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Bioorganic & Medicinal Chemistry 12 (2004) 3987–3994
Bioorganic & Medicinal Chemistry

Synthesis and in vitro antitumor activity of ring C and D-substituted phenanthrolin-7-one derivatives, analogues of the marine pyridoacridine alkaloids ascididemin and meridine

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Received 30 April 2004; revised 28 May 2004; accepted 2 June 2004
Available online 24 June 2004

Abstract—A series of cycle C and D-substituted phenanthrolin-7-ones, analogues of the marine pyridoacridines meridine and ascididemin have been synthesized on the basis of Diels–Alder reactions involving quinoline-5,8-dione and 2- (or un)-substituted-*N*,*N*-dimethylhydrazones. All the compounds were evaluated for in vitro cytotoxic activity against 12 distinct human cancer cell lines. They all exhibit cytotoxic activity with IC_{50} values at least of micromolar order.

1. Introduction

A considerable amount of attention has been focused on the marine pyridoacridine alkaloids due to their potentially valuable biological activity.[1] The important cytotoxic properties of ascididemin **1** and meridine **2**, two members of this group have made them good candidates to design new and useful anticancer drugs.[2] In a preliminary work, we have been interested in phenanthrolin-7-ones derivatives **3**, analogues of the two natural products.[3] These compounds mainly substituted on ring A, except one with a methoxy group as R_2 (ring C substitution), exhibited good cytotoxic activity with IC_{50} values at least of micromolar order (Fig. 1).

We report herein the continuation of this research in which we investigated if the introduction of substituents in cycles C and D was able to increase the antitumor potential.

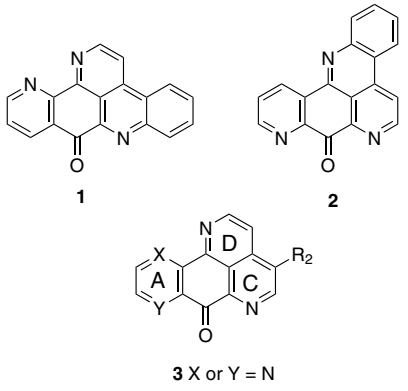

Figure 1.

2. Chemistry

As previously described phenanthrolin-7-one derivatives were generally prepared in two steps: hetero-Diels–Alder reaction of quinoline-5,8-diones with aldehyde-*N*,*N*-dimethylhydrazones to give the two regio-diazaanthraquinone adducts and subsequent ring D annelation via Bracher's methodology[4] involving DMF–DEA to form

Keywords: Marine alkaloids; Pyridoacridine; Ascididemin; Meridine.
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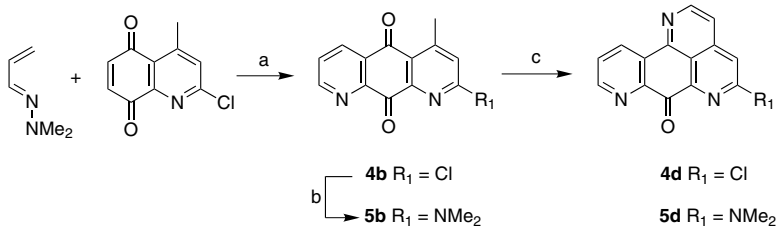
doi:10.1016/j.bmc.2004.06.006

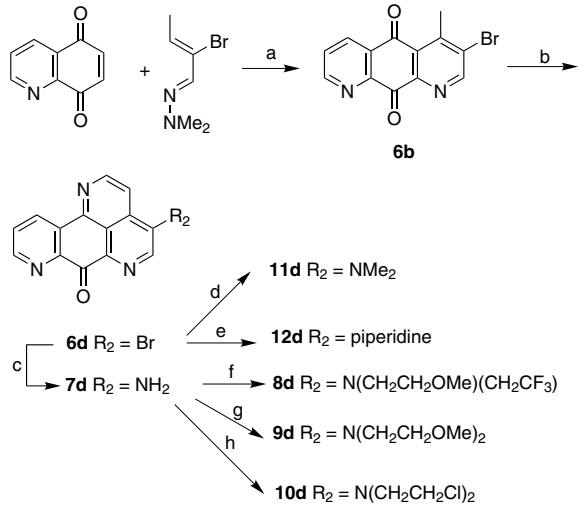


Scheme 1. Reagents and conditions: (a) (1) $CHCl_3$, Ac_2O, rt, 18 h; (2) MnO_2, $CHCl_3$, rt, overnight. (b) $Me_2NH{\cdot}HCl$, NaOH, THF/H_2O, reflux, 1 h. (c) (1) DMF–DEA, DMF, N_2, reflux, 1 h; (2) NH_4Cl, MeOH, reflux, 30 min.

