Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 2845–2853

BIOORGANIC & MEDICINAL CHEMISTRY

# Synthesis and Cytotoxic Evaluation of Analogues of the Marine Pyridoacridine Amphimedine

Christine Brahic,[a] Francis Darro,[b] Mirabelle Belloir,[a] Jean Bastide,[a] Robert Kiss[c] and Evelyne Delfourne[a,*]

[a]*Centre de Phytopharmacie, UMR-CNRS 5054, Université de Perpignan, 52 Avenue de Villeneuve, 66860 Perpignan Cedex, France*

[b]*Laboratoire Louis Lafon, Centre de Recherches, 19 avenue du Pr Cadiot, BP 22, 94701 Maisons-Alfort Cedex, France*

[c]*Laboratoire d'Histopathologie, Faculté de Médecine, Université Libre de Bruxelles, 808 Route de Lennik, 1070 Brussels, Belgium*

Received 15 March 2002; accepted 7 May 2002

**Abstract**—4-Substituted-7H-pyrido-[4,3,2-*de*][1,8] or [1,9]-phenanthroline-7-ones and 9-methyl-1,4-diazanaphtacene-3,10-dione, analogues of the marine pyridoacridine amphimedine were synthesised from isoquinoline-5,8-dione. The first compounds were obtained starting from a Diels–Alder reaction whereas the synthesis of the last compound was initiated by a reaction of condensation with 2-aminoacetophenone. The different tetra- and pentacyclic compounds were evaluated for in vitro cytotoxic activities against six distinct human cancer cell lines. All the compounds exhibit cytotoxic activity with $IC_{50}$ values (i.e., the drug concentration inhibiting the mean growth value of the six cell lines by 50%) $< 10^{-7}$ M for two of them. 

## Introduction

The pyridoacridines form an important class of marine metabolites, many of which exhibit significant cytotoxic activity.[1] Amphimedine **1**, isolated in 1983 from an *Amphimedon* sp. sponge, was the first example of this type of alkaloid from a marine organism.[2] Recently, Ireland's group published the isolation of two structurally related compounds, neoamphimedine **2**[3] and deoxyamphimedine **3**[4] (Fig. 1).

Three total synthesis of amphimedine[5] have been reported in literature, with the first ones based on an aza-Diels–Alder reaction involving a 5,8-quinolinedione derivative and a 2-aza-diene. This Diels–Alder strategy was also employed in the synthesis of the marine metabolites meridine[6] **4** or cystodamine[7] **5**, the diene being in these cases a 1-aza-diene. To our knowledge, only a limited number of studies related to aza-Diels–Alder reactions with isoquinoline-5,8-dione have been reported so far.[8] Diels–Alder cycloadditions between 1-aza-dienes and isoquinoline-5,8-dione constitute a good access to diaza-1,7- or 1,8-anthracenediones. More generally, isoquinolinedione could be a good starting material in synthetic routes to derivatives of amphimedine.

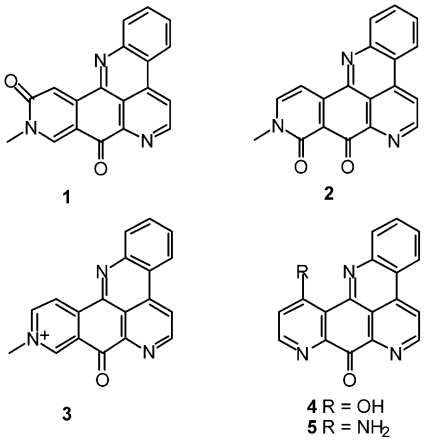



**Figure 1.**

*Corresponding author. Tel.: +33-468-66-2251; fax: +33-468-66-2223; e-mail: delfourn@univ-perp.fr

0968-0896/02/$ - see front matter 
PII: S0968-0896(02)00148-7

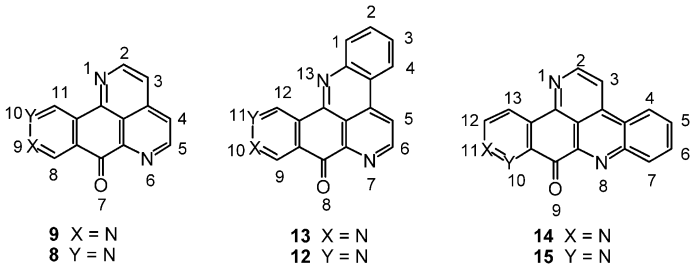



**Figure 2.**

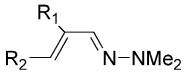
As part of our ongoing programme to investigate new anticancer drugs,[9] we survey compounds comprising the 7H-pyrido[4,3,2-*de*][1,8 or 1,9-phenanthroline]-7-one moiety and give the first account of their biological properties (Fig. 2).

## Chemistry

The tetra- and pentacyclic compounds **8**, **9**, **12** and **13** were considered according to the retrosynthetic analysis outlined in Scheme 1.

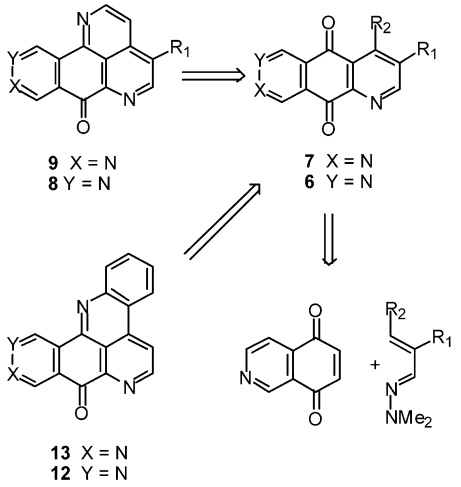
The diazaanthraquinones **6** and **7** would be key intermediates prepared by Diels–Alder reaction between isoquinoline-5,8-dione and various 1-aza-dienes. The different dienes involved in these reactions, that is crotonaldehyde-*N*,*N*-dimethylhydrazone,[10] 2-methoxy-2-butenal-*N*,*N*-dimethyl hydrazone,[11] 2-fluoro-2-butenal-*N*,*N*-dimethylhydrazone[12] and 4-(2′-trifluoroacetamidophenyl)-1-dimethylamino-1-azadiene[13] were synthesised according to procedures described previously. The [4+2] cycloadditions between isoquinoline-5,8-dione and 1-azadienes were carried out in toluene, at room temperature under inert atmosphere. These conditions were those selected by Nebois and Fillion for a similar reaction.[8b] In all reactions, two regioisomers were obtained for which the structure assignments will be further discussed. The percentage of each isomer reported in Table 1 was determined from the $^1$H NMR spectra of reaction mixture. The different 1-aza-dienes led to an equivalent ratio of the two regioisomers. The dihydroazaquinone intermediates were subsequently oxidized by $MnO_2$ to give the corresponding anthraquinones **6**(**a**–**d**) and **7**(**a**–**d**) (Schemes 2 and 3). No attempts to improve the yields of these reactions were done.

