many compounds synthesized have been evaluated for their ability to trap cleavable complexes using purified calf thymus topoisomerase II (13,18). The cleavage efficiency and cleavage site specificity of the cleavable complexes have also been determined.

The results of both the cytotoxic and the DNA topoisomerase assays are shown in Table II. A representative cleavage pattern is shown in Fig. 3. All batracylin analogues stimulated topoisomerase II-mediated DNA cleavage with a cleavage pattern different from that of VM-26. However, the cleavage patterns produced by various batracylin analogues were indistinguishable.

Among the 10,12-dihydroisoindolo[1,2-*b*]quinazoline-12-one series, the 7,8-methylenedioxy- (**1d**), 9-{[2-(dimethylamino)ethyl}aminocarbonyl}- (**1h**), 9-[2-(dimethylamino)ethoxycarbonyl] (**1i**), 2-amino- (**1p**), 2{[2-dimethylamino)ethyl]aminocarbonyl}- (**1s**), 1,2,3,4-tetrabromo- (**1t**), and 2,3-methylenedioxy-8-nitro- (**1y**) derivatives exhibited good inhibitory activities against HL-60 cell growth. In the DNA topoisomerase II-mediated cleavage efficacy assays, the following were found to be

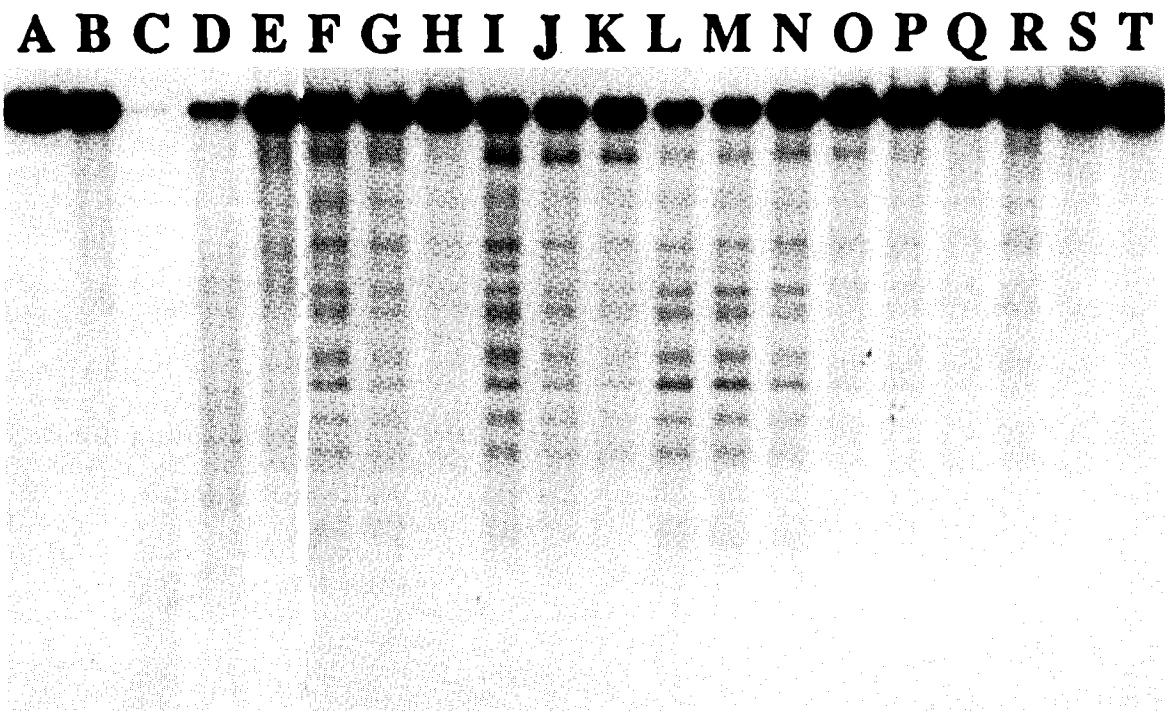


Fig. 3. Batracyclin analogues induce site-specific DNA cleavage in the presence of mammalian topoisomerase II: 3′ end-labeled YEPG DNA and calf thymus DNA topoisomerase II were used in the topoisomerase cleavage assay as described under Materials and Methods. Lane A, DNA control, no drug, no enzyme. Lane B, no drug. Lanes C–E, 0.5, 0.05, and 0.005 μg/mL *VM-26*, respectively. Lanes F–H, 50, 5, and 0.5 μg/mL *Bactracylin* (**1c**), respectively. Lanes I–K, 50, 5, and 0.5 μg/mL compound **1d**, respectively. Lanes L–N, 50, 5, and 0.5 μg/mL compound **1p**, respectively. Lanes O–Q, 50, 5, and 0.5 μg/mL compound **1o**, respectively. Lanes R–T, 50, 5, and 0.5 μg/mL compound **1e**, respectively.

active: the parent compound **1a** and the 7,8-methylenedioxy- (**1d**), 9-substituted (**1h** and **1i**), 3-methyl- (**1k**), 2-amino- (**1p**), 2-amino-7,8-methylenedioxy- (**1ab**), 4-aza- (**10**), and 7,8-dimethoxy-1,4,4a,10,12,12a-hexahydro- (**11b**) derivatives. The benz[4,5]isoquinolino[1,2-*b*]quinazolines **13a** and **13b** are inactive. Although several compounds in this series possess activity in both assay systems, the topo II-mediated DNA cleavage activity did not always correlate with the activity exhibited by the HL-60 cell line inhibition, which infers that topoisomerase inhibition may not represent the mechanism of observed cytotoxicity action for compounds selected in the present study. None of the observed biological activities are comparable with existing anticancer agents such as VM-26 (21). The activity of batracylin is also not high in these tests. Nevertheless, the fact that both 2-amino substituted compounds **1p** and **1ab** exhibited reasonably good activity against both HL-60 cells and topo II-mediated DNA cleavage action suggested that side-chain substitutions on the amino function at this position may furnish compounds with the desired biological activity. Work along this line, together with our total drug design program based on a specific "2-phenylnaphthalene"-type structural feature proposed in our laboratory (22), is currently in progress.

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