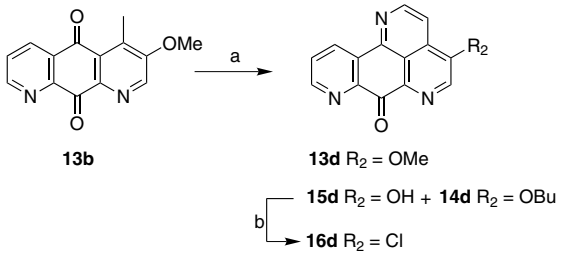
an intermediate enamine, which is then cyclized with ammonium chloride in ethanol. As indicated in Scheme 1, the reaction of 2-chloro-4-methylquinoline-5,8-dione with 2-propenal-*N*,*N*-dimethylhydrazone led exclusively to the adduct **4b**, which was converted into the dimethylamino derivative **5b** by the action of dimethylamine in the mixture THF/H_2O.

These two compounds gave, respectively, the tetracyclic compounds **4d** and **5d**. In the same way, the bromo-compound **6b** was obtained by the reaction of quinoline-5,8-dione with 2-bromo-2-butenal-*N*,*N*-dimethylhydrazone and was transformed into the bromotetracyclic derivative **6d** (Scheme 2).

Action of sodium azide on this last compound resulted in the replacement of the bromo atom by an amino group (compound **7d**). Different conditions were assayed in order to make reductive alkylation of this compound. The reaction of **7d** with methoxyacetaldehyde dimethylacetal and subsequent addition of $NaBH_3CN$ (95 °C for 2 h) gave compound **8d** whereas the same reaction, but



Scheme 2. Reagents and conditions: (a) (1) Toluene, Ac_2O, rt, 14 h; (2) 85% MnO_2, 8 h. (b) (1) DMF–DEA, DMF, N_2, 120 °C, 30 min; (2) NH_4Br, AcOH, 110 °C, 30 min. (c) NaN_3, DMF, 80 °C, 30 min. (d) $Me_2NH{\cdot}HCl$, NaOH, THF/H_2O 2:1, reflux, 1.5 h. (e) Piperidine, THF/H_2O 2:1, 80 °C, 40 min. (f) TFA, 2-methoxyacetaldehyde dimethylacetal, $NaBH_3CN$, 95 °C, 2 h. (g) 2-Methoxyacetaldehyde, TFA, $NaBH_3CN$, rt, 35 min. (h) 50% aq chloroacetaldehyde, TFA, $NaBH_3CN$, rt, 30 min.



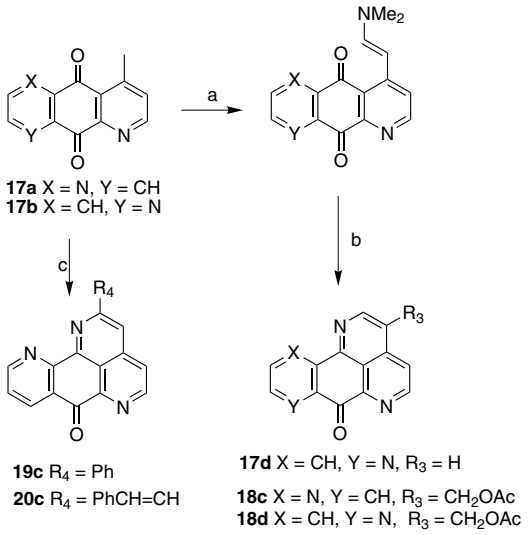
Scheme 3. Reagents and conditions: (a) (1) DMF–DEA, DMF, N_2, 120 °C, 1 h; (2) NH_4Cl, MeOH, reflux, 30 min for **13d**; NH_4Cl, BuOH, reflux, 16 h for **14d** and **15d**. (b) $POCl_3$, reflux, 2 h.

involving 2-methoxyacetaldehyde (instead of its dimethylacetal derivative), at room temperature, led to compound **9d**. The structure of **8d** could be explained by the formation in a first step of the trifluoroacetamide derivative (resulting from the reaction of the amine **7d** with trifluoroacetic acid), which reacts in a second step with 2-methoxyacetaldehyde dimethylacetal and $NaBH_3CN$. Compound **10d** was obtained when **7d** was reacted with chloroacetaldehyde and $NaBH_3CN$. The two other amines **11d** and **12d** were obtained directly from the bromo-derivative **6d** by the action, respectively, of dimethylamine and piperidine. Compound **13d** was synthesized from 2-methoxy-6-methylpyrido[4,3-*g*]quinoline-5,10-dione **13b** as previously described,[3] **14d** and **15d** were also synthesized from **13b** with butanol instead of acetic acid or methanol in Bracher's methodology (Scheme 3).