**Table 1.** Regioselectivity of cycloadditions of the different 1-aza-dienes with isoquinoline-5,8-dione

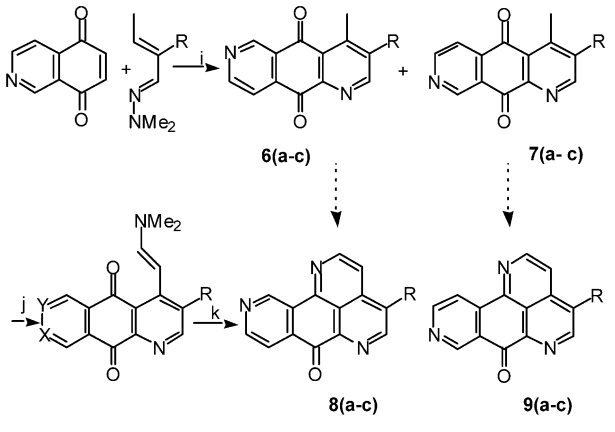


| $R_2$ | $R_1$ | Isomers **6** (%) | Isomers **7** (%) | Yield (%) |
|---|---|---|---|---|
| Me | H | **6a** (80) | **7a** (20) | 36 |
| Me | OMe | **6b** (70) | **7b** (30) | 39 |
| Me | F | **6c** (80) | **7c** (20) | 36 |
| $C_6H_4(NHCOCF_3)$ | H | **6d** (70) | **7d** (30) | 3 |

The formation of the last cycle from compounds **6a**–**c** and **7a**–**c** was accomplished by means of a one pot annelation method developed by Bracher.[14] The reaction of dimethylformamide diethyl acetal with the methyldiazaanthraquinones gave quantitatively (yields determined by $^1$H NMR) the enamine intermediates which were treated with $NH_4Cl$ in refluxing ethanol (or methanol) to yield the tetracyclic compounds **8a**–**c** and **9a**–**c** (Scheme 2).



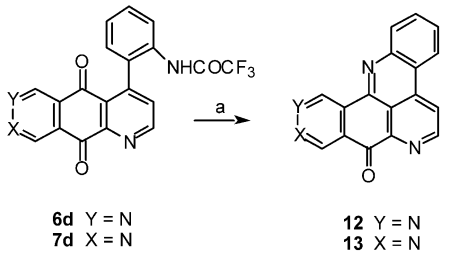

**Scheme 1.**




**Scheme 2.** (i) Toluene, dark, $N_2$, rt/$MnO_2$, $CHCl_3$, rt; (j) DMF-DEA, DMF, $N_2$, 120 °C; (k) $NH_4Cl$, EtOH, reflux.

The two additional tetracyclic compounds **10** and **11** were obtained as minor products in the reaction of formation of **8c**. A possible explanation for the formation of **10** is the nucleophilic substitution of the fluorine atom by the dimethylamine released from the enamine intermediate during the cyclisation step. Compound **11** could result of addition of ethanol to the enamine before the cyclisation step (Fig. 3).

The diazaanthraquinones **6d** and **7d**, formed in very low yields in the Diels–Alder reaction, were cyclised in alkaline conditions to give the corresponding pentacyclic compounds **12** and **13** (Scheme 3).




**Scheme 3.** (a) $CHCl_3$, 1 N NaOH, rt.

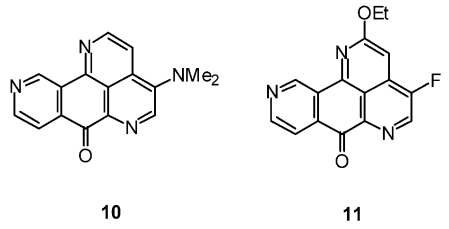


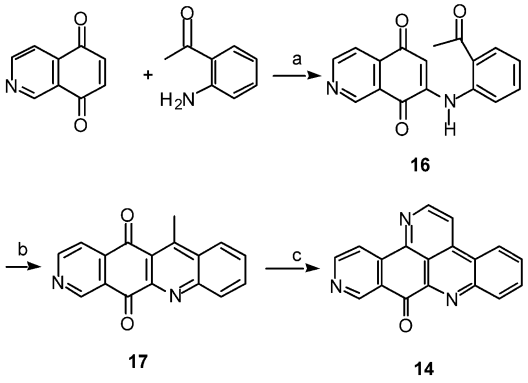

**Figure 3.**

The pentacyclic compound **14** is an analogue of the marine pyridoacridine ascididemine **15**,[15] it was synthesized according to the synthetic procedure reported by Bracher for this last alkaloid[14] (Scheme 4).

Isoquinoline-5,8-dione was converted regioselectively to the diaryl amine **16** by oxidative amination with 2-aminoacetophenone in the presence of $CeCl_3$ and air (Scheme 1). Cyclisation of **16** to the tetracyclic quinone **17** proceeded on heating with concentrated $H_2SO_4$ in glacial acetic acid. The formation of ring E was accomplished by the method previously described for compounds **8** and **9**.

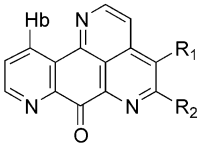
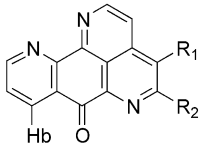
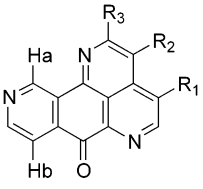
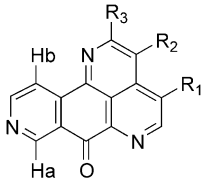
## Structural assignment

The structure of each isomer obtained in the Diels–Alder reaction was assigned comparatively with related




**Scheme 4.** (a) $CeCl_3$, EtOH, rt, overnight; (b) cc $H_2SO_4/CH_3COOH$, reflux, 1 h; (c) DMF-DEA, DMF, $N_2$, 120 °C, 30 min, $NH_4Cl$, MeOH, reflux, 30 min.

**Table 2.** $^1H$ NMR chemical shift (ppm) of the protons $H_1$ and $H_4$ of the different tetra- and pentacyclic compounds

| |  | |  | | Ref |
|---|---|---|---|---|---|
| | $H_b$ | | $H_b$ | | |
| $R_1=R_2=H$ | 9.17 | | 8.77 | | 16 |
| $R_1=OMe$, $R_2=H$ | 9.19 | | 8.78 | | 16 |
| $R_1$, $R_2=$  | 9.27 | | 8.80 | | 15/17 |
| |  | |  | | |
| | $H_a$ | $H_b$ | $H_a$ | $H_b$ | |
| $R_1$, $R_2$, $R_3=H$ | 10.14 | 8.21 | 9.66 | 8.63 | |
| $R_1=OMe$, $R_2$, $R_3=H$ | 10.14 | 8.22 | 9.67 | 8.63 | |
| $R_1=F$, $R_2$, $R_3=H$ | 10.15 | 8.22 | 9.66 | 8.63 | |
| $R_1=H$, $R_2$, $R_3=$  | 10.30 | 8.23 | 9.70 | 8.81 | |

compounds reported in literature or univocaly synthesised by our group in previous studies.[15–17] The two isomers have the same difference in their structural environment both in the compounds of reference and in the isoquinolinic derivatives described in this paper.

In the case of the quinolinic derivatives reported in Table 2, a notable difference in the $^1$H NMR chemical shift of the proton $H_4$ can be observed between the two isomers. The proton close to the iminoquinone function in isomers **8** shows a deshielding effect of around 0.4 ppm related to the same proton in isomers **9**. This deshielding effect may be due to the position of this proton towards the D-pyridine ring. In the case of isoquinolinic derivatives synthesised in this work, we have the same relative position of proton and pyridine rings. The data (Table 2) indicate a deshielding of 0.41–0.58 ppm between the protons $H_a$ and $H_b$ in the two isomers; by analogy with the relationship previously established, these deshielded protons are close to the iminoquinone moiety. Thus, the structure **8** and **9** can be assigned to compounds **a** to **c** as well as the structures **12** and **13**. It has to be noted that a long range constant is systematically observed between protons $H_a$ and $H_b$ in isomers **9** but not in isomers **8**.

