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Studies on quinones. Part 44: Novel angucyclinone *N*-heterocyclic analogues endowed with antitumoral activity ☆

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ABSTRACT

In the search for new potentially anticancer drugs, series of angucyclinone aza-analogues containing pyridine and pyridopyridazine rings have been designed and synthesized by a highly efficient sequence involving a one-pot step for the synthesis of tricyclic quinone intermediate and highly regiocontrolled cycloaddition reactions with polarized 1,3-dienes. The new *N*-heterocyclic angular quinones were evaluated in vitro on normal human fibroblasts and on a panel of four distinct human cancer cell lines. All tested compounds showed high to moderate antitumor activity. Among the compounds, those with one and two pyridine moieties fused to the quinone system have shown the best effect. Structure–activity relationships established the main structural requirement for the activity of the new potential anticancer drugs.

1. Introduction

Angucyclines/angucyclinones are a class of antibiotics, isolated from several strains of *Streptomyces*, that display a broad spectrum of pharmacological properties, including antiviral, antifungal, antitumor, and enzyme inhibitor activity.[2,3] This group of antibiotics is, after the tetracyclines and anthracyclines, the third class of natural antibiotics featuring a carbotetracyclic skeleton. Several of these antibiotics possesses a unique benz[*a*]anthraquinone structure with a carbonyl group at C-1 and an oxygenated substituent at C-6 {i.e., (−)-rabelomycin **1**[4] and (+)-hatomarubigin A **2**[5]} and C-6,8 {i.e. (−)-tetrangomycin **3**[6] and (+)-rubiginone B_2 **4**[7]}(Fig. 1). Because of the interest in the biological activities associated with these compounds,[2,8] the synthesis of benz[*a*]anthraquinones occupies a position of importance in quinone chemistry.[9–15]

Bioisosteric replacements to produce better new drugs are well documented in medicinal chemistry.[16,17] In fact, bioisosterism prompted successful lead modification in many instances. Replacing an atom or a group of atoms by other appropriate moieties produces a number of physico-chemical modifications, including changes in size, shape, electronic distribution, solubility, phase distribution, reactivity, hydrogen bonding ability, which are likely to affect drug pharmacodynamics, pharmacokinetics and metabolism to a remarkable extent. This is indeed the case for a family of promising compounds, the aza-anthracenediones derived from the introduction of one or more nitrogens into the carbocyclic ring structure of the anthraquinone-based drugs.[18]The synthesis of aza-analogues of the benz[*a*]anthraquinone chromophore of angucyclinones has received relatively little attention. In this respect, Guingant et al.[19,20] have reported the synthesis of angucyclinone 5-aza-analogues by a method that involves cycloaddition reactions of 2-bromo-1,4-naphthoquinone derivatives with a push-pull heterodiene.

As part of our continuous interest in the synthesis and biological evaluation of quinones[1,21–29] we have recently reported studies on isoquinolinquinone-containing polycyclic compounds.[24] The efficient and regioselective access to these compounds along, with their promising antitumoral activities on several tumor cell lines in preliminary trials encouraged us to extend our studies to the synthesis[30] and biological evaluation of two series of angular *N*-heterocyclic quinones structurally related to the benz[*a*]anthraquinone chromophore of some angucyclinones.

Since the carbonyl group on the angularly fused ring appears in several angucyclinones on the 1-position (ring A), such as in angucyclinones **1**–**4**, this substituent was retained throughout the present study. The effect due to replacement of the benz[*a*]anthraquinone ABD carbocyclic rings by *N*-heterocyclic rings such as pyridine and piridazine, and the introduction of substituents such as methyl and hydroxyl groups into the corresponding angucyclinone aza-analogues was examined. Among the variety of the new angucyclinone aza-analogues, some members displayed significant activity, and they could be potentially useful in the design of novel chemotherapeutic drugs.

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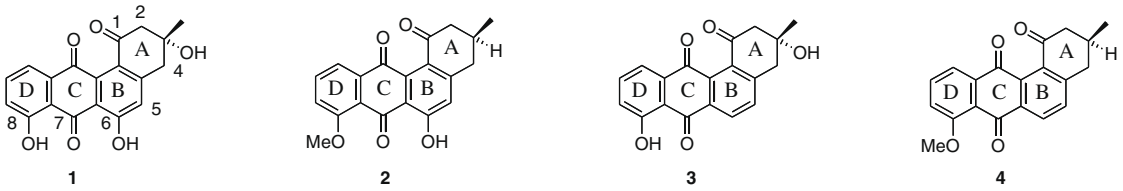


Figure 1. Structures of rabelomycin **1**, hatomarubigin A **2**, tetrangomycin **3,** and rubiginone B_2 **3**.

2. Chemistry

Based on our previous results on the synthesis of benzisoquinolinquinones from activated benzoquinones and methyl aminocrotonate,[24] we first planned to prepare a series of angucyclinone 5-aza analogues using the retrosynthetic strategy shown in Scheme 1.

The following starting substances were employed in this study: 2,5-dihydroxybenzaldehyde **5a**; 2,5-dihydroxyacetophenone **5b**; 3-amino-2-cyclohexen-1-one **6a**, and 3-amino-5,5-dimethyl-2-cyclohexen-1-one **6b.**

After considerable experimentation to prepare the dihydroxyphenanthridines required as precursors of phenanthridinequinones **8**, from enaminones **6** and activated quinones **7** (Scheme 1), we developed a simple and straightforward procedure to synthesize the target tricyclic quinones **8** by using a one-pot procedure. In this synthetic method the activated benzoquinones **7a**,**b**, generated in situ from the corresponding hydroquinones **5a**,**b** with silver (I) oxide, react with enaminone **6a**,**b** in an ionic [3+3] process to give the corresponding dihydroxyphenanthridines, which by a further *in situ* oxidation yielded phenanthridinequinones **8a**–**d** in 65–79% yields (Scheme 2).

After successfully synthesizing quinones **8a**–**d** we turned our attention to carrying out the Diels–Alder reaction of these dienophiles with 1-(*E*)-trimethylsilyloxybuta-1,3-diene according to the retrosynthetic strategy shown in Scheme 1. Surprisingly, the cycloaddition of quinone **8a** and the diene proceeded smoothly in dichloromethane at room temperature, yielding adduct **9a** (Scheme 2) as the sole regioisomer, as was evidenced by TLC and ^{1}H NMR. In a similar manner, cycloaddition of quinones **8b**–**d** with the silyloxybutadiene provided access to the corresponding adducts **9b**–**d** and no regioisomers were detected in the reaction mixture (Scheme 2). The structures of compounds **9** were established by 2D NMR techniques.

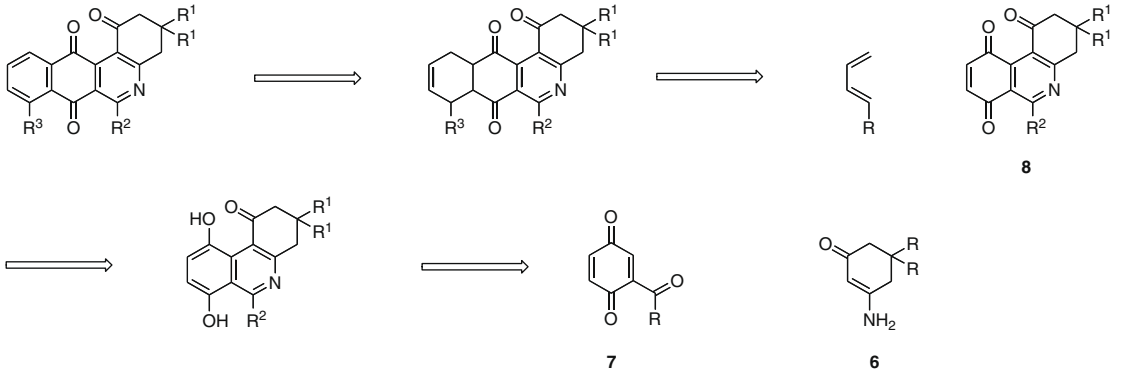
The remarkable regioselectivity of the cycloaddition of quinones **8a**–**d** and the unsymmetrical diene led us to analyses the cycloaddition reaction of quinone **8a** with the diene in terms of frontier molecular orbital (FMO) theory.[31] Theoretical calculations of the primary LUMO coefficients of dienophile **8a** (0.3967 for C-8 and 0.3141 for C-9) and the primary HOMO coefficients of the diene (−0.5016 for C-4 and 0.4646 for C-1), performed using the semiempirical PM3 method, led to the prediction that adduct **9a** should be the less favoured regioisomer in this cycloaddition. Accordingly, the regiochemistry of the cycloadditions of dienophiles **8a–d** with trimethylsyloxybutadiene proceed in the opposite manner from that predicted by FMO theory. These facts could be attributed to steric and/or electronic factors associated with the fused cyclohexenone ring in compounds **8a**–**d**, which determine the regiochemical control of these cycloadditions.[30b]

Taking into account the high regioselectivity of the Diels–Alder reaction of quinones **8a** with the polarized silyloxybutadiene, we studied the cycloaddition of quinone **8a** with 1-dimethylamino-3-methyl-1-azabuta-1,3-diene **10**. The cycloaddition gave in 53% yield the tetracyclic quinone **11** as the single regioisomer (Scheme 1). The structure of **11** was established by 2D NMR techniques.