The chloro-derivative **16d** was prepared by the action of $POCl_3$ on the hydroxy-derivative **15d**.

All the cycle D-substituted derivatives were obtained from the already known tricyclic Diels–Alder adducts **17a** and **17b**[3] according to methods previously reported by Lindsay et al. for the synthesis of ring-E analogues of ascididemin.[5]

These compounds were first reacted with DMF–DEA in DMF to form the corresponding intermediate enamines and Eschenmoser's salt was added to give, respectively, **18c** and **18d** whereas, as seen before, reaction of **17b** with NH_4Cl in ethanol gave **17d** (Scheme 4). Reaction of **17a** with benzaldehyde or cinnamaldehyde in presence of NH_4Cl gave **19c** and **20c**, respectively.



Scheme 4. Reagents and conditions: (a) DMF–DEA, DMF, N_2, 115 °C, 30 min; (b) NH_4Cl, MeOH, reflux, 30 min for **17d**; DMF, Eschenmoser's salt, NH_4Cl, AcOH, 115 °C, 30 min for **18c** and **18d**; (c) NH_4Cl, AcOH, N_2, benzaldehyde or cinnamaldehyde, reflux, 15 h or 4 h.

3. Pharmacology

3.1. In vitro determination of the drug-induced inhibition of human cancer cell line growth

For each of the compounds under study, six concentrations were tested on 12 distinct human cancer cell lines including various histopathological types (glioblastomas and breast, colon, lung, prostate and bladder cancers). We made use of the colorimetric MTT assay, which indirectly assesses the effect of potentially anticancer compounds on overall growth of adherent cell lines.[6] The IC_{50} values, that is the concentration, which reduced the mean growth value of the 12 cell lines by 50%, were determined for each drug, as compared to the mean control growth value. Table 1 illustrates the individual IC_{50} values of the different compounds obtained for each of the 12 cell lines under study.

4. Discussion

The present study was focused on rings D and C modified phenanthrolin-7-ones derivatives. In vitro data obtained showed that most of the synthesized compounds have cytotoxic properties with IC_{50} values in the micromolar–nanomolar range. Compounds **9d** and/or **12d** exhibited interesting IC_{50} values of nanomolar order towards the glioblastoma, bladder, breast, lung and prostate cell lines. The potency of most compounds towards J-82 (bladder), T-47D (breast) and A-549 (lung) cell lines was moderate with only two compounds **12d** and **13d** having a higher cytotoxic activity towards one of these cell lines T-47D ($IC_{50} = 6$ nM **12d**) and A-549 ($IC_{50} = 50$ nM **13d**), respectively. Compound **13d** was the less selective with IC_{50} values between 50 and 90 nM for nine cell lines. For the 11 R_2-substituted compounds, no clear-cut relationship emerged for cytotoxicity and physical parameters (polarity, hydrophobicity, steric parameters). The most active compounds are substituted by an electron-donating group without active hydrogen.

5. Experimental section

5.1. Chemistry

^1H 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

Table 1. Characterization of the in vitro cytotoxic-related antitumor effects (IC_{50} value $\times 10^{-6}$ M) of the compounds listed

Compounds	Cell lines											
	U-87MG	U-373MG	SW1088	T24	J82	HCT-15	LoVo	MCF7	T-47D	A-549	A-427	PC-3
4d	5	5	7	6	>10	6	6	7	>10	6	5	>10
6d	2	5	5	6	6	4	5	4	5	7	4	6
7d	3	5	4	4	>10	6	6	2	9	5	7	7
8d	0.9	0.4	0.1	0.08	4	0.7	0.2	0.3	0.9	6	2	0.7
9d	0.9	0.7	0.009	0.009	>10	0.9	0.8	0.5	>10	1	0.08	0.007
10d	0.09	0.05	0.05	0.02	0.5	0.08	0.03	0.02	0.6	0.5	0.4	0.05
11d	0.7	0.6	0.5	0.5	0.7	0.9	4	0.5	5	0.6	0.3	0.6
12d	0.08	0.3	0.002	0.0004	5	0.09	0.06	0.009	0.006	0.7	0.006	0.0004
13d	0.05	0.07	0.08	0.06	0.8	0.09	0.7	0.06	6.0	0.05	0.07	0.08
14d	2	0.6	4	0.7	4	3	4	0.8	4	6	0.6	0.5
15d	>10	>10	>10	>10	>10	>10	>10	>10	>10	8	>10	>10
16d	6	5	5	5	3	6	6	2	4	5	3	6
17d	6	4	5	0.8	5	0.6	2	0.6	0.8	3	0.7	0.9
18c	0.6	0.8	0.5	0.6	0.6	0.9	0.8	0.6	1	4	4	4
18d	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

The IC_{50} value constitutes the concentration of the compound, which inhibits the growth of the human cancer cells by 50% as compared to the control value.