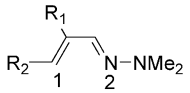
These results are in accordance with the theoretical prediction of the regioselectivity of the Diels–Alder reaction. Indeed, this regioselectivity can be rationalised by FMO theory. The hetero-Diels–Alder reaction of the different dienes and isoquinoline-5,8-dione is a normal $\Pi_s^4 + \Pi_s^2$ process with a HOMOdiene-LUMOdienophile control. The HOMOdiene-LUMOdienophile gaps for crotonaldehyde-*N*,*N*-dimethylhydrazone, 2-methoxy-2-butenal-*N*,*N*-dimethyl hydrazone, 2-fluoro-2-butenal-*N*,*N*-dimethylhydrazone and 4-(2′-trifluoroacetamidophenyl)-1-dimethylamino-1-azadiene are 6.884, 6.956, 7.148 and 7.002 eV, respectively, calculated by using the PM3 semi-empirical molecular orbital method.[18] If the sole atoms considered are those which are bonded, the favored addition was that associating the atom having the larger coefficient for the HOMO of the diene with the atom having the larger coefficient for the LUMO of the dienophile (Table 3).

The major isomer was the structure **6**, nevertheless, the difference between the coefficients of isoquinoline-5,8-dione being low (0.3159 vs 0.3233), the two regioisomers were formed.

Although a sole regioisomer, analogue of ascididemin was obtained, the $^1$H NMR chemical shift of protons $H_a$ and $H_b$ (9.68 and 8.70, respectively) are consistent with structure **14** for the compound synthesised.

Two authors have previously described the Diels–Alder addition of 2-butenal-*N*,*N*-dimethylhydrazone[8d] and 2-ethoxy-2-butenal-*N*,*N*-dimethylhydrazone[8c] with isoquinoline-5,8-dione. They reported the formation of two isomers with a majority one, and they had in both reactions, the same regioselectivity as that we observed in this work. They performed the structural assignment of the major isomer from the reactivity of dienophile

**Table 3.** HOMO and LUMO data of the different 1-aza-dienes and isoquinoline-5,8-dione calculated by PM-3 method[18]



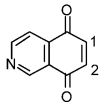

| $R_2$ | $R_1$ | HOMO (eV) | $c_1$ | $c_2$ |
|---|---|---|---|---|
| Me | H | −8.677 | 0.3715 | 0.3196 |
| Me | OMe | −8.449 | 0.4021 | 0.3130 |
| Me | F | −8.941 | 0.4058 | 0.2968 |
| $C_6H_4(NHCOCF_3)$ | H | −8.795 | 0.3622 | 0.2946 |
|  | | LUMO (eV) | $c_1$ | $c_2$ |
| | | −1.793 | 0.3159 | 0.3233 |

**Table 4.** Characterization of the in vitro cytotoxic-related antitumor effects $IC_{50}$ (μM)

| Compounds | Cell lines | | | | | |
|---|---|---|---|---|---|---|
| | U-87MG | U-373MG | J82 | HCT-15 | LoVo | A549 |
| **8a** | 2.2 | 0.8 | 2.4 | 0.7 | 2.5 | 0.9 |
| **9a** | 0.5 | 0.3 | 0.8 | 0.3 | 0.8 | 3.1 |
| **8b** | 0.5 | 0.7 | 3.1 | 0.5 | 2.9 | 0.8 |
| **9b** | 0.8 | 0.8 | >10 | 1.3 | 4.2 | 1.8 |
| **8c** | 3.2 | 3.0 | 3.0 | 2.8 | 2.5 | >10 |
| **9c** | 6.9 | 4.4 | 6.9 | 3.6 | 5.1 | >10 |
| **6d** | 0.06 | 0.03 | 0.7 | 0.05 | 0.08 | 0.15 |
| **7d** | 0.02 | 0.1 | 0.2 | 0.04 | 0.04 | 0.06 |
| **12** | 0.1 | 0.2 | 2.9 | 0.2 | 0.4 | 0.2 |
| **13** | 0.1 | 0.05 | 1.4 | 0.3 | 0.05 | 1.8 |
| **14** | 1 | 2.5 | 3 | 0.9 | 2.8 | 0.7 |

controlled by the position of the ring nitrogen atom relative to the carbonyl group. In the same way, the regioselectivity of the reaction of isoquinoline-5,8-dione with amines described previously[19] was based mainly on the speculation that the C-5 carbonyl group, which is *para* to the nitrogen, is more electron deficient than the C-8 carbonyl group. In this work, the regioselectivity of both these two types of reaction was confirmed by NMR data.

## Biological Results

The cytotoxic activity of the tetra- and pentacyclic compounds **8**, **9** (**a**–**c**), **6d**, **7d**, **12**, **13** and **16** was tested on six distinct human cancer cell lines including various histopathological types (glioblastomas, colon, lung, and bladder cancers). The colorimetric MTT assay, which indirectly assesses the effect of potentially anticancer compounds on overall growth of adherent cell lines[20] was used. The $IC_{50}$ values, that is the concentration which reduced the mean growth value of the six cell lines by 50%, was determined for each drug, as compared to the mean control growth value. Table 4 illustrates the individual $IC_{50}$ values of the different

compounds obtained for each of the six cell lines under study. All those compounds present cytotoxic activity. The derivatives with the most close structure to the natural product amphimedine (**12** and **13**) exhibited $IC_{50}$ values $<10^{-7}$ M and are the more active compounds of this series with the intermediates **6d** and **7d**. The substituted tetracycles **8b**, **8c** and **9b**, **9c** were sensibly less cytotoxic than the unsubstituted ones **8a** and **9a**. The difference in activity between the two isomers was relatively weak. More generally, the same cytotoxicity was observed from a cell line to another, marking the poor selectivity of these compounds.

In conclusion, we have synthesised three different types of pyridoacridine derivatives from isoquinoline-5,8-dione; the compounds which exhibit the better potency against cancer cells were the amphimedine analogues.

## Experimental

### Chemical synthesis

$^1$H and $^{13}$C NMR spectra were obtained with a JEOL 400 MHz spectrometer with the chemical shifts of the remaining protons of the deuterated solvents serving as internal standards. IR spectra were obtained with a Perkin-Elmer (1600 series FTIR) spectrometer. Mass spectra (MS) were recorded on an automass Unicam spectrometer. Reagents were purchased from commercial sources and used as received. Chromatography was performed on silicagel (15–40 μm) using the solvent systems indicated below. The purity of the different ascidiidemin analogues was evaluated on an analytical chromatographic system consisted of a ZorbaxNH$_2$, 5 μm column (150 × 4.6 mm), isooctane/MeOH/EtOH 80:10:10 at 1.5 mL/min flow rate.

### Diels–Alder reactions of isoquinoline-5,8-dione with the different dienes

**General method A.** To a solution of isoquinoline-5,8-dione in toluene was added dropwise under nitrogen atmosphere a solution of diene in toluene. The reaction media was stirred in the dark, at room temperature for different times. After concentration to dryness, the crude product was filtered on silicagel to give the Diels–Alder adducts. $MnO_2$ 85% and $CHCl_3$ were added, and the mixture was stirred at room temperature. After filtration over Celite, the crude product was purified on flash-chromatography silicagel column to give the expected products.

**4-Methyl-1,7-diazaanthraquinone (6a) and 4-methyl-1,6-diazaanthraquinone (7a).** Method A was used and involved isoquinolinedione (3.2 g, 20 mmol) in toluene (240 mL), crotonaldehyde-dimethylhydrazone (3.38 g, 30 mmol) in toluene (15 mL), stirring 45 h. Filtration was done on silicagel ($CH_2Cl_2$/MeOH 95:5). $MnO_2$ 85% (20.5 g, 0.2 mol) and $CHCl_3$ (200 mL) were added, and the solution was stirred at room temperature for 4 h 30 min. Flash-chromatography ($CH_2Cl_2$/MeOH 99:1) gave the expected tricyclic compounds.