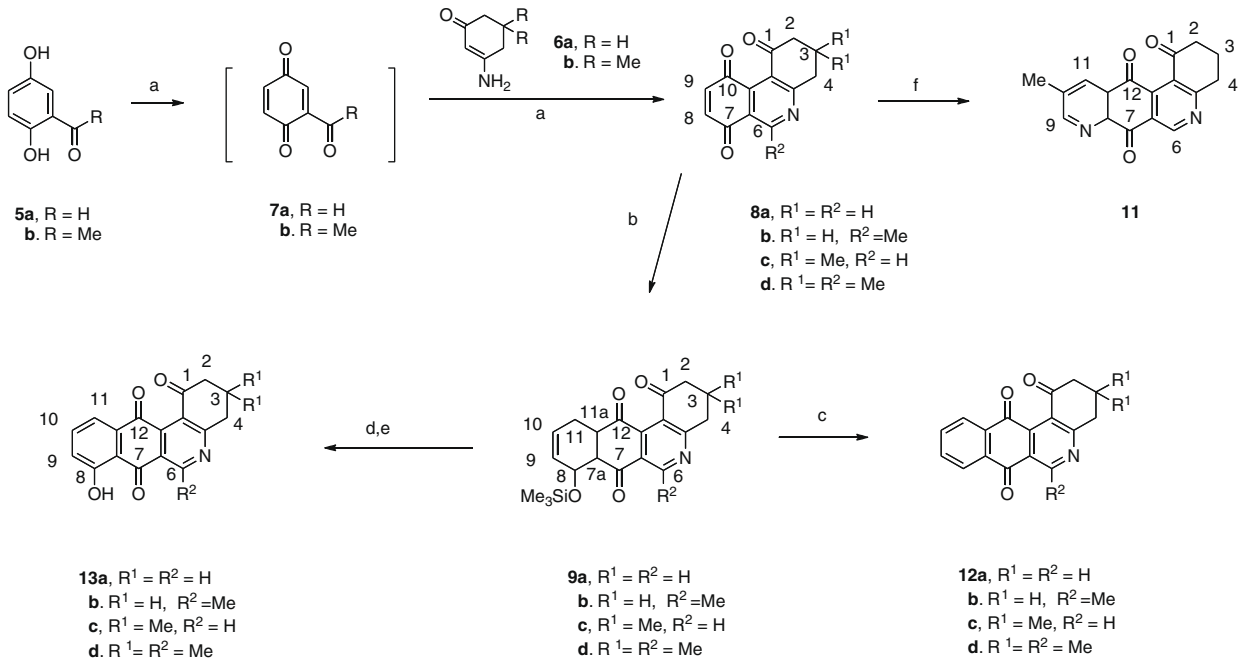
Diels–Alder adducts **9a**–**d** were converted into the corresponding benzo[*j*]phenanthridinequinones **12a**–**d** in 63–95% yield by reaction with hydrochloric acid. On the other hand, mild hydrolysis of adducts **9a**–**d** with hydrochloric acid followed by oxidation with PCC of the alcohol intermediates produced the corresponding 8-hydroxy benzo[*j*]phenanthridinequinones **13a**–**d** in 66–84% yields (Scheme 2). It is worth mentioning that compounds **13** had the same relative location of the carbonyl (C-1) and hydroxyl groups (C-8) on the angular tetracyclic framework as that of some angucyclinones (*i.e.*, (−)-rabelomycin **1** and (+)-halomarubigin A **2**).

The successful results obtained in the synthesis of angucyclinone 5-aza-analogues **12** and **13** were used for the synthesis of a second series of angucyclinone aza-analogues. This new series was designed by replacement of the AB rings of the angucyclinone chromophore with a pyridopyrimidine moiety. This *N*-heterocyclic moiety was considered relevant due to its presence in some antitumoral compounds.[32–34]

The synthesis of the required tricyclic quinone precursor **15** was achieved using our efficient one-pot procedure described previously for the synthesis of phenanthridinquinones **8**. Thus, treatment of hydroquinones **5a**,**b**, aminouracil **14**, and silver (I) oxide in dichloromethane at room temperature yielded the correspond-



Scheme 1. Retrosynthetic route to angucyclinone 5-aza-analogues.

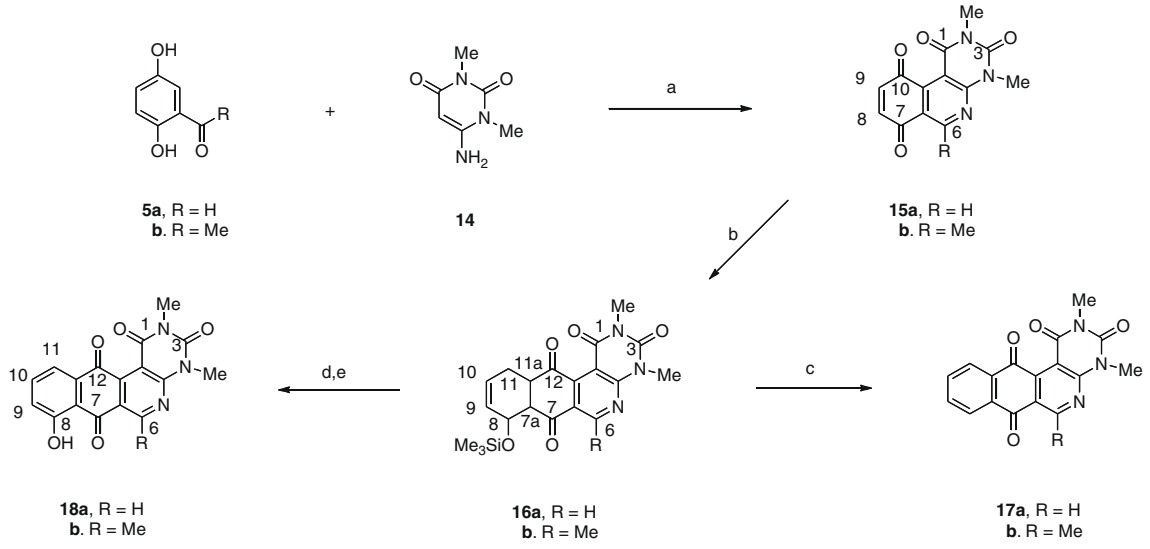


Scheme 2. Reagents and conditions: (a) Ag_2O, $MgSO_4$, CH_2Cl_2, rt; (b) $Me_3SiO{-}CH{=}CH{-}CH{=}CH_2$, CH_2Cl_2, rt; (c) HCl aq THF, H_2O, rt; (d) HCl aq THF, H_2O, 5 °C; (e) PCC, AcONa, CH_2Cl_2, rt; (f) $Me_2N{-}N{=}CH{-}C(Me){=}CH_2$ (**10**), MeCN, rt.

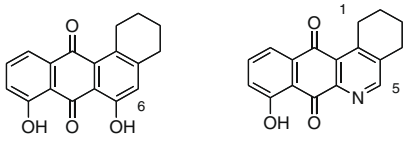
ing pyrimido[4,5-*c*]-isoquinolinequinones **15a** and **15b** in 61% and 86% yield, respectively. The cycloadditions of quinones **15** with the silyloxydiene were performed at room temperature to give the corresponding adducts **16a** and **16b** as the single detectable regioisomers in 99% and 65% yield, respectively (Scheme 3). The structures of these compounds were established unambiguously by 2D NMR techniques. Diels–Alder adducts **16a**,**b** were converted into the corresponding angucyclinone AB-pyrido[2,3-*d*]pyrimidine analogues **17a**,**b** in 63% and 72% yield by reaction with hydrochloric acid. On the other hand, mild hydrolysis of adducts **16a**,**b** with hydrochloric acid followed by oxidation of the alcohol intermediates with PCC gave the corresponding hydroxyquinones **18a**,**b** in 95% and 96% yield (Scheme 2).