Six concentrations ranging from 10 μm to 0.1 nM were assayed on 12 different human cancer cell lines for each compound under study. The drug-induced effects at cell line growth level were determined.

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 ascididemin analogues was evaluated on two analytical chromatographic systems. System I consisted of a, 5 μm column (250 mm × 4.6 mm), CH_3CN/H_2O/TFA (see composition) at 1 mL/min flow rate, 260 nm and the system II consisted of a Kromasil SI 5 μm 100 A column (250 mm × 4.6 mm), isooctane/EtOH (see composition) at 1 or 2 mL/min flow rate, 250 nm.

5.1.1. 2-Bromo-2-butenal-*N*,*N*-dimethylhydrazone. To a solution of 2-bromo-2-butenal[7] (5 g, 33.55 mmol) and acetic acid (1 mL) in toluene (38 mL) was added dropwise dimethylhydrazine (3.8 mL, 50.33 mmol). The reaction mixture was warmed at 70 °C for 1 h. The organic layer was washed with H_2O (2 × 30 mL) and dried over $MgSO_4$. After concentration over vacuum the expected diene was obtained as a yellow oil (3.9 g, 60%). ^1H NMR ($CDCl_3$) 1.91 (d, 3H, $J = 6.8$ Hz); 2.91 (s, 6H); 6.12 (q, 1H, $J = 6.8$ Hz); 6.83 (s, 1H). ^{13}C NMR ($CDCl_3$) 17.17; 43.07 (2C); 125.12; 127.66; 131.44.

5.1.2. 2-Chloro-4-methylpyrido[3,2-*g*]quinoline-5,10-dione (4b). A mixture of 2-chloro-4-methylquinoline-5,8-dione (1.1 g, 5.30 mmol), 2-propenal-*N*,*N*-dimethylhydrazone (575 mg, 5.87 mmol) and acetic anhydride (1.5 mL, 15.9 mmol) in $CHCl_3$ (60 mL) was stirred at room temperature for 18 h. After concentration, 85% MnO_2 (7.25 g, 70.9 mmol) and $CHCl_3$ (230 mL) were added and the mixture was stirred overnight at room temperature. The reaction media was filtered over Celite and the filtrate was concentrated over vacuum. Purification on flash chromatography (CH_2Cl_2/MeOH 99:1) gave the expected compound as a brown solid (606 mg, 44%), mp 226 °C. ^1H NMR ($CDCl_3$) 2.89 (d, 3H, $J = 0.8$ Hz); 7.58 (d, 1H, $J = 0.8$ Hz); 7.78 (dd, 1H, $J = 4.4$ and 8.1 Hz); 8.60 (dd, 1H, $J = 8.1$ and 1.9 Hz); 9.15 (dd, 1H, $J = 4.4$ and 1.9 Hz). ^{13}C NMR ($CDCl_3$) 22.67; 127.26; 128.44; 130.70; 131.95; 135.56; 147.67; 150.12; 154.58; 155.54; 156.68; 178.95; 183.24. IR ($CHCl_3$) 1676, 1705 cm^{-1}.

5.1.3. 2-Dimethylamino-4-methylpyrido[3,2-*g*]quinoline-5,10-dione (5b). A mixture of compound **4b** (250 mg, 0.97 mmol), dimethylamine, HCl (395 mg, 4.85 mmol), NaOH (193 mg, 4.83 mmol) in THF/H_2O (7 mL/3.5 mL) was refluxed for 1 h. The reaction media was concentrated and CH_2Cl_2/MeOH 95:5 (60 mL) were added. The organic layer was recovered, washed with H_2O and dried over $MgSO_4$. After concentration over vacuum the crude product was purified by flash chromatography (CH_2Cl_2/MeOH 99:1) to give the expected compound as an orange solid (115 mg, 45%), mp 248 °C. ^1H NMR ($CDCl_3$) 2.77 (d, 3H, $J = 0.7$ Hz); 3.31 (s, 6H); 6.58 (d, 1H, $J = 0.7$ Hz); 7.67 (dd, 1H, $J = 4.6$ and 8.0 Hz); 8.54 (dd, 1H, $J = 8.0$ and 1.9 Hz); 9.02 (dd, 1H, $J = 4.6$ and 1.9 Hz). ^{13}C NMR ($CDCl_3$) 23.58; 38.00 (2C); 111.96; 119.18; 127.80; 131.16; 135.03; 148.09; 150.84; 151.54; 154.07; 159.73; 181.49; 182.34. IR ($CHCl_3$) 1651, 1699 cm^{-1}.