**(6a).** Brown solid (1.3 g, 29%), mp $>260$ °C. $^1$H NMR ($CDCl_3$) 2.92 (s, 3H); 7.56 (dd, 1H, $J=4.8$ and 0.7 Hz); 8.12 (dd, 1H, $J=5.2$ and 0.7 Hz); 8.93 (d, 1H, $J=4.8$ Hz); 9.12 (d, 1H, $J=5.2$ Hz); 9.55 (d, 1H, $J=0.7$ Hz). $^{13}$C NMR ($CDCl_3$) 22.83; 118.87; 126.46; 128.99; 131.89; 137.32; 149.62; 149.91; 151.90; 153.85; 155.36; 181.51; 184.12. IR ($CHCl_3$) 1698; 1678 cm$^{-1}$. MS ($m/z$) 224 (42); 210 (100); 196 (26); 182 (49); 168 (10).

**(7a).** Brown solid (0.3 g, 7%), mp 245 °C. $^1$H NMR ($CDCl_3$) 2.91 (s, 3H); 7.55 (dd, 1H, $J=4.8$ and 0.7 Hz); 8.05 (dd, 1H, $J=5.2$ and 0.7 Hz); 8.96 (d, 1H, $J=4.8$ Hz); 9.13 (d, 1H, $J=5.2$ Hz); 9.62 (d, 1H, $J=0.7$ Hz). $^{13}$C NMR ($CDCl_3$) 22.83; 119.06; 125.43; 129.06; 131.47; 138.77; 149.59; 149.92; 151.91; 154.21; 155.75; 181.26; 184.13. IR ($CHCl_3$) 1692; 1680 cm$^{-1}$. MS ($m/z$) 224 (100); 196 (18); 168 (26); 140 (25).

**3-Methoxy-4-methyl-1,7-diazaanthraquinone (6b) and 3-methoxy-4-methyl-1,6-diazaanthraquinone (7b).** Method A was used and involved isoquinolinedione (715 mg, 4.5 mmol) in toluene (55 mL), 2-methoxy-2-butenal-dimethylhydrazone (950 mg, 6.75 mmol) in toluene (15 mL), stirring 16 h. Filtration was done on silicagel ($CH_2Cl_2$/MeOH 99:1). $MnO_2$ 85% (3.2 g, 31,5 mmol) and $CHCl_3$ (30 mL) were added, and the solution was stirred for 6 h. The crude product was washed with ether before flash-chromatography ($CH_2Cl_2$/MeOH 99:1) which gave the expected tricyclic compounds.

**(6b).** Ochre solid (309 mg, 27%), mp $>260$ °C. $^1$H NMR ($CDCl_3$) 2.77 (s, 3H); 4.11 (s, 3H); 8.10 (dd, 1H, $J=5.9$ and 0.7 Hz); 8.65 (s, 1H); 9.09 (d, 1H, $J=5.9$ Hz); 9.52 (d, 1H, $J=0.7$ Hz). $^{13}$C NMR ($CDCl_3$) 13.02; 56.96; 118.91; 127.14; 129.63; 136.78; 137.83; 138.98; 142.85; 149.85; 155.21; 157.49; 180.75; 184.81. IR ($CHCl_3$) 1688; 1674 cm$^{-1}$. MS ($m/z$) 254 (100); 236 (7); 223 (13); 211(20); 183 (18).

**(7b).** Ochre solid (132 mg, 12%), mp $>260$ °C. $^1$H NMR ($CDCl_3$) 2.75 (s, 3H); 4.13 (s, 3H); 8.02 (d, 1H, $J=5.1$ Hz); 8.68 (s, 1H); 9.10 (d, 1H, $J=5.1$ Hz); 9.61 (s, 1H). $^{13}$C NMR ($CDCl_3$) 13.05; 56.96; 119.04; 125.60; 129.59; 137.07; 139.02; 139.31; 142.80; 149.87; 155.38; 157.11; 180.48; 184.73. IR ($CHCl_3$) 1684 cm$^{-1}$. MS ($m/z$) 254 (100); 223 (6); 211 (18); 183 (21).

**3-Fluoro-4-methyl-1,7-diazaanthraquinone (6c) and 3-fluoro-4-methyl-1,6-diazaanthraquinone (7c).** Method A was used and involved isoquinolinedione (1.7 g, 10.7 mmol) in toluene (130 mL), 2-fluoro-2-butenal-dimethylhydrazone (820 mg, 6.3 mmol) in toluene (5 mL), the reaction was stirred for 32 h. The mixture of Diels–Alder adducts was stirred with silicagel (30 g) in AcOEt (100 mL) at room temperature for 1 h. The silicagel was filtered off and the solution was concentrated to dryness. $MnO_2$ 85% (5.5 g, 54 mmol) and $CHCl_3$ (60 mL) were added, and the solution was stirred overnight. Flash-chromatography ($CH_2Cl_2$) gave the expected tricyclic compounds.

**(6c).** Yellow-brown solid (432 mg, 29%), mp 170 °C. $^1$H NMR ($CDCl_3$) 2.85 (s, 3H); 8.13 (d, 1H, $J=5.1$ Hz);

8.86 (s, 1H); 9.14 (d, 1H, $J=5.1$ Hz); 9.55 (s, 1H). $^{13}$C NMR ($CDCl_3$) 12.58 (d, $J=5$ Hz); 119.03; 126.63; 130.33; 137.31; 137.63 (d, $J=15$ Hz); 142.70 (d, $J=28$ Hz); 145.77 (d, $J=7$ Hz); 149.92; 155.64; 160.65 (d, $J=263$ Hz); 180.24; 183.88. IR ($CHCl_3$) 1698, 1677 cm$^{-1}$. MS (*m*/*z*) 242 (100); 214 (64); 186 (71); 158 (52).

**(7c).** Yellow-brown solid (108 mg, 7%), mp 216 °C. $^1$H NMR ($CDCl_3$) 2.80 (s, 3H); 8.01 (d, 1H, $J=5.1$ Hz) ; 8.85 (s, 1H); 9.12 (d, 1H, $J=5.1$ Hz); 9.58 (s, 1H). $^{13}$C NMR ($CDCl_3$) 12.55 (d, $J=5$ Hz); 118.99; 125.21; 130.26; 137.5 3 (d, $J=14$ Hz); 137.77; 142.86 (d, $J=27$ Hz); 145.56 (d, $J=7$ Hz); 149.98; 155.74; 160.23 (d, $J=253$ Hz);179.91; 183.90. IR ($CHCl_3$) 1712 cm$^{-1}$. MS (*m*/*z*) 242 (100); 214 (15); 185 (19); 158 (33); 131 (31).

**4 - (2 - Trifluoroacetamidophenyl)pyrido[4,3 - *g*]quinoleine-5,10-dione (6d) and 4-(2-trifluoroacetamidophenyl)pyrido[3,4]quinoleine-5,10-dione (7d).** A mixture of isoquinolinedione (3.5 g, 22 mmol), 4-(2-trifluoroacetamido phenyl)-1-dimethylamino-1-aza-1,3-butadiene (6.9 g, 24.2 mmol), acetic anhydride (7.2 mL) and 10% Pd/C (4.6 g) in $CH_3CN$ (250 mL) was refluxed under nitrogen atmosphere and in the dark for 29 h. The reaction media was filtered over Celite and concentrated to dryness. The crude product was purified by flash chromatography ($CH_2Cl_2$/MeOH 99:1) to give the expected tricyclic compounds.