3. Biological results and discussion

The newly synthesized angular tri- and tetracyclic quinones together with the angucyclinone aza-analogue **19**[35] and angucyclinone **20**,[36] (Fig. 2) compounds used as angucyclinone-type reference drugs (Fig. 2), were evaluated for in vitro anticancer activity against normal MRC-5 human lung fibroblasts and several human tumor cells: AGS gastric adenocarcinoma cell lines, SK-MES-1 lung cancer cells, J82 bladder carcinoma cells, and HL-60 leukemia cells in 72-h drug exposure assays. The cytotoxicity of the compounds was measured using a conventional microculture tetrazolium reduction assay.[37] The average IC_{50} values (μg/mL) are collected in Table 1.



Scheme 3. Reagents and conditions: (a) Ag_2O, $MgSO_4$, CH_2Cl_2, rt; (b) $Me_3SiO{-}CH{=}CH{-}CH{=}CH_2$, CH_2Cl_2, rt; (c) HCl aq THF, H_2O, rt; (d) HCl aq THF, H_2O, 5 °C; (e) PCC, AcONa, CH_2Cl_2, rt.



19 20

Figure 2. Structure of compounds **19** and **20**.

Comparison of the IC_{50} values obtained with the phenanthridinequinones **8a**,**b** and pyrimidoisoquinolinequinones **15a**,**b** clearly indicates that replacement of the cyclohexenone ring in **8a**,**b** by a uracil moiety decreases the antitumoral potency. Furthermore, the IC_{50} values obtained with compounds **8a**– **c** reveals that the introduction of a methyl group in the 6-position of the phenanthridinequinone chromophore, as in **8b**, induces an increase of the antitumor potency on SK-MES-1 and J82 cell lines. On the contrary, the introduction of a *gem*-dimethyl group into the 3-position of the chromophore, as in **8c**, markedly decreases the antitumor activity. Concerning compounds **15a**,**b**, the IC_{50} values indicates that the presence of a methyl group in the 6-position does not affect the potency of the pyrimidoisoquinolinequinone chromophore. Comparison of the IC_{50} values for angucyclinone derivative **19** and the angucyclinone 6-aza-analogue **20** indicates that the replacement of the CHOH group located in the 6-position of the benz[*a*]anthraquinone chromophore of **19** by a nitrogen atom induces an increase of the antitumor activity. Interestingly, comparison of the IC_{50} values for compounds **19**, **20,** and **13a** indicates that the introduction of a nitrogen atom into the B-ring and the functionalisation of the ring A of the benz[*a*]anthraquinone chromophore of **19** are apparently crucial. In fact, insertion of a nitrogen atom in the 5-position combined with the introduction of a carbonyl group in the 1-position of the benzophenanthridinequinone chromophore, as in **13a**, markedly enhanced the antitumor potential to reach low IC_{50} values of 1.2–11.3 μM.

Comparison of the IC_{50} values of the aza-analogues **12a** and **13a** reveals that removal of the hydroxyl group in **13a** induces a significant decrease of the cytotoxic potency. Furthermore, the potency of **13a** decreases when a methyl group is introduced into the 6-position of the benzo[*j*]phenanthridinequinone chromophore, as in compound **13b**. The cytotoxic potency of compounds **13b**–**d** compared to that of **13a** indicates that the presence of methyl groups in the 3- and/or 6-positions of rings A and B do not have a significant influence on the potency of the benzophenanthridinequinone chromophore.

The results obtained with **11**, an angucyclinone BD-dipyridoanalogue, was remarkable. In fact, compounds **11** exhibited the highest antitumor activity against all the cell lines, with IC_{50}-values in the range 1.2–2.4 μM, comparable to those shown by the reference drug etoposide (IC_{50} 0.36–3.9 μM). This indicates that the insertion of a nitrogen atom into the 8-position of the benzo[*j*]phenanthridinequinone chromophore increases dramatically the antitumor activity.

In relation to the members of the second series of angucyclinone analogues, the data of Table 1 indicate that replacement of the carbocyclic AB-rings of angucyclinone **19** by a pyridopyrimidine moiety enhanced the cytotoxic potency (i.e., compound **18a**) to reach IC_{50} values 5.9–15.9 μM. It is noteworthy that the **18a**,**b** members of this series of aza-analogues were found to be less active on the tested cancer cell lines compared to the members of the first series **13a**,**b**.

It is worth mentioning that comparison of the potency of congeners **17a,b** and **18a,b** indicate that insertion of a hydroxyl group in the 8-position does not improve the antitumor potency of the benzo[*g*]pyrimido[4,5-*c*]isoquinoline-1,3,7,12-tetraone pharmacophore. On the other hand, introduction of a methyl group at the 6-position of the chromophore induces a significative decrease of the antitumor potency, as in **17b**, or supression of the activity, as in **18b**.

The results arising from the biological evaluation of the *N*-isosters of angucyclinones reveals that replacement of the carbocyclic rings of the benz[*a*]anthraquinone chromophore by pyrido and pyridopyridazino moieties improves the anticancer potency of the carbocyclic chromophore. Therefore these new angucyclinone *N*-bioisosteres represent potential candidates for further in vivo evaluation studies.

4. Conclusions

In conclusion, we have described a simple and flexible strategy for preparing angucyclinone B, AB, and BD-aza-analogues. The reported synthesis involves easily available precursors and an efficient Michael addition-heterocyclization, Diels–Alder, and oxidative aromatization reaction sequence. By this procedure benzo[*j*]phenanthridine-1,7,12-trione; benzo[*g*]pyrimido[4,5-*c*]isoquinoline-1,3,7,12-tetraone, and pyrido[3,2-*b*]phenanthridine-1,7,12-trione derivatives were synthesized, showing the broad applicability of this synthetic method. Taking into the account the accessibility to acylhydroquinones by photo-Friedel–Crafts acylation of 1,4-quinones with aldehydes,[38,39] this approach may be used to make a variety of angucyclonone aza-analogues. Furthermore, the synthesized compounds exhibited remarkable antitumor activities in in vitro assays. Structure–activity relationships within the series of benzo[*j*]phenanthridine-1,7,12-trione and benzo[*g*]pyrimido[4,5-*c*]isoquinoline-1,3,7,12-tetraone revealed that methyl and hydroxy substituents in the 6- and 8-positions modify the potency of the corresponding chromophores. The members of the series of benzo[*j*]phenanthridine-1,7,12-trione; benzo[*g*]pyrimido[4,5-*c*]isoquinoline-1,3,7,12-tetraone and a pyrido[3,2-*b*]phenanthridine-1,7,12-trione exhibit moderate to high cytotoxic activity towards cancer cells, and therefore represent promising leads for the development of anticancer agents. Further experiments will be done to identify their physiological target and improve the potency and selectivity of both series of compounds.

5. Experimental

5.1. Chemistry

5.1.1. Materials and methods

All reagents were of commercial quality, reagent grade, and were used without further purification. Melting points (mp) were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. IR spectra were recorded on a Bruker vector 22-FT spectrophotometer using KBr discs, and wavenumbers are given in cm^{-1}. Proton nuclear magnetic resonance (^1H NMR) spectra were measured at 200 and 400 MHz on Bruker AM-200 and AM-400 spectrometers. Chemical shifts are expressed in ppm downfield relative to TMS (δ scale), and coupling constants (J) are reported in Hz. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were measured at 50 and 100 MHz on Bruker AM-200 and AM-400 spectrometers. Silica gel (70–230 and 230–400 mesh) and TLC aluminium foil 60 F254 (Merck, Darmstadt) were used for preparative column and analytical TLC, respectively. Tetracyclic quinones **19** and **20** were prepared by previously reported procedures.[35,36]

5.1.2. 3,4-Dihydro-2*H*-phenanthridine-1,7,10-trione (8a)

A suspension of **5a** (138 mg, 0.10 mmol), enaminone **6a** (114 mg, 1.05 mmol), Ag_2O (924 mg, 4.00 mmol), anhydrous $MgSO_4$ (0.5 g), and CH_2Cl_2 (20 mL) was stirred at rt for 4 h. The mixture was filtered and washed with CH_2Cl_2. Removal of the solvent under reduced pressure followed by column chromatography