5.1.4. 3-Bromo-4-methylpyrido[3,2-*g*]quinoline-5,10-dione (6b). A mixture of quinoline-5,8-dione (10 g, 62.9 mmol), 2-bromo-2-butenal-*N*,*N*-dimethylhydrazone (18 g, 94.2 mol) and acetic anhydride (60 mL, 0.63 mmol) in toluene (140 mL) was stirred at room temperature for 14 h. MnO_2 (85%, 64 g, 0.62 mol) was added and the mixture was stirred for 8 h. The reaction medium was filtered over Celite and the filtrate was concentrated under vacuum. Flash chromatography (CH_2Cl_2/MeOH 95:5) gave the expected compound, which was washed with diethyl ether, beige solid (3.7 g, 20%), mp > 260 °C. ^1H NMR ($CDCl_3$) 3.03 (s, 3H); 7.79 (dd, 1H, $J = 8.1$ and 4.4 Hz); 8.61 (dd, 1H, $J = 1.8$ and 8.1 Hz); 9.15 (dd, 1H, $J = 4.4$ and 1.8 Hz); 9.16 (s, 1H). ^{13}C NMR ($CDCl_3$) 21.30; 128.46; 129.46; 130.98; 131.36; 135.78; 147.74; 148.45; 150.57; 155.56; 156.16; 179.91; 183.58. IR ($CHCl_3$) 1679, 1698 cm^{-1}.

5.1.5. 5-Chloro-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (4d). A mixture of compound **4b** (210 mg, 0.82 mmol) and DMF–DEA (0.52 mL, 3.03 mmol) in DMF (4 mL) was refluxed, under nitrogen atmosphere for 1 h. After concentration, NH_4Cl (2 g, 37.4 mmol) and methanol (180 mL) were added and the mixture was refluxed for 30 min. After concentration, a solution of $NaHCO_3$ (100 mL) was added and the mixture was extracted with CH_2Cl_2 (4 × 100 mL). After drying over $MgSO_4$, the organic layers were concentrated and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH 98:2) to give the tetracyclic compound as a brown solid (22 mg 10%), mp > 260 °C. ^1H NMR ($CDCl_3$) 7.72 (d, 1H, $J = 5.9$ Hz); 7.77 (dd, 1H, $J = 4.8$ and 8.1 Hz); 8.07 (s, 1H); 8.92 (d, 1H, $J = 5.9$ Hz); 9.07 (dd, 1H, $J = 1.9$ and 4.8 Hz); 9.16 (dd, 1H, $J = 8.1$ and 1.9 Hz). ^{13}C NMR ($CDCl_3$) 118.24; 118.91; 123.98; 128.29; 132.60; 133.88; 140.90; 147.40; 147.94; 148.21; 150.21; 152.10; 153.31; 179.40. IR ($CHCl_3$) 1696 cm^{-1}. MS: m/z 269 (53); 267 (100); 239 (72); 204 (51); 177 (29). t_R is 4.55 min (99.5% purity), using system I (CH_3CN/H_2O/TFA 50:50:0.1), and t_R is 15.35 min (98.1% purity), using system II (isooctane/EtOH 80:20), flow rate 1 mL/min.

5.1.6. 5-Dimethylamino-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (5d). A mixture of compound **5b** (100 mg, 0.37 mmol) and DMF–DEA (0.3 mL, 1.75 mmol) in DMF (1.5 mL) was refluxed, under nitrogen atmosphere for 1 h. NH_4Cl (150 mg, 2.8 mmol) and H_2O (13 mL) were added and the mixture was warmed at 100 °C for 1 h. H_2O (15 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 30 mL). After drying over $MgSO_4$, the organic layers were concentrated and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH 99:1) to give the tetracyclic compound as a purple solid (3.2 mg, 3%), which decomposed before melting. ^1H NMR ($CDCl_3$) 3.34 (s,