**(6d).** Brown solid (145 mg, 1.7%), mp 232 °C. $^1$H NMR ($CDCl_3$): 7.21 (dd, 1H, $J=1.5$ and 7.7 Hz, $C_1$–H); 7.48 (ddd, 1H, $J=1.5$; 7.7 and 7.7 Hz, $C_2$–H); 7.60 (ddd, 1H, $J=1.4$; 8.1 and 7.7 Hz, $C_3$–H); 7.62 (d, 1H, $J=5.1$ Hz, $C_5$–H); 7.70 (d, 1H, $J=8.1$ and 1.4 Hz, $C_4$–H); 7.90 (br.s, 1H, NH); 8.15 (dd, 1H, $J=0.8$ and 4.8 Hz, $C_9$–H); 9.14 (d, 1H, $J=5.1$ Hz, $C_6$-H); 9.16 (d, 1H, $J=4.8$ Hz, $C_{10}$–H); 9.38 (d, 1H, $J=0.8$ Hz, $C_{11}$–H). $^{13}$C NMR ($CDCl_3$): 115.51 (q, $J=367$ Hz); 119.16; 125.67; 125.97; 127.83; 128.15; 128.93; 129.99; 131.55; 131.65; 134.52; 137.40; 148.28; 149.23; 149.76; 154.78; 155.47 (q, $J=72$ Hz); 155.80; 180.73; 183.12. IR ($CHCl_3$): 1736, 1698, 1679 cm$^{-1}$. MS (*m*/*z*) 397 (45); 328 (34); 285 (100); 216 (9). $t_R$ is 14.71 min (100% purity).

**(7d).** Beige solid (67 mg, 0.8%), mp >260 °C. $^1$H NMR ($CDCl_3$): 7.21 (d, 1H, $J=7.4$ Hz, $C_1$–H); 7.47 (dd, 1H, $J=7.4$ and 7.7 Hz, $C_2$–H); 7.59–7.62 (m, 2H, $C_3$–H and $C_5$–H); 7.73 (d, 2H, $J=8.1$ Hz, $C_4$–H + NH); 7.85 (d, 1H, $J=4.8$ Hz, $C_{12}$–H); 9.09 (d, 1H, $J=4.8$ Hz, $C_6$–H); 9.18 (d, 1H, $J=4.8$ Hz, $C_{11}$–H); 9.63 (s, 1H, $C_9$–H). $^{13}$C NMR ($CDCl_3$): 115.45 (q, $J=287$ Hz); 118.92; 125.30; 125.43; 127.95; 128.05; 129.00; 130.04; 131.08; 131.44; 133.96; 138.22; 148.03; 149.25; 150.04; 155.12; 155.14 (q, $J=37$ Hz); 155.94; 180.44; 183.22. IR ($CHCl_3$): 1736, 1693 cm$^{-1}$. $t_R$ is 16.62 min (97% purity).

### Formation of tetracyclic compounds

**General method B.** A solution of tricyclic intermediate and dimethylformamide diethylacetal (DMF-DEA) in DMF was heating at 120 °C under nitrogen atmosphere. After concentration to dryness, $NH_4Cl$ and ethanol were added and the mixture was refluxed for additional time. The solvent was removed over vacuum, water was added and the mixture was extracted four times with $CH_2Cl_2$. The organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified to give the expected tetracyclic derivative.

**7H - Pyrido[4,3,2 - *de*][1,9]phenanthroline - 7 - one (8a).** Method B was used and involved tricyclic compound **6a** (100 mg, 0.446 mmol), DMF-DEA (0.27 mL, 1.56 mmol) in DMF (1 mL), heating at 120 °C for 15 min. $NH_4Cl$ (685 mg, 12.9 mmol) and absolute EtOH (90 mL) were added and the reaction was refluxed for 30 min. Flash chromatography ($CH_2Cl_2$/MeOH 99:1) gave the expected tetracyclic compound as a yellow solid (42 mg, 40%), mp >260 °C. $^1$H NMR ($CDCl_3$) 7.80 (d, 1H, $J=5.8$ Hz, $C_3$–H); 8.01 (d, 1H, $J=5.5$ Hz, $C_4$–H); 8.21 (d, 1H, $J=5.2$ Hz, $C_8$–H); 8.96 (d, 1H, $J=5.8$ Hz, $C_2$–H); 9.03 (d, 1H, $J=5.2$ Hz, $C_9$–H); 9.19 (d, 1H, $J=5.5$ Hz, $C_5$–H); 10.14 (s, 1H, $C_{11}$–H). $^{13}$C NMR ($CDCl_3$) 119.74; 119.76; 119.95; 124.36; 128.42; 136.93; 138.81; 147.30; 147.65; 148.63; 148.88; 149.88; 152.68; 181.75. IR ($CHCl_3$) 1689 cm$^{-1}$. MS (*m*/*z*) 233 (100); 204 (24); 178 (16); 151 (36). $t_R$ is 4.13 min (100% purity).

**7H - Pyrido[4,3,2 - de][1,8]phenanthroline - 7 - one (9a).** Method B was used and involved tricyclic compound **7a** (140 mg, 0.63 mmol), DMF-DEA (0.37 mL, 2.19 mmol) in DMF (1 mL), heating at 120 °C for 15 min. $NH_4Cl$ (960 mg, 17.9 mmol) and absolute EtOH (90 mL) were added and the reaction was refluxed for 30 min. Flash chromatography ($CH_2Cl_2$/MeOH 99:1) gave the expected tetracyclic compound as a yellow solid (41 mg, 28%), mp >260 °C. $^1$H NMR ($CDCl_3$) 7.87 (d, 1H, $J=5.8$ Hz, $C_3$–H); 8.00 (d, 1H, $J=5.5$ Hz, $C_4$–H); 8.63 (dd, 1H, $J=5.2$ and 0.7 Hz, $C_{11}$–H); 8.98 (d, 1H, $J=5.8$ Hz, $C_2$–H); 9.05 (d, 1H, $J=5.2$ Hz, $C_{10}$–H); 9.20 (d, 1H, $J=5.5$ Hz, $C_5$–H); 9.66 (d, 1H, $J=0.7$ Hz, $C_8$–H). $^{13}$C NMR ($CDCl_3$) 118.24; 120.72; 121.08; 123.79; 126.26; 138.79; 141.86; 147.48; 147.72; 148.96; 149.36; 150.73; 155.21; 181.66. IR ($CHCl_3$) 1683 cm$^{-1}$. MS (*m*/*z*) 233 (100); 204 (25); 178 (25); 151 (26). $t_R$ is 4.49 min (92% purity).

**4-Methoxy-7H-pyrido[4,3,2-*de*][1,9]phenanthroline-7-one (8b) and 4-methoxy-7H-pyrido[4,3,2-*de*][1,8]phenanthroline-7-one (9b).** Method B was used and involved a mixture of tricyclic compound **6b** and **7b** (250 mg, 0.98 mmol), DMF-DEA (0.63 mL, 3.44 mmol) in DMF (2.5 mL), heating at 120 °C for 75 min. $NH_4Cl$ (1.5 g, 35.5 mmol) and absolute EtOH (250 mL) were added and the reaction was refluxed for 30 min. Flash-chromatography ($CH_2Cl_2$/MeOH 98:2) gave the expected tetracyclic compounds as a yellow solid (170 mg, 69%). The two isomers were separated by successive chromatographies ($CH_2Cl_2$/MeOH 98:2).