Table 1
In vitro antitumor activity of angucyclinone *N*-analogues and their tricyclic precursors

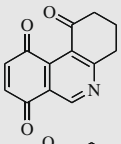
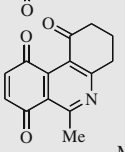
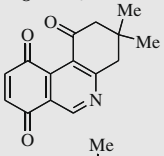
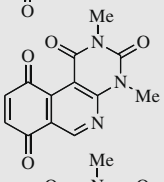
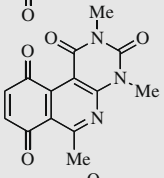
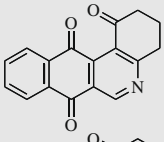
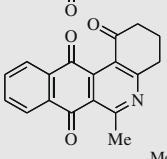
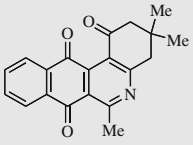
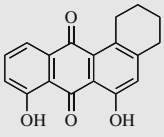
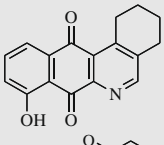
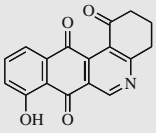
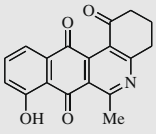
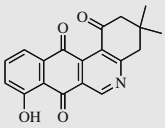
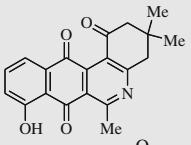
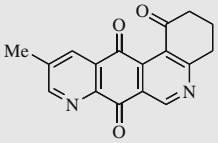
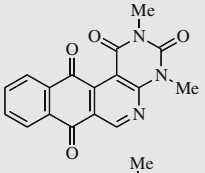
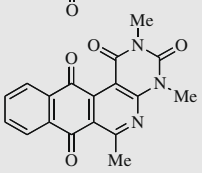
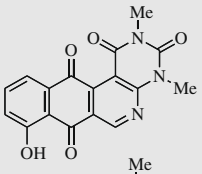
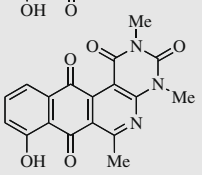
Compound	Structure	(IC_{50} ± SEM)[a] (μM)				
		MRC-5[b]	AGS[c]	SK-MES-1[d]	J82[e]	HL-60[f]
8a		20.2 ± 1.1	19.4 ± 1.0	25.7 ± 1.3	38.8 ± 1.7	15.8 ± 0.7
8b		20.5 ± 0.9	22 ± 1.3	3.5 ± 0.2	5.6 ± 0.3	46.5 ± 2.5
8c		39.9 ± 2.2	75.2 ± 3.9	>100	95.3 ± 4.4	43.6 ± 2.5
15a		72.8 ± 3.4	82.1 ± 4.4	67.5 ± 3.6	78.6 ± 3.9	31.5 ± 1.7
15b		67.1 ± 3.5	50.3 ± 2.7	70.2 ± 3.3	72.4 ± 3.8	26.3 ± 1.5
12a		41.3 ± 2.2	19.0 ± 0.9	51.6 ± 2.7	62.6 ± 3.4	73.2 ± 3.8
12b		21.5 ± 1.3	9.5 ± 0.5	11.9 ± 0.7	7.7 ± 0.3	45.2 ± 2.4
12d		15.9 ± 0.8	4.5 ± 0.3	13.5 ± 0.8	18.9 ± 0.9	17.5 ± 1.0
19		>100	41.6 ± 2.3	73.7 ± 3.5	69.9 ± 3.4	>100
20		63.6 ± 3.1	36.8 ± 1.7	36.8 ± 2.1	20.5 ± 1.2	45.8 ± 2.4
13a		11.3 ± 0.6	1.6 ± 0.1	3.7 ± 0.2	9.2 ± 0.6	5.6 ± 0.3
13b		17.3 ± 0.9	4.5 ± 0.3	10.2 ± 0.6	17.5 ± 0.7	23.9 ± 1.3

Table 1 (*continued*)

Compound	Structure	(IC_{50} ± SEM)[a] (μM)				
		MRC-5[b]	AGS[c]	SK-MES-1[d]	J82[e]	HL-60[f]
13c		6.5 ± 0.4	11.6 ± 0.5	3.6 ± 0.2	3.9 ± 0.3	2.3 ± 0.1
13d		5.5 ± 0.3	7.4 ± 0.4	4.6 ± 0.2	15.7 ± 0.7	11.8 ± 0.7
11		0.73 ± 0.04	1.2 ± 0.08	1.7 ± 0.1	1.8 ± 0.09	2.4 ± 0.1
17a		12.6 ± 0.7	3.5 ± 0.2	8.9 ± 0.5	8.7 ± 0.3	20.4 ± 1.1
17b		>100	19.1 ± 1.2	5.2 ± 0.3	24.9 ± 1.4	>100
18a		6.8 ± 0.2	5.9 ± 0.3	8.1 ± 0.5	9.2 ± 0.6	15.9 ± 0.7
18b		>100	70.0 ± 3.8	>100	>100	>100
	Etoposide	3.9 ± 0.21	0.36 ± 0.02	2.5 ± 0.15	2.8 ± 0.18	0.80 ± 0.04

[a] Data represent mean values (±SEM) for six independent determination.
[b] Human lung fibroblasts cells.
[c] Human gastric adenocarcinoma cell line.
[d] Human lung cancer cell line.
[e] Human bladder carcinoma cell line.
[f] Human leukemia cell line.

(CH_2Cl_2/EtOAc 60:40) yielded pure quinone **8a** (157 mg, 69% yield) as a yellow solid, mp 127–129 °C. IR (KBr): 1697, 1667 (C═O); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.26 (quint., J = 6.5 Hz, 2H, 3-H), 2.90 (t, J = 6.5 Hz, 2H, 2-H), 3.20 (t, J = 6.5 Hz, 2H, 4-H), 6.93 (d, J = 10.5 Hz, 1H, 8- or 9-H), 7.09 (d, J = 10.5 Hz, 1H, 9- or 8-H), 9.31 (s, 1H, 6-H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 21.4, 33.4, 39.1, 125.1, 127.8, 136.6, 136.8, 139.9, 150.7, 169.2, 183.4, 183.6, 197.1. Anal. Calcd for $C_{13}H_9NO_3$: C, 68.72; H, 3.99; N, 6.60. Found: C, 68.68; H, 3.91; N, 6.48.

5.1.3. 6-Methyl-3,4-dihydro-2*H*-phenanthridine-1,7,10-trione (8b)

The same procedure as for compound **8a**: Compound **5b** (152 mg, 1.00 mmol), enaminone **6a** (114 mg, 1.05 mmol), Ag_2O (924 mg, 4.00 mmol), anhydrous $MgSO_4$ (0.5 g), and CH_2Cl_2 (20 mL), reaction time 3 h, column flash chromatography (CH_2Cl_2/EtOAc 60:40). Compound **8b** was obtained as a yellow solid (188 mg, 78% yield), mp 134–136 °C. IR (KBr): 1705, 1665 (C═O); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.26 (quint., J = 6.5 Hz, 2H, 3-H), 2.86 (t, J = 6.5 Hz, 2H, 2-H), 2.96 (s, 3H, 6-CH_3), 3.11 (t, J = 6.5 Hz, 2H, 4-H), 6.85 (d, J = 10.5 Hz, 1H, 8- or 9-H,), 7.04 (d, J = 10.5 Hz, 1H, 9- or 8-H,); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 21.5, 26.2, 33.2, 39.1, 123.7, 126.9, 138.0 (2C), 138.6 (2C), 163.2, 167.1, 184.7, 197.5. Anal. Calcd for $C_{14}H_{11}NO_3$: C, 69.70; H, 4.60; N, 5.81. Found: C, 69.77; H, 4.60; N, 5.29.