6H); 6.82 (s, 1H); 7.43 (d, 1H, $J = 5.8$ Hz); 7.68 (dd, 1H, $J = 4.4$ and 8.0 Hz); 8.52 (d, 1H, $J = 5.8$ Hz); 9.00 (dd, 1H, $J = 1.4$ and 4.4 Hz); 9.07 (dd, 1H, $J = 8.0$ and 1.4 Hz). ^{13}C NMR ($CDCl_3$) 38.37 (2C); 100.36; 113.99; 119.07; 127.62; 132.97; 133.57; 140.96; 146.06; 146.92; 147.85; 149.31; 152.37; 158.76; 181.15. IR ($CHCl_3$) 3686; 1687; 1613 cm^{-1}. MS: m/z 276 (100); 261 (20); 247 (15); 233 (83); 205 (59); 178 (48). t_R is 9.81 min (96.2% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 12.02 min (100% purity), using system II (isooctane/EtOH 80:20), flow rate 1 mL/min.

5.1.7. 4-Bromo-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (6d). A mixture of compound **6b** (100 mg, 0.33 mmol), DMF–DEA (0.2 mL, 1.12 mmol) in DMF (1 mL) was warmed at 120 °C, under nitrogen atmosphere for 30 min. After concentration, NH_4Br (0.97 g, 9.9 mmol) and acetic acid (1.8 mL) were added and the mixture was warmed at 110 °C for 30 min. After concentration, H_2O (10 mL) was added and the mixture was extracted with $CHCl_3$ (3×20 mL). After drying over $MgSO_4$, the organic layers were concentrated and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH 98:2) to give the expected tetracyclic compound as a red solid (63 mg, 61%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 7.78 (dd, 1H, $J = 4.6$ and 8.2 Hz); 8.04 (d, 1H, $J = 5.8$ Hz); 9.03 (d, 1H, $J = 5.8$ Hz); 9.07 (dd, 1H, $J = 1.5$ and 4.6 Hz); 9.19 (dd, 1H, $J = 8.2$ and 1.5 Hz); 9.34 (s, 1H). ^{13}C NMR ($CDCl_3$) 119.06; 120.21; 124.03; 128.22; 132.60; 134.10; 138.61; 146.64; 147.67; 148.69; 150.11; 150.63; 153.34; 180.24. IR ($CHCl_3$) 1692 cm^{-1}. MS: m/z 313 (12); 311 (10); 232 (61); 204 (78); 177 (35). t_R is 4.79 min (99.3% purity), using system I (CH_3CN/H_2O/TFA 50:50:0.1), and t_R is 11.6 min (97.1% purity), using system II (isooctane/EtOH 80:20), flow rate 1 mL/min.

5.1.8. 4-Amino-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (7d). A mixture of compound **6d** (0.3 g, 0.96 mmol) and NaN_3 (0.6 g, 9.23 mmol) in DMF (20 mL) was warmed at 80 °C for 30 min. After concentration, the crude product was purified by flash chromatography (LiChroprep RP-8, 40–63 μm MeOH/H_2O 1:1) to give the expected amino-derivative as a purple solid (60 mg, 25%), mp > 260 °C. ^{1}H NMR (DMSO-d_6) 7.83 (dd, 1H, $J = 4.1$ and 7.7 Hz); 7.95 (br s, 2H); 8.28 (d, 1H, $J = 5.8$ Hz); 8.43 (s, 1H); 8.86 (d, 1H, $J = 5.8$ Hz); 8.96 (dd, 1H, $J = 1.2$ and 4.1 Hz); 9.13 (dd, 1H, $J = 7.7$ and 1.2 Hz). ^{13}C NMR (DMSO-d_6) 116.85; 120.20; 124.33; 127.61; 132.23; 133.56; 133.59; 134.60; 144.78; 145.64; 147.79; 148.86; 152.63; 177.99. IR (KBr) 3379; 3174; 1654; 1636 cm^{-1}. t_R is 6.10 min (95.9% purity), using system I (CH_3CN/H_2O/TFA 20:80:0.1), and t_R is 11.70 min (96.6% purity), using system II (isooctane/EtOH 70:30), flow rate 1 mL/min.