**(8b).** Yellow solid (118 mg, 65%) mp >260 °C. $^1$H NMR ($CDCl_3$) 4.27 (s, 3H); 8.07 (d, 1H, $J=5.9$ Hz, $C_3$–H); 8.22 (d, 1H, $J=5.2$ Hz, $C_8$–H); 8.71 (s, 1H, $C_5$–H); 8.96 (d, 1H, $J=5.9$ Hz, $C_2$–H); 9.00 (d, 1H, $J=5.2$ Hz,

$C_9$–H); 10.14 (s, 1H, $C_{11}$–H). $^{13}C$ NMR ($CDCl_3$) 57.07; 114.90; 119.80; 128.73; 129.48; 130.27; 137.38; 140.26; 146.88; 148.53; 148.91; 152.39; 153.40; 174.07; 180.61. IR ($CHCl_3$) 1674 $cm^{-1}$. MS (*m*/*z*) 263 (82); 248 (62); 220 (100); 192 (32). $t_R$ is 3.74 min (98% purity).

**(9b).** Yellow solid (63 mg, 81%), mp >260 °C. $^1H$ NMR ($CDCl_3$) 4.27 (s, 3H); 8.15 (d, 1H, $J$ = 5.9 Hz, $C_3$–H); 8.63 (dd, 1H, $J$ = 5.5 and 0.8 Hz, $C_{11}$–H); 8.71 (s, 1H, $C_5$–H); 8.97 (d, 1H, $J$ = 5.9 Hz, $C_2$–H); 9.04 (d, 1H, $J$ = 5.5 Hz, $C_{10}$–H); 9.67 (d, 1H, $J$ = 0.8 Hz, $C_8$–H). $^{13}C$ NMR ($CDCl_3$) 57.14; 116.31; 118.16; 121.08; 126.56; 129.34; 130.41; 140.36; 141.98; 146.96; 148.37; 150.72; 152.95; 154.64; 180.65. IR ($CHCl_3$) 1674 $cm^{-1}$. MS (*m*/*z*) 263 (20); 248 (13); 220 (8). $t_R$ is 3.88 min (99% purity).

**4-Fluoro-7H-pyrido[4,3,2-*de*][1,9]phenanthroline-7-one (8c), 4-dimethylamino-7H-pyrido[4,3,2-*de*][1,9]phenanthroline-7-one (10) and ethoxy-4-fluoro-7H-pyrido[4,3,2-*de*][1,9]phenanthroline-7-one (11).** Method B was used and involved tricyclic compound **6c** (80 mg, 0.33 mmol), DMF-DEA (0.2 mL, 1.16 mmol) in DMF (0.7 mL), heating at 120 °C for 15 min. $NH_4Cl$ (507 mg, 9.48 mmol) and absolute EtOH (80 mL) were added and the reaction was refluxed for 30 min. Flash chromatography ($CH_2Cl_2$/MeOH 99:1) gave the expected tetracyclic compound and two side products.

**(8c).** Yellow solid (24 mg, 29%), mp >260 °C. $^1H$ NMR ($CDCl_3$) 8.00 (d, 1H, $J$ = 5.8 Hz, $C_3$–H); 8.22 (d, 1H, $J$ = 5.2 Hz, $C_8$–H); 9.01 (d, 1H, $J_{H\text{-}F}$ = 0.8 Hz, $C_5$–H); 9.04 (d, 1H, $J$ = 5.2 Hz, $C_9$–H); 9.05 (d, 1H, $J$ = 5.8 Hz, $C_2$–H); 10.15 (s, 1H, $C_{11}$–H). $^{13}C$ NMR ($CDCl_3$) 112.91; 119.93; 120.81; 128.25; 129.06 (d, $J$ = 20 Hz); 135.27 (d, $J$ = 24 Hz); 137.09; 143.65; 147.92; 148.65; 149.81; 153.04; 156.11 (d, $J$ = 288 Hz); 180.19. IR ($CHCl_3$) 1684 $cm^{-1}$. MS (*m*/*z*) 251 (100); 223 (36); 196 (24); 169 (25). $t_R$ is 3.58 min (100% purity).

**(10).** Red solid (6 mg, 7%), mp >260 °C. $^1H$ NMR ($CDCl_3$) 3.36 (s, 6H); 7.94 (d, 1H, $J$ = 5.9 Hz); 8.23 (dd, 1H, $J$ = 5.1 and 0.7 Hz); 8.60 (s, 1H); 8.84 (d, 1H, $J$ = 5.9 Hz); 8.96 (d, 1H, $J$ = 5.1 Hz); 10.14 (d, 1H, $J$ = 0.7 Hz). $^{13}C$ NMR ($CDCl_3$) 29.68; 44.05; 117.66; 119.50; 120.84; 128.66; 129.37; 135.11; 137.50; 137.60; 144.65; 147.39; 148.47; 149.27; 151.87; 180.06. IR ($CHCl_3$) 3690; 1660 $cm^{-1}$. MS (*m*/*z*) 251 (100); 223 (36); 196 (24); 169 (25).

**(11).** Brown solid (7 mg, 7%), mp 196 °C. $^1H$ NMR ($CDCl_3$) 1.56 (t, 3H, $J$ = 7 Hz); 4.69 (q, 2H, $J$ = 7 Hz); 7.24 (s, 1H); 8.18 (dd, 1H, $J$ = 5.0 and 0.7 Hz); 8.76 (s, 1H); 9.01 (d, 1H, $J$ = 5.0 Hz); 10.03 (s, 1H). $^{13}C$ NMR ($CDCl_3$) 14.65; 63.75; 95.91; 117.93; 120.03; 128.08; 132.74 (d, $J$ = 18 Hz); 133.66 (d, $J$ = 24 Hz); 137.19; 144.08; 148.37; 149.18; 153.03; 155.71 (d, $J$ = 277 Hz); 163.61; 180.49. IR ($CHCl_3$) 1689; 1618 $cm^{-1}$. MS (*m*/*z*) 295 (20); 239 (44).

**4-Fluoro-7H-pyrido[4,3,2-*de*][1,8]phenanthroline-7-one (9c).** Method B was used and involved tricyclic compound **7c** (40 mg, 0.17 mmol), DMF-DEA (0.1 mL, 0.58 mmol) in DMF (0.35 mL), heating at 120 °C for 15 min. $NH_4Cl$ (254 mg, 4.74 mmol) and absolute EtOH (40 mL) were added and the reaction was refluxed for 30 min. Flash chromatography ($CH_2Cl_2$/MeOH 99:1) gave the expected tetracyclic compound as a yellow solid (10 mg, 23%), mp >260 °C. $^1H$ NMR ($CDCl_3$) 8.07 (d, 1H, $J$ = 5.8 Hz, $C_3$–H); 8.63 (dd, 1H, $J$ = 5.5 and 0.7 Hz, $C_{11}$–H); 9.01 (d, 1H, $J_{H\text{-}F}$ = 1.4 Hz, $C_5$–H); 9.06 (d, 1H, $J$ = 5.8 Hz, $C_2$–H); 9.07 (d, 1H, $J$ = 5.5 Hz, $C_{10}$–H); 9.66 (d, 1H, $J$ = 0.7 Hz, $C_8$–H). $^{13}C$ NMR ($CDCl_3$) 114.23; 118.19; 121.49; 126.14; 129.04 (d, $J$ = 17 Hz); 135.14 (d, $J$ = 23 Hz); 141.54; 143.65; 147.88; 149.07; 150.79; 155.15; 155.60 (d, $J$ = 274 Hz); 180.11. IR ($CHCl_3$) 1721, 1684 $cm^{-1}$. MS (*m*/*z*) 251 (100); 222 (17); 196 (26); 169 (24). $t_R$ is 3.75 min (94% purity).