5.1.4. 3,3-Dimethyl-2*H*-3,4-dihydrophenanthridine-1,7,10-trione (8c)

The same procedure as for compound **8a**: Compound **5a** (138 mg, 1.0 mmol), enaminone **6b** (139 mg, 1.0 mmol), Ag_2O (464 mg, 2 mmol), anhydrous $MgSO_4$ (400 mg), and CH_2Cl_2 (20 mL), reaction time 2 h. Column flash chromatography

(CH_2Cl_2/EtOAc 60/40). Compound **8c** was obtained as a yellow solid (178 mg, 70% yield), mp 126.5–127 °C. IR (KBr): 1694, 1678 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.16 (s, 6H, 3-2×Me), 2.78 (s, 2H, 2-H), 3.15 (s, 2H, 4-H), 6.93 (d, J = 10.5 Hz, 1H, 8- or 9-H), 7.10 (d, J = 10.5 Hz, 1H, 9- or 8-H), 9.32 (s, 1H, 6-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.7, 33.5, 47.6, 53.2, 125.0, 126.8, 136.6, 138.6, 140.0, 151.1, 167.7, 183.3, 183.6, 196.6. Anal. Calcd for $C_{15}H_{13}NO_3$: C, 70.58; H, 5.13; N, 5.49. Found: C, 70.38; H, 5.23; N, 5.32.

5.1.5. 3,3,6-Trimethyl-2*H*-3,4-dihydrophenanthridine-1,7,10-trione (8d)

Same procedure as for compound **8a**: compound **5b** (152 mg, 1.0 mmol), 3-amino-5,5-dimethyl-2-cyclohexen-1-one **6b** (139 mg, 1.05 mmol), Ag_2O (924 mg, 4.0 mmol), anhydrous $MgSO_4$ (420 mg), and CH_2Cl_2 (20 mL), reaction time 2 h. Column flash chromatography (CH_2Cl_2/EtOAc 60:40). Compound **8d** was obtained as a yellow solid (175 mg, 60% yield), mp 131–132 °C. IR (KBr):1668, 1610 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.15 (s, 6 H, 2×Me), 2.75 (s, 2H, 2-H), 2.97 (s, 3 H, 6-Me), 3.06 (s, 2H, 4-H), 6.86 (d, J = 10.5 Hz, 1H, 8- or 9-H), 7.05 (d, J = 10.5 Hz, 1H, 9- or 8-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.2, 28,3, 33.5, 47.5, 53.3, 123.6, 126.1, 139.0, 138.5, 141.6, 163.5, 165.7, 184.8, 184.9, 197.0. Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.22; H, 5.50; N, 5.19.

5.1.6. 8-(Trimethylsilyloxy)-3,4,7a,8,11,11a-hexahydro-2*H*-benzo[*j*]phenanthridine-1,7,12-trione (9a)

A solution of quinone **8a** (265 mg, 1.17 mmol), 1-trimethylsilyloxy-1,3-butadiene (199 mg; 1.40 mmol), and CH_2Cl_2 (20 mL), was left at rt for 24 h. The mixture was evaporated at reduced pressure and the residue was column chromatographed (CH_2Cl_2/AcOEt 90:10) to give adduct **9a** (232 mg, 54% yield) as a yellow oil, used without further purification. IR (KBr): 1731, 1693 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ *2.20–2.40* (m, 3H, 3- and 11-H), 2.60–2.80 (m, 1H, 11-H′), 2.85–3.25 (m, 4H, 2- and 4-H), 3.35 (dd, J = 3.9; 3.9 Hz, 1H, 7a-H), 3.68 (dd, J = 6.5; 6.5 Hz, 1H,11a-H), 4.43 (dd, J = 3.9-3.9 Hz, 1H, 8-H), 5.7 (m, 1H, 9- or 10-H), 5.9 (m, 1H, 10- or 9-H), 9.22 (s,1H, 6-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.6, 22.4, 33.2, 38.9, 43.7, 54.5, 65.7, 126.1, 127.5, 128.9, 130.3, 145.4, 151.1, 168.4, 194.3, 197.3, 197.5.

5.1.7. 6-Methyl-8-(trimethylsilyloxy)-3,4,7a,8,14,11a-hexahydro-2*H*-benzo[*j*] phenanthridine-1,7,12-trione (9b)

Same procedure as for **9a:** quinone **8b (**160 mg, 0.66 mmol), 1-trimethylsilyloxy-1,3-butadiene (113 mg, 0.80 mmol), and CH_2Cl_2 (20 mL), reaction time 24 h. Adduct **9b** was obtained as a yellow oil (105 mg, 42% yield) used without further purification. IR (KBr): 1729, 1693 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ *2.20–2.40* (m, 3H, 3- and 11-H), 2.40–2.65 (m, 1H, 11-H′), 2.84 (s, 3H, Me), 2.80–3.20 (m, 4H, 2- and 4-H), 3.25 (dd, J = 4.0; 4.0 Hz, 1H, 7a-H), 3.64 (dd, J = 6.6; 6.6 Hz, 1H, 11a-H), 4.35 (dd, J = 4.1; 4.1 Hz, 1H, 8-H), 5.7 (m, 1H, 9- or 10-H), 5.9 (m, 1H, 10- or 9-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.4, 22.3, 26.5, 33.2, 39.1, 43.6, 55.0, 65.7, 126.9, 128.7, 137.9, 138.6, 147.9, 163.3, 166.1, 195.0, 197.9, 198.4.

The structure of compounds **9** were assigned on the basis of the HMQC/HMBC experiments. The HMBC spectrum of **9a** shows $^2J_{C,H}$ coupling between the C-12 carbon (δ 195.0 ppm) with the protons at C-11a (δ 3.64 ppm) and $^4J_{C,H}$ interaction for the C-7 carbon (δ 197.9 ppm) with the protons of the methyl group at C-6 (δ 2.84 ppm).

5.1.8. 8-Trimethylsilyloxy-3,3-dimethyl-2*H*-3,4,7a,8,11,11a-hexahydro-benzo[*j*]phenanthridine-1,7,12-trione (9c)

Same procedure as for **9a:** quinone **8c** (144 mg, 0.53 mmol) 1-trimethylsilyloxy-1,3-butadiene (91 mg, 0.64 mmol), and CH_2Cl_2 (20 mL), reaction time 24 h. Adduct **9c** was obtained as a yellow oil (218 mg, 99% yield) used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ *−0.30* (s, 9H, 8-$OSiMe_3$), 1.03 (s, 3H, 3-Me), 1.17 (s, 3H, 3-Me′), 2.14 (m, 1H, 11-Ha), 2.49 (d, J = 15.1 Hz, 1H, 4-H), 2.86 (d, J = 15.1 Hz, 1H, 4-H′), 3.01 (m, 1H, 11-Hb), 3.05 (s, 1H, 2-H), 3.07 (s, 1H, 2-H′), 3.31 (m, 1H, 7a-H), 3.67 (t, J = 6.5, 6.5 Hz, 1H, 11a-H), 4.42 (t, J = 4.3, 4.3 Hz, 1H, 8-H), 5.71 (m, 1H, 10-H), 5.92 (m, 1H, 9-H), 9.21 (s, 1H, 6-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ *−0.48,* 22.3, 27.5, 29.5, 33.2, 43.7, 47.3, 52.9, 54.5, 65.6, 126.0, 126.5, 128.9, 130.2, 145.3, 151.4, 166.7, 194.1, 196.9, 197.2. Anal. Calcd for $C_{22}H_{27}NO_4Si$: C, 66.47;H, 6.85; N, 3.52. Found: C, 66.02; H, 7.22; N, 3.17.

5.1.9. 8-Trimethylsilyloxy-3,3,6-trimethyl-2*H*-3,4,7a,8,11,11a-hexahydro-benzo[*j*]phenanthridine-1,7,12-trione (9d)

Same procedure as for **9a:** Quinone **8d** (145 mg, 0.51 mmol) 1-trimethylsilyloxy-1,3-butadiene (79 mg, 0.55 mmol), and CH_2Cl_2 (20 mL), reaction time 24 h. Adduct 9 d was obtained as a yellow oil (140 mg, 65% yield) used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ *−0.28* (s, 9H, 8-$OSiMe_3$), 1.02 (s, 3H, 3-Me), 1.17 (s, 3H, 3-Me′), 2.12 (m, 1H, 11-H), 2.45 (d, J = 15.0 Hz, 1H, 4-H), 2.85 (d, J = 15.0 Hz, 1H, 4-H′), 2.90 (s, 3H, 6-Me), 2.97 (m, 1H, 11-H′), 2.98 (s, 1H, 2-H), 3.00 (s, 1H, 2-H′), 3.28 (dd, J = 4.3, 6.2 Hz, 1H, 7a-H), 3.70 (t, J = 6.7, 6.7 Hz, 1H, 11a-H), 4.41 (t, J = 4.6, 4.6 Hz, 1H, 8-H), 5.71 (m, 1H, 10-H), 5.91 (m, 1H, 9-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ *−0.42,* 22.2, 26.5, 27.5, 29.6, 33.2, 43.5, 47.1, 52.9, 54.9, 65.6, 125.7, 126.1, 128.6, 128.7, 147.8, 163.6, 164.4, 194.9, 197.4, 198.3. Anal. Calcd for $C_{23}H_{29}NO_4Si$: C, 67.12; H, 7.10; N, 3.40. Found: C, 66.56; H, 7.64; N, 3.07.