5.1.9. 4-[(2′-Methoxyethyl)(2″,2″,2″-trifluoroethyl)amino]-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (8d). To a solution of compound **7d** (142 mg, 0.57 mmol) in TFA (4.2 mL) was first added 2-methoxyacetaldehyde dimethylacetal (0.38 mL, 2.9 mmol). After 5 min, $NaBH_3CN$ (0.53 g, 8.4 mmol) was subsequently added and the reaction media was warmed at 95 °C for 2 h. After cooling down, the mixture was made alkaline with 5 N NaOH and extracted with CH_2Cl_2/MeOH 95:5 (3×100 mL). The organic layers were dried over $MgSO_4$ and were concentrated over vacuum. Purification by flash chromatography (CH_2Cl_2/MeOH 98:2) gave the expected tetracyclic compound as a brown solid (13 mg, 6%), which decomposes before melting. ^{1}H NMR ($CDCl_3$) 3.29 (s, 3H); 3.50 (t, 2H, $J = 5.0$ Hz); 3.76 (t, 2H, $J = 5.0$ Hz); 4.22 (q, 2H, $J_{H-F} = 8.8$ Hz); 7.75 (dd, 1H, $J = 4.8$ and 8.1 Hz); 8.01 (d, 1H, $J = 5.9$ Hz); 8.92 (d, 1H, $J = 5.9$ Hz); 8.94 (s, 1H); 9.03 (dd, 1H, $J = 1.8$ and 4.8 Hz); 9.18 (dd, 1H, $J = 8.1$ and 1.8 Hz). ^{13}C NMR ($CDCl_3$) 54.53 (q, $J = 33$ Hz); 54.94; 59.04; 70.52; 116.54; 120.29; 124.95 (q, $J = 280$ Hz); 128.04; 132.96; 134.03; 134.66; 142.69; 143.52; 145.12; 147.01; 147.69; 149.97; 152.92; 180.12. IR ($CHCl_3$) 1684 cm^{-1}. MS (m/z) 388 (29); 343 (100); 262 (11); 204 (22). t_R is 5.09 min (95% purity), using system I (CH_3CN/H_2O/TFA 50:50:0.1), and t_R is 7.87 min (95.9% purity), using system II (isooctane/EtOH 80:20), flow rate 2 mL/min.

5.1.10. 4-Bis-(2′methoxyethyl]amino)-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (9d). 2-Methoxyacetaldehyde dimethylacetal (0.96 g, 8 mmol) in 0.5 N HCl (14.4 mL, 7.2 mmol) was warmed at 50 °C for 30 min. Compound **7d** (200 mg, 0.8 mmol) and TFA (6 mL) were added and the mixture was stirred at room temperature for 25 min. $NaBH_3CN$ (0.2 g, 3.17 mmol) was added and the mixture was stirred for an additional 35 min. The mixture was made alkaline with 1 N NaOH (130 mL), extracted with CH_2Cl_2 (2×120 mL) and the organic layers were washed with H_2O (60 mL). They were dried over $MgSO_4$ and concentrated over vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to give the tetracyclic compound as a red solid (81 mg, 28%), which decomposes before melting. ^{1}H NMR ($CDCl_3$) 3.33 (s, 6H); 3.67 (t, 4H, $J = 5.5$ Hz); 3.83 (t, 4H, $J = 5.5$ Hz); 7.70 (dd, 1H, $J = 4.4$ and 8.0 Hz); 8.15 (d, 1H, $J = 5.9$ Hz); 8.76 (s, 1H); 8.80 (d, 1H, $J = 5.9$ Hz); 9.02 (dd, 1H, J = 1.8 and 4.8 Hz); 9.17 (dd, 1H, $J = 8.0$ and 1.8 Hz). ^{13}C NMR ($CDCl_3$) 52.93 (2C); 59.16 (2C); 70.08 (2C); 117.71; 120.50; 127.45; 131.77; 132.78; 133.77; 138.72; 139.57; 145.35; 146.42; 148.04; 149.32; 152.69; 179.85. IR ($CHCl_3$) 1674; 1602 cm^{-1}. MS (m/z) 364 (27); 319 (100); 287 (15); 261 (98). t_R is 6.67 min (98.5% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 15.01 min (97.2% purity), using system II (isooctane/EtOH 80:20), flow rate 2 mL/min.