**Benzo[*b*]pyrido[4,3,2-*de*][1,9]phenanthroline-8-one (12).** A solution of compound **6d** (100 mg, 0.25 mmol) in $CHCl_3$ (75 mL) and 1 N NaOH (3.3 mL) was stirred at room temperature for 2 h 30 min. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (3 × 100 mL). The combined organic layers were dried over $MgSO_4$ and concentrated over vacuum to give the expected pentacyclic compound as a yellow solid (65 mg, 91%), mp >260 °C. $^1H$ NMR ($CDCl_3$): 7.85 (ddd, 1H, $J$ = 7.7, 7.0 and 1.4 Hz, $C_3$–H); 7.97 (dd, 1H, $J$ = 8.1, 7.0 and 1.1 Hz, $C_2$–H); 8.23 (d, 1H, $J$ = 5.1 Hz, $C_9$–H); 8.40 (dd, 1H, $J$ = 8.1 and 1.1 Hz, $C_1$–H); 8.62 (dd, 1H, $J$ = 7.7 and 1.4 Hz, $C_4$–H); 8.70 (d, 1H, $J$ = 5.5 Hz, $C_5$–H); 9.04 (d, 1H, $J$ = 5.1 Hz, $C_{10}$–H); 9.35 (d, 1H, $J$ = 5.5 Hz, $C_6$–H); 10.30 (s, 1H, $C_{12}$–H). $^{13}C$ ($CDCl_3$) NMR 118.47; 119.48; 120.28; 121.67; 122.92; 128.53; 129.32; 131.69; 132.09; 137.12; 138.29; 145.63; 146.70; 147.66; 149.03; 150.35; 152.42; 181.97. IR ($CHCl_3$): 1685 $cm^{-1}$. MS (*m*/*z*) 283 (100); 254 (4); 228 (8).

**Benzo[*b*]pyrido[4,3,2-*de*][1,8]phenanthroline-8-one (13).** The same procedure as for compound **12** involving compound **7d** (32 mg, 0.081 mmol) in $CHCl_3$ (25 mL) and 1 N NaOH (1.1 mL) was used. The aqueous layer was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated over vacuum to give the expected pentacyclic compound as a yellow solid (22 mg, 96%), mp >260 °C. $^1H$ NMR ($CDCl_3$) 7.87 (ddd, 1H, $J$ = 7.1; 8.0 and 1.1 Hz, $C_3$–H); 7.99 (ddd, 1H, $J$ = 8.4; 7.1 and 1.1 Hz, $C_2$–H); 8.41 (dd, 1H, $J$ = 8.4 and 1.1 Hz, $C_1$-H); 8.65 (dd, 1H, $J$ = 8.0 and 1.1 Hz, $C_4$–H); 8.68 (d, 1H, $J$ = 5.5 Hz, $C_5$–H); 8.81 (dd, 1H, $J$ = 5.1 and 0.8 Hz, $C_{12}$–H); 9.09 (d, 1H, $J$ = 5.1 Hz, $C_{11}$–H); 9.36 (d, 1H, $J$ = 5.5 Hz, $C_6$–H); 9.70 (d, 1H, $J$ = 0.8 Hz, $C_9$–H). $^{13}C$ NMR ($CDCl_3$): 118.28; 119.02; 119.77; 122.23; 123.02; 126.44; 129.82; 131.82; 132.07; 138.07; 141.89; 145.48; 146.83; 147.13; 150.37; 150.62; 154.75; 181.69. IR ($CHCl_3$): 1684 $cm^{-1}$. MS (*m*/*z*) 283 (100); 254 (29); 227 (14).

**5-(2-Acetylphenylamino)isoquinoline-3,6-dione (16).** A solution of isoquinoline-5,8-dione (1 g, 6.29 mmol) in ethanol (60 mL) was added dropwise to a solution of cerium(III) chloride $CeCl_3$ (3 g, 12.59 mmol) and aminoacetophenone (1.7 g, 12.59 mmol) in ethanol (25 mL). The reaction media was stirred at room temperature overnight, hydrolysed by 10% acetic acid (100 mL) and

extracted with $CH_2Cl_2$ (4 × 200 mL). The organic layers were dried over $MgSO_4$ and concentrated to dryness. The crude product was purified by flash chromatography ($CH_2Cl_2$) to give the expected product as a red solid (190 mg, 51%), mp 204 °C. RMN $^1H$ ($CDCl_3$) 2.69 (s, 3H); 6.80 (s, 1H); 7.23 (ddd, 1H, $J = 1.5$, 6.6 and 8.4 Hz); 7.60 (ddd, 1H, $J = 1.5$, 6.6 and 8.1 Hz); 7.62 (dd, 1H, $J = 8.1$ and 1.5 Hz), 7.90 (dd, 1H, $J = 0.8$ and 5.0 Hz); 7.97 (dd, 1H, $J = 1.5$ and 8.4 Hz); 11.33 (s, 1H). $^{13}C$ NMR ($CDCl_3$): 28.51; 106.19; 118.54; 120.95; 123.67; 124.17; 125.97; 132.41; 134.21; 138.23; 139.64; 143.85; 148.58; 156.21; 181.22; 182.83; 201.51. IR ($CHCl_3$) 1684, 1664, 1641 $cm^{-1}$.

**9-Methyl-1,4-diazanaphtacene-3,10-dione (17).** To a solution of tetracyclic compound **16** (0.5 g, 1.71 mmol) in acetic acid (12 mL), was added dropwise a solution of concentrated sulfuric acid (2 mL) in acetic acid (10 mL). The reaction media was refluxed for 1 h and after cooling was poured into ice. The mixture was neutralized by $K_2CO_3$ and extracted with $CH_2Cl_2$ (4 × 50 mL). The organic layers were dried over $MgSO_4$ and concentrated to dryness. Flash chromatography ($CH_2Cl_2$/MeOH 99:1) gave the expected tetracyclic compound as a green solid (248 mg, 53%), mp >260 °C. $^1H$ NMR ($CDCl_3$): 3.31 (s, 3H); 7.81 (ddd, 1H, $J = 1.0$; 7.0 and 8.4 Hz); 7.96 (ddd, 1H, $J = 1.0$, 7.0 and 8.4 Hz); 8.11 (dd, 1H, $J = 0.7$ and 5.1 Hz); 8.40 (dd, 1H, $J = 1.0$ and 8.4 Hz); 8.48 (dd, 1H, $J = 1.0$ and 8.4 Hz); 9.14 (d, 1H, $J = 5.1$ Hz); 9.66 (d, 1H, $J = 0.7$ Hz). $^{13}C$ NMR ($CDCl_3$): 16.75; 119.49; 125.17; 125.57; 126.24; 129.65; 129.98; 132.53; 133.06; 140.21; 147.64; 148.91; 150.18; 152.78; 155.72; 181.53; 184.27. IR ($CHCl_3$) 1690, 1675 $cm^{-1}$.