5.1.10. 10-Methyl-3,4,7a,8,11,11a-hexahydro-2*H*-5,8-diazabenzo[*a*]anthracene-1,7,12-trione (11)

A solution of quinone **8a** (74 mg, 0.33 mmol), azadiene **10**[24] (44 mg, 3.93 mmol), Ac_2O (0.1 mL) and MeCN (20 mL) was left in darkness at room temperature for 72 h. The mixture was evaporated under reduced pressure and the residue was column chromatographed (CH_2Cl_2/EtOAc 60:40) to give compound **11** (65 mg, 53% yield) as a red solid, mp 176–178 °C. IR (KBr): 1702, 1685 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.30 (quint., J = 6.5 Hz, 2H, 3-H), 2.60 (s, 3H, Me), 2.97 (t, J = 6.5 Hz, 2H, 2-H), 3.27 (t, J = 6.5 Hz, 2H, 4-H), 8.30 (d, J = 2.1 Hz,1H, 11-H), 9.20 (d, J = 2.1 Hz, 1H, 9-H), 9.61 (s, 1H, 6-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.0, 21.5, 33.3, 39.1, 126.2, 128.3, 131.7, 135.3, 139.6, 140.1, 145.4, 152.1, 156.3, 169.4, 180.3, 182.6, 197.3. Anal. Calcd for $C_{17}H_{12}N_2O_3$: C, 69.86; H, 4.14; N, 9.58. Found: C, 69.82; H, 4.10; N, 9.53.

The HMBC spectrum of **11** shows $^3J_{C,H}$ couplings between the C-12 carbon (δ 180.3 ppm) with the proton at C-11 (δ 8.30 ppm) and for the C-7 carbon (δ 182.6 ppm) with the proton at C-6 (δ 9.61 ppm).

5.1.11. 3,4-Dihydro-2*H*-benzo[*j*]phenanthridine-1,7,12-trione (12a)

To a stirred solution of **9a** (52 mg, 0.13 mmol) in THF (20 mL) was added hydrochloric acid (30%, 4 drops) and the mixture was left at rt for 1 h. It was then diluted with water, neutralized with aqueous $NaHCO_3$, and extracted with CH_2Cl_2. The extract was washed with water (3 × 10 mL) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was column chromatographed (CH_2Cl_2/EtOAc 60:40) to give **12a** (23 mg, 63% yield), mp 213-216 °C. IR (KBr): 1697, 1680,1664 (C=O);[1] H NMR ($CDCl_3$, 400 MHz): δ 2.28 (quint., J = 6.5 Hz, 2H, 3-H), 2.93 (t, J = 6.5 Hz, 2H, 2-H), 3.21 (t, J = 6.5 Hz, 2H, 4-H), 7.80–8.26 (m, 4H, arom.), 9.48 (s,1H, 6-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.5, 33.3, 39.1, 126.6, 126.9, 127.6, 128.6, 132.2, 134.5, 134.7, 134.8, 140.9, 151.6, 169.0, 181.8, 182.8, 197.7. Anal. Calcd for $C_{17}H_{11}NO_3$: C, 73.64; H, 4.00; N, 5.05. Found: C, 73.58; H, 4.07; N, 5.10.

5.1.12. 6-Methyl-3,4-dihydro-2*H*-benzo[*j*]phenanthridine-1,7,12-trione (12b)

Same procedure as for **12a**: adduct **9b** (187 mg, 0.47 mmol), THF (20 mL), hydrochloric acid (30%, 7 drops), reaction time 1 h. Column chromatography (CH_2Cl_2/EtOAc 60:40). Compound **12b** was obtained as a yellow solid (99 mg, 72% yield), mp 188 °C (d). IR (KBr): 1692, 1671 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz: δ 2.26 (quint., J = 6.5 Hz, 2H, 3-H), 2.89 (t, 2H, J = 6.5 Hz, 2-H), 3.05 (s, 3H, Me), 3.12 (t, 2H, J = 6.5 Hz, 4-H), 7.77–8.17 (m, 4H, arom.); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 21.5, 26.9, 33.1, 39.1, 126.7, 127.0, 127.6, 133.6, 134.0, 134.1, 134.4, 135.2, 144.1, 164.6, 166.8, 183.3, 184.2, 198.0. Anal. Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50; N, 4.81. Found: C, 74.43; H, 4.38; N, 4.80.

5.1.13. 3,3-Dimethyl-3,4-dihydro-2*H*-benzo[*j*]phenanthridine-1,7,12-trione (12c)

Same procedure as for **12a**: Adduct **9c** (64 mg, 0.15 mmol), THF (20 mL), hydrochloric acid (30%, 0.2 mL), reaction time 1 h. Column chromatography (CH_2Cl_2/AcOEt 90:10). Compound **12c** was isolated as a yellow solid (44.3 mg, 89% yield), mp 129–131 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.18 (s, 6H, 3-2 × Me), 2.82 (s, 2H, 2-H), 3.17 (s, 2H, 4-H), 7.82–8.20 (m, 4H, arom.), 9.48 (s, 1H, 6-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 28.7, 33.5, 47.4, 53.2, 126.3, 126.6, 127.3, 127.4, 132.0, 134.2, 134.6, 134.7, 140.8, 151.8, 167.4, 181.6, 182.7, 196.9. Anal. Calcd for $C_{19}H_{15}NO_3$: C, 74.74; H, 4.95; N, 4.59. Found: C, 74.63; H, 4.66; N, 4.89.

5.1.14. 3,3,6-Trimethyl-3,4-dihydro-2*H*-benzo[*j*]phenanthridine-1,7,12-trione (12d)

Same procedure as for **12a**: Adduct **9d** (67 mg, 0.18 mmol), THF (20 mL), hydrochloric acid (30%, 0.2 mL), reaction time 1 h. Column chromatography (CH_2Cl_2/AcOEt 90:10) gave **12d** as a yellow solid (49 mg, 92% yield), mp 170.5–171.5 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.17 (s, 6H, 3-2 × Me), 2.79 (s, 2H, 2-H), 3.06 (s, 3H, 6-Me), 3.08 (s, 2H, 4-H), 7.78–8.16 (m, 4H, arom.); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.8, 28.8, 33.5, 47.4, 53.2, 125.0, 126.5, 126.6, 126.9, 133.5, 134.0, 134.1, 134.2, 144.1, 164.3, 165.3, 183.2, 184.3, 197.3. Anal. Calcd for $C_{20}H_{17}NO_3$: C, 75.22; N, 5.37; N, 4.39. Found: C, 75.10; H, 4.46; N, 5.22.

5.1.15. 8-Hydroxy-3,4-dihydro-2*H*-benzo[*j*]phenanthridine-1,7,12-trione (13a)

A solution of adduct **9a** (74 mg, 0.2 mmol), aqueous THF (THF/H_2O 90:10, 15 mL), and hydrochloric acid (5%, 0.16 mL) was left at rt for 90 min. The mixture was diluted with water (20 mL), neutralized with $NaHCO_3$, and extracted with CH_2Cl_2 (30 mL). The organic extract was washed with water (3 × 10 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (20 mL). The solution was added to a stirred suspension of PCC (0.4 g, 1.8 mmol), anhydrous AcONa (54 mg, 0.66 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred for 1 h at rt. The mixture was column chromatographed ($CHCl_3$/MeOH 90:10) to give pure **13a** as a yellow solid (39 mg, 67 % yield), mp 180–181 °C. IR (KBr): 3448 (O—H), 1702, 1680 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.32 (quint., J = 6.5 Hz, 2H, 3-H), 2.97 (t, J = 6.5 Hz, 2H, 2-H), 3.25 (t, J = 6.5 Hz, 2H, 4-H), 7.34–7.71 (m, 3H, arom.), 9.60 (s, 1H, 6-H), 12.10 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.4, 33.3, 39.0, 115.1, 120.0, 124.6, 126.5, 128.7, 134.5, 137.5, 141.7, 151.2, 162.2, 169.6, 182.0, 187.0, 197.5.