5.1.11. 4-[Bis-(2′chloroethyl)amino]-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (10d). To a solution of compound **7d** (0.25 g, 1 mmol) in TFA (7.5 mL) was first added chloroacetaldehyde (50% in H_2O) (0,77 mL, 6 mmol). After 5 min, $NaBH_3CN$ (0.25 g, 4 mmol) was added and the reaction media was stirred at room temperature for 30 min. The mixture was made alkaline with 1 N NaOH and extracted with CH_2Cl_2/MeOH 95:5

(3×100 mL). The organic layers were washed over $MgSO_4$ and concentrated over vacuum. Purification by flash chromatography (CH_2Cl_2/MeOH 95:5) gave the expected compound as a red solid (133 mg, 36%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 3.70 (t, 4H, $J = 6$ Hz); 3.96 (t, 4H, $J = 6.0$ Hz); 7.73 (dd, 1H, $J = 4.6$ and 8.1 Hz); 7.99 (d, 1H, $J = 5.9$ Hz); 8.86 (s, 1H); 8.91 (d, 1H, $J = 5.9$ Hz); 9.05 (dd, 1H, $J = 1.6$ and 4.6 Hz); 9.19 (dd, 1H, $J = 8.1$ and 1.6 Hz). ^{13}C NMR ($CDCl_3$) 41.30 (2C); 54.87 (2C); 116.69; 120.49; 127.76; 132.87; 133.89; 134.01; 141.28; 142.20; 144.31; 146.61; 147.98; 150.12; 153.00; 179.95. IR ($CHCl_3$) 1681 cm^{-1}. t_R is 5.07 min (99.6% purity), using system I (CH_3CN/H_2O/TFA 50:50:0.1), and t_R is 7.16 min (98.8% purity), using system II (isooctane/EtOH 80:20), flow rate 2 mL/min.

5.1.12. 4-Dimethylamino-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (11d). A solution of compound **6d** (14 mg, 0.052 mmol), dimethylamine, HCl (24 mg, 0.29 mmol) and NaOH (13 mg, 0.32 mmol) in THF/H_2O (2 mL/1 mL) was refluxed for 1.5 h. After concentration, H_2O (15 mL) was added and the mixture was extracted with $CHCl_3$ (3×20 mL). The organic layers were dried over $MgSO_4$ and concentrated over vacuum. The crude product was purified by flash chromatography ($CHCl_3$/MeOH, 95:5) to give the expected dimethylamino-derivative as a red solid (9 mg, 63%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 3.34 (s, 6H); 7.71 (dd, 1H, $J = 4.4$ and 8.1 Hz); 7.96 (d, 1H, 6.0 Hz); 8.62 (s, 1H); 8.83 (d, 1H, $J = 6.0$ Hz); 9.04 (dd, 1H, $J = 1.5$ and 4.4 Hz); 9.19 (dd, 1H, $J = 1.5$ and 8.1 Hz). ^{13}C NMR ($CDCl_3$) 44.06 (2C); 117.89; 120.40; 127.22; 129.69; 132.59; 133.68; 135.30; 138.51; 144.67; 146.98; 148.14; 149.16; 152.66; 179.55. IR ($CHCl_3$) 1666 cm^{-1}. MS: m/z 276 (100); 249 (11); 204 (1). t_R is 10.41 min (97.2% purity), using system I (CH_3CN/H_2O/TFA 20:80:0.1), and t_R is 22.38 min (98.8% purity), using system II (isooctane/EtOH 80:20), flow rate 2 mL/min.

5.1.13. 4-(1′Piperidino)-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (12d). A mixture of compound **6d** (50 mg, 0.16 mmol) and piperidine (1.16 mL, 1.92 mmol) in THF/H_2O (2.4 mL/1.2 mL) was warmed at 80 °C for 40 min. H_2O (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3×30 mL). The organic layers were dried over $MgSO_4$ and concentrated over vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to give the expected compound as an ochre solid (36 mg, 59%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 1.75–1.80 (m, 4H); 1.88–1.94 (m, 2H); 3.46 (t, 4H, $J = 5.5$ Hz); 7.72 (dd, 1H, $J = 4.4$ and 8.0 Hz); 7.85 (d, 1H, $J = 5.9$ Hz); 8.69 (s, 1H); 8.85 (d, 1H, $J = 5.9$ Hz); 9.04 (dd, 1H, $J = 1.8$ and 4.4 Hz); 9.18 (dd, 1H, $J = 8.0$ and 1.4 Hz). ^{13}C NMR ($CDCl_3$) 24.16; 26.06 (2C); 53.78 (2C); 117.38; 120.40; 127.52; 131.97; 132.80; 133.77; 137.44; 140.19; 145.58; 147.66; 148.14; 149.54; 152.79; 179.92. IR ($CHCl_3$) 1676; 1602 cm^{-1}. MS (m/z) 316 (97); 315 (100); 274 (7); 260 (16). t_R is 4.23 min (99.5% purity), using system I (CH_3CN/H_2O/TFA 50:50:0.1), and t_R is 6.20 min (99.3% purity), using system II (isooctane/EtOH 80:20), flow rate 2 mL/min.

5.1.14. 4-