**9H-Quino[4,3,2-*de*][1,7]phenanthrolin-9-one (14).** Method B using tetracyclic compound **17** (150 mg, 0.55 mmol), dimethylformamide diethylacetal (0.33 mL, 1.92 mmol) in DMF (3.3 mL), the reaction was warmed at 120 °C under nitrogen atmosphere for 30 min. $NH_4Cl$ (0.88 g, 17.1 mmol) and methanol (18 mL) were added and the reaction was refluxed for 30 min. The crude product was purified by flash-chromatography ($CH_2Cl_2$/MeOH 98:2) to give the pentacyclic compound as a yellow solid (35 mg, 23%), mp >260 °C. $^1H$ NMR ($CDCl_3$): 7.96 (ddd, 1H, $J = 1.1$, 7.3 and 8.2 Hz); 8.04 (ddd, 1H, $J = 1.4$, 7.3 and 8.2 Hz); 8.56 (d, 1H, $J = 5.7$ Hz); 8.66 (dd, 1H, $J = 8.2$ and 1.1 Hz); 8.69 (dd, 1H, $J = 8.2$ and 1.4 Hz); 8.70 (dd, 1H, $J = 5.1$ and 0.7 Hz); 9.06 (d, 1H, $J = 5.1$ Hz); 9.16 (d, 1H, $J = 5.7$ Hz); 9.68 (d, 1H, $J = 0.7$ Hz). $^{13}C$ NMR ($CDCl_3$): 117.27; 118.21; 118.48; 122.89; 123.28; 126.32; 130.86; 132.03; 133.32; 138.02; 142.48; 145.98; 146.17; 148.42; 149.15; 150.79; 155.25, 181.80. IR ($CHCl_3$) 1653, 1690 $cm^{-1}$. MS ($m/z$): 283 (100); 254 (27); 228 (8). $t_R$ is 7.05 min (95% purity).

### In vitro characterization of drug-induced effects with respect to human cancer cell line growth

Six human tumor cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). These included two glioblastomas (U-373 MG and U-87 MG), two colon (HCT-15 and LoVo), one non-small-cell-lung (A549), and one bladder (J82) cancer models. The ATCC numbers of these cell lines are HTB 14 (U-87 MG), HTB 17 (U-373 MG), CCL225 (HCT-15), CCL229 (LoVo), CCL 185 (A549), and HTB1 (J82). The cells were cultured at 37 °C in sealed (airtight) Falcon plastic dishes (Nunc, Gibco, Belgium) containing Eagle's minimal essential medium (MEM, Gibco) supplemented with 5% fetal calf serum (FCS). All the media were supplemented with a mixture of 0.6 mg/mL glutamine (Gibco), 200 IU/mL penicillin (Gibco), 200 IU/mL streptomycin (Gibco) and 0.1 mg/mL gentamycin (Gibco). The FCS was heat-inactivated for 1 h at 56 °C.

The six cell lines were incubated for 24 h in 96-microwell plates (at a concentration of 40,000 cells/mL culture medium) to ensure adequate plating prior to cell growth determination, which was carried out by means of the colorimetric MTT assay, as detailed previously.[21,22] This assessment of cell population growth is based on the capability of living cells to reduce the yellow product MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; (Sigma, St Louis, MO, USA) to a blue product, formazan, by a reduction reaction occurring in the mitochondria. The number of living cells is directly proportional to the intensity of the blue, which is quantitatively measured by spectrophotometry on a DIAS microplate reader (Dynatech Laboratories, Guyancourt, France) at a 570 nm wavelength (with a reference of 630 nm). Each experiment was carried out in sextuplicate. Nine concentrations ranging from $10^{-5}$ to $10^{-9}$ M were assayed for each of the compounds under study.

## References and Notes

1. (a) Molinski, T. *F. Chem. Rev*. **1993**, *93*, 1825. (b) Ding, Q.; Chichak, K.; Lown, J. W. *Current Med. Chem*. **1999**, *6*, 1.
2. Schmitz, F. J.; Agarwal, S. K.; Gunasekera, S. P. *J. Am. Chem. Soc*. **1983**, *105*, 4835.
3. De Guzman, F. S.; Carte, B.; Troupe, N.; Faulkner, D. J.; Harper, M. K.; Concepcion, G. P.; Mangalindan, G. C.; Matsumoto, S. S.; Barrows, L. R.; Ireland, C. M. *J. Org. Chem*. **1999**, *64*, 1400.
4. Tasdemir, D.; Marshall, K. M.; Mangalindan, G. C.; Concepcion, G. P.; Barrows, L. R.; Harper, M. K.; Ireland, C. M. *J. Org. Chem*. **2001**, *66*, 3246.
5. (a) Echevarren, A. M.; Stille, J. K. *J. Am. Chem. Soc*. **1988**, *110*, 4051. (b) Kubo, A.; Nakahara, S. *Heterocycles* **1988**, *27*, 2095. (c) Prager, R. H.; Tsopelas, C. *Heterocycles* **1989**, *29*, 847.
6. Kitahara, Y.; Tamura, F.; Kubo, A. *Chem. Pharm. Bull*. **1994**, *42*, 1363.
7. Kitahara, Y.; Tamura, F.; Kubo, A. *Tetrahedron Lett*. **1997**, *38*, 4441.
8. (a) Potts, K. T.; Walsh, E. B.; Bhattacharjee, D. *J. Org. Chem*. **1987**, *52*, 2285. (b) Nebois, P.; Fillion, H. *Tetrahedron Lett*. **1991**, *32*, 1307. (c) Nebois, P.; Carneiro do Nascimento, S.; Boitard, M.; Bartoli, M. H.; Fillion, H. *Pharmazie* **1994**, *49*, 819. (d) Bouaziz, Z.; Nebois, P.; Fillion, H.; Luche, J. L.; Jenner, G. H. *Tetrahedron* **1995**, *51*, 4057.
9. Delfourne, E.; Darro, F.; Bontemps-Subielos, N.; Decaestecker, C.; Bastide, J.; Frydman, A.; Kiss, R. *J. Med. Chem*. **2001**, *44*, 3275.
10. Severin, T.; Wanninger, G.; Lerche, H. *Chem. Ber*. **1984**, *117*, 2875.

11. Kitahara, Y.; Onikura, H.; Shibano, Y.; Watanabe, S.; Mikami, Y.; Kubo, A. *Tetrahedron* **1997**, *53*, 6001.
12. Ghosh, S.; Schlosser, M. *J. Fluorine Chem.* **1994**, *67*, 53.
13. Blanco, M. M.; Avendano, C.; Menendez, J. C. *Synlett* **2000**, 689.
14. Bracher, F. *Heterocycles* **1989**, *29*, 2093.
15. Kobayashi, J.; Cheng, J. F.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Nozoe, S. *Tetrahedron Lett.* **1988**, *29*, 1177.
16. Delfourne, E.; Bontemps-Subielos, N.; Darro, F.; Bastide, J.; Kiss, R.; Frydman, A. International Patent Application WO0055160, 21 September 2000.
17. Alvarez, M.; Feliu, W.; Joule, J. A.; Fernandez-Puentes, J. L. *Eur. J. Org. Chem.* **2000**, 849.
18. Stewart, J. J. P. *MOPAC 2000*; Fujitsu Limited: Tokyo, Japan, 1999. Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209.
19. Ryu, C. K.; Lee, I. K.; Jung, S. H.; Kang, H. Y.; Lee, C. O. *Med. Chem. Res.* **2000**, *10*, 40.
20. Weinstein, J. N.; Kohn, K. W.; Grever, M. R.; Viswanadhan, V. N.; Rubinstein, L. V.; Monks, A. P.; Scudiero, D. A.; Welch, L.; Koutsoukos, A. D.; Chiausa, A. J.; Paull, K. D. *Science* **1992**, *258*, 447.
21. Pauwels, O.; Kiss, R.; Pasteels, J. L.; Atassi, G. *Pharm. Res.* **1995**, *12*, 1011.
22. Camby, I.; Salmon, I.; Danguy, A.; Pasteels, J. L.; Brotchi, J.; Martinez, J.; Kiss, R. *J. Natl. Cancer Inst.* **1996**, *88*, 594.