The spectral properties of **13a** are in full agreement to those reported in the literature.[19]

5.1.16. 8-Hydroxy-6-methyl-3,4-dihydro-2*H*-benzo[*j*]phenanthridine-1,7,12-trione (13b)

Same procedure as for **13a**: (a) Adduct **9b** (62 mg, 0.17 mmol), aqueous THF (THF: H_2O 90:10, 15 mL), hydrochloric acid (5%, 0.16 mL), reaction time 2 h. (b) PCC (0.4 g, 1.86 mmol), anhydrous AcONa (55 mg, 0.66 mmol) in CH_2Cl_2 (50 mL). Column chromatography ($CHCl_3$/MeOH 90:10) gave **13b** as a yellow solid (41 mg, 82% yield), mp 195–196 °C. IR (KBr): 3447 (O—H), 1707, 1675 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.26 (quint., J = 6.7 Hz, 2H, 3-H), 2.89 (t, J = 6.7 Hz, 2H, 2-H), 3.06 (s, 3H, Me), 3.13 (t, 2H, J = 6.7 Hz, 4-H), 7.28-7.60 (m, 3H, arom.), 12.33 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.5, 27.3, 33.1, 39.0, 114.6, 116.0, 119.0, 125.0, 127.9, 134.0, 136.8, 144.2, 162.0, 164.0, 167.3, 183.3, 189.0, 197.6. Anal. Calcd for $C_{18}H_{13}NO_4$: C, 70.35; H, 4.26; N, 4.56. Found: C, 70.00; H, 4.20; N, 4.34.

5.1.17. 3,3-Dimethyl-8-hydroxy-1,2,3,4-tetrahydro-1*H*-benzo[*j*]phenanthridine-1,7,12-trione (13c)

Same procedure as for **13a**: (a) adduct **9c** (72 mg, 0.17 mmol), hydrochloric acid (5%, 0.2 mL) and aqueous THF (THF–H_2O 90:10, 10 mL), reaction time 90 min. (b) PCC (0.4 g, 1.86 mmol), anhydrous AcONa (63.2 mg, 0.66 mmol), CH_2Cl_2 (30 mL). Column chromathography (CH_2Cl_2/EtOAc 90:10) gave compound **13c** as a yellow solid (49.5 mg, 83% yield), mp 186.5 °C (d). ^{1}H NMR (400 MHz, $CDCl_3$,): δ 1.17s, 3H, Me, 1.19s, 3H, Me′), 2.81 (s, 3H, 2-H), 3.17 (s, 2H, 4-H), 7.31-7.69 (m, 3H, arom.), 9.52 (s, 1H, 6-H), 12.11 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.8, 33.6, 47.7, 53.3, 115.2, 120.0, 124.5, 126.4, 127.8, 134.8, 137.5, 141.3, 151.6, 162.2, 168.2, 187.1, 196.9. Anal. Calcd for $C_{19}H_{15}NO_4$: C, 71.02; H, 4.71; N, 4.36. Found: C, 70.76; N, 4.89; N, 4.30.

5.1.18. 3,3,6-Trimethyl-8-hydroxy-1,2,3,4-tetrahydro-1*H*-benzo[*j*]phenanthridine-1,7,12-trione (13d)

Same procedure as for **13a**: (a) Adduct **9c** (105 mg, 0.25 mmol), HCl (5%, 3 drops,), aqueous THF (THF/H_2O 90:10. 6 mL), reaction time 1 h. (b) PCC (412 mg, 1.91 mmol), anhydrous AcONa (95 mg, 1.16 mmol), CH_2Cl_2 (30 mL), reaction time 90 min. Column chromatography (CH_2Cl_2/EtOAc 90:10) gave **13d** as an orange solid (65.5 mg, 78% yield), mp 166–167 °C (d). IR (KBr): 3449 (O—H), 1708 (s), 1678 (s), 1636 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$,): δ 1.15 (s, 6H, 3-2Me), 2.77 (s, 2H, 2-H), 3.07 (s, 5H, 4-H + 6-Me), 7.28–7.60 (m, 3H, arom.), 12.30 (s, 1H, OH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 27.33, 28.80, 47.5, 53.3, 116.1, 119.0, 124.5, 125.0, 127.0, 134.3, 136.8, 144.4, 162.0, 164.5, 166.0, 183.6, 189.0, 197.0. Anal. Calcd for $C_{20}H_{17}NO_4$: C, 71.63; H, 5.11; N, 4.18. Found: C, 71.45; H, 4.94; N, 4.21.

5.1.19. 2,4-Dimethyl-2*H*,4*H*-pyrimido[4,5-*c*]-isoquinoline-1,3,7,10-tetraone (15a)

A suspension of **5a** (138 mg, 1 mmol), 6-amino-1,3-dimethyluracil **14** (155 mg, 1 mmol), Ag_2O (695 mg, 3 mmol mmol), $MgSO_4$ (300 mg), and CH_2Cl_2 (20 mL) was vigorously stirred at rt for 3 h. The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was flash chromatographed (CH_2Cl_2/AcOEt 90:10) to give pure **15a** (166 mg, 61% yield) as yellow crystals, mp 203.5–205.5 °C (d). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.51 (s, 3H, 4-Me), 3.79 (s, 3H, 2-Me), 6.88 (d, J = 10.5 Hz, 1H, 8-H), 7.15 (d, J = 10.5 Hz, 1H, 9-H), 9.30 (s, 1H, 6-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.1, 30.5, 106.3, 122.6, 136.3, 140.6, 142.9, 150.8, 153.1, 154.8, 158.2, 182.1, 182.8. Anal. Calcd for $C_{13}H_9N_3O_4$: C, 57.57; H, 3.34; N, 15.49. Found: C, 57.56; H, 3.00; N, 15.41.

5.1.20. 2,4,6-Trimethyl-2*H*,4*H*-pyrimido[4,5-*c*]-isoquinoline-1,3,7,10-tetraone (15b)

Same procedure as for **15a:** Compound **5b** (152 mg, 1 mmol), 6-amino-1,3-dimethyluracil **14** (155 mg, 1 mmol), Ag_2O (928 mg, 4 mmol), $MgSO_4$ (300 mg), and CH_2Cl_2 (20 mL), reaction time 1 h. Flash chromatography on silica gel (CH_2Cl_2/EtOAc 90:10). Compound **15b** was isolated as yellow crystals (244 mg, 86% yield),

mp 197.5–198.5 °C (d). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.99 (s, 3H, 2-Me), 3.47 (s, 3H, 4-Me), 3.75 (s, 3H, 6-Me), 6.83 (d, J = 10.5 Hz, 1H, 8- or 9-H), 7.13 (d, J = 10.5 Hz, 1H, 9- or 8-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.6, 28.9, 30.1, 105.2, 121.1, 138.1, 138.4, 145.8, 150.9, 152.3, 158.3, 166.2, 183.4, 184.2. Anal. Calcd for $C_{14}H_{11}N_3O_4$: C, 58.95; H, 3.89; N, 14.73. Found: C, 58.96; H, 3.69; N, 14.61.

5.1.21. 8-Trimethylsilyloxy-2,4-dimethyl-7a,8,11,11a-tetrahydro-benzo[*g*]pyrimido[4,5-*c*]isoquinoline-1,3,7,12-tetraone (16a)

A solution of quinone **15a** (144 mg, 0.53 mmol), 1-trimethylsilyloxy-1,3-butadiene (91 mg, 0.64 mmol), and CH_2Cl_2 (20 mL), was left at rt for 24 h in darkness. The mixture was evaporated under reduced pressure and the residue triturated with *n*-hexane (4 × 20 mL) to give adduct **16a** (218 mg, 99% yield), mp 158.9 °C (d). ^{1}H NMR (400 MHz, $CDCl_3$): δ *−0.23* (s, 9H, $OSiMe_3$), 2.19 (m, 1H, 11-H), 3.05 (m, 1H, 11-H′), 3.35 (m, 1H, 11a-H), 3.45 (s, 3H, 4-Me), 3.77 (m, 1H, 7a-H), 3.80 (s, 3H, 2-Me), 4.52 (m, 1H, 8-H), 5.81 (m, 1H, 9-H), 6.02 (m, 1H, 10-H), 9.27 (s, 1H, 6-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ *−0.30,* 22.4, 28.9, 30.4, 44.2, 54.6, 65.8, 105.6, 125.9, 127.5, 128.8, 149.1, 150.93, 153.6, 154.1, 158.6, 193.4, 195.8. Anal. Calcd for $C_{20}H_{23}N_3O_5Si$: C, 58.09; H, 5.61; N, 10.16. Found: C, 57.90; H, 5.42; 9.97.

5.1.22. 8-Trimethylsilyloxy-2,4,6-trimethyl-7a,8,11,11a-tetrahydro-benzo[*g*]pyrimido[4,5-*c*]isoquinoline-1,3,7,12-tetraone (16b)

Same procedure described for **16a**: Adduct **15b** (145 mg, 0.51 mmol), 1-trimethylsilyloxy-1,3-butadiene (79 mg, 0.55 mmol), CH_2Cl_2 (20 mL), reaction time 24 h. Adduct **16b** was obtained in 65% yield (140.5 mg), mp 161.5–162.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ *−0.21* (s, 9H, $OSiMe_3$), 2.18 (m, 1H, 11-H), 2.96 (s, 3H, 6-Me), 3.04 (m, 1H, 11-H′), 3.32 (m, 1H, 11a-H), 3.43 (s, 3H, 4-Me), 3.74 (s, 3H, 2-Me), 3.76 (m, 1H, 7a-H), 4.50 (m, 1H, 8-H), 5.76 (m, 1H, 9-H), 6.00 (m, 1H, 10-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ −0.2, 22.3, 27.0, 28.8, 30.0, 43,80, 54.9, 65.9, 104.7, 126.0, 126.1, 128.7, 151.2, 151.5, 151.7, 158.9, 166.6, 194.4, 196.9. Anal. Calcd for $C_{21}H_{25}N_3O_5Si$: C, 59.00; H, 5.89; N, 9.83. Found: C, 59.21; H, 5.90; N, 9.71.

5.1.23. 2,4-Dimethyl-*2H*,*4H*-benzo[g]pyrimido[4,5-*c*]isoquinoline-1,3,7,12-tetraone (17a)

A solution of adduct **16a** (64 mg, 0.15 mmol), hydrochloric acid (30%, 0.2 mL) and THF (10 mL) was left at rt for 1 h. The mixture was diluted with water (20 mL), neutralized with $NaHCO_3$ and the solution was extracted with CH_2Cl_2 (30 mL). The organic extract was washed with water (3 × 10 mL), dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was column chromatographed (CH_2Cl_2/EtOAc 90:10) to give pure **17a** (44 mg, 89% yield) as a yellow solid; mp 245–246 °C. **).** ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.51 (s, 3H, 4-Me), 3.80 (s, 3H, 2-Me), 7.83 (m, 2H, 9- and 10-H), 8.16 (m, 1H, 8-H), 8.22 (m, 1H, 11-H), 9.51 (s, 1H, 6-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.1, 30.5, 106.6, 123.9, 126.7, 127.3, 132.1, 134.2, 134.8, 135.9, 145.4, 150.8, 154.0, 154.7, 158.4, 180.6, 182.8. Anal. Calcd for $C_{17}H_{11}N_3O_4$: C, 63.55; H, 3.45; N, 13.08. Found: C, 63.43; H, 3.51; N, 13.10.

5.1.24. 2,4,6-Trimethyl-2*H*,4*H*-benzo[*g*]pyrimido[4,5-*c*]isoquinoline-1,3,7,12-tetraone (17b)

Same procedure as for 17a: Adduct **16b** (67 mg, 0.18 mmol), hydrochloric acid (3%, 0.2 mL), THF (10 mL). Column chromatography (CH_2Cl_2/EtOAc 90:10). Compound **17b** was isolated as a yellow solid (49 mg, 93% yield); mp 251–252 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.10 (s, 3H, 6-Me), 3.49 (s, 3H, 4-Me), 3.77 (s, 3H, 2-Me), 7.79 (m, 2H, 9- and 10-H), 8.09 (m, 1H, 8-H), 8.15 (m, 1H, 11-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.4, 29.0, 30.2, 105.6, 122.7, 126.4, 126.9, 133.5, 134.1, 135.9, 148.5, 151.1, 152.4, 158.7, 167.1, 182.1, 184.5. Anal. Calcd for $C_{18}H_{13}N_3O_4$: C, 64.47; H, 3.91; N, 12.53. Found: C, 64.55; H, 3.56; N, 12.47.

5.1.25. 8-Hydroxy-2,4-dimethyl-2*H*,4*H*-benzo[*g*]pyrimido[4,5-*c*]isoquinoline-1,3,7,12-tetraone (18a)

A solution of adduct **18a** (72 mg, 0.17 mmol), aqueous THF (THF/H_2O 90/10, 10 mL), and hydrochloric acid (5%, 0.2 mL) was left at rt for 1.5 h. The mixture was diluted with water (20 mL), neutralized with $NaHCO_3$, and extracted with CH_2Cl_2 (30 mL). The organic extract was washed with water (3 × 10 mL) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (10 mL). The solution was added to a stirred suspension of PCC (0.4 g, 1.86 mmol), AcONa (63 mg, 0.66 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred at rt for 1 h and then column flash chromatographed (CH_2Cl_2:AcOEt 90:10) to give pure **18a** (50 mg, 83% yield) as a yellow solid; mp 271.5–272.5 °C (d). NMR (400 MHz, $CDCl_3$): δ 3.53 (s, 3H, 4-Me), 3.81 (s, 3H, 2-Me), 7.33 (dd, J = 7.5, 2.1 Hz, 1H, 9-H), 7.70 (m, 2H, 10- and 11-H), 9,54 (s, 1H, 6-H), 12.05 (s, 1H, 8-OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.8, 33.6, 47.7, 53.3, 53.3, 115.2, 120.0, 124.5, 126.4, 127.8, 134.8, 137.5, 141.3, 151.6, 162.2, 168.2, 187.1, 196.9. Anal. Calcd for $C_{17}H_{11}N_3O_5$: C, 60.54; H, 3.29; N, 12.46. Found: C, 60.46; H, 3.00; N, 12.38.

5.1.26. 8-Hydroxy-2,4,6-trimethyl-2*H*,4*H*-benzo[*g*]pyrimido[4,5-*c*]isoquinoline-1,3,7,12-tetraone (18b)

Same procedure as for **18a**: (a) adduct **16b** (65 mg, 0.15 mmol), aqueous THF (THF/water 90:10, 10 mL), and hydrochloric acid (5%, 1.4 mL); (b) PCC (0.4 g, 1.86 mmol), AcONa (63 mg, 0.66 mmol) in CH_2Cl_2 (20 mL). Flash chromatography (CH_2Cl_2/AcOEt 90:10) yielded **18b** as a yellow solid (49 mg, 92% yield); mp 232–234 °C (d). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.13 (s, 3H, 2-Me), 3.50 (s, 3H, 4-Me), 3.79 (s, 3H, 6-Me), 7.30–7.60 (m, 3H, 9-, 10-and 11-H) ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.8, 29.1, 30.2, 105.9, 115.9, 118.8, 122.5, 124.3, 135.2, 136.8, 148.9, 151.0, 152.6, 158.5, 161.9, 167.3, 183.8, 187.6. Anal. Calcd for $C_{18}H_{13}N_3O_5$: C, 61.54; H, 3.73; N, 11.96. Found: C, 61.77; H, 3.25; N, 11.89.

5.2. Biology: in vitro assays[39]

The cell lines used in this work were obtained from the American Type Culture Collection (ATCC, Manasas, VA, USA). They included MRC-5 normal human lung fibroblasts (CCL-171), AGS human gastric adenocarcinoma cells (CRL-1739), HL-60 human leukemia cells (CCL-240), SK-MES-1 human lung cancer cells (HTB-58), and J82 human bladder carcinoma cells (HTB-1). Cells were grown in the following media: MRC-5, SKMES- 1, and J82 in MEM; AGS cells in Ham F-12; and HL-60 in RPMI. The MEM medium was supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, and 1.5 g/L sodium hydrogencarbonate. RPMI contained 1 mM sodium pyruvate and 2 g/L sodium hydrogencarbonate. All media were supplemented with 10% heat-inactivated FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin in a humidified incubator with 5% CO_2 in air at 37 °C. For the experiments, cells were plated at a density of 50,000 cells/mL in 96-well plates. One day after seeding, the cells were treated with the medium containing the compounds at concentrations ranging from 0 to 100 μM for 3 days, and finally the MTT reduction assay was carried out. The compounds were dissolved in DMSO (1% final concentration) and complete medium. Untreated cells were used as controls. Each experiment was carried out in sextuplicate. Etoposide, used as a positive control, was tested in the same way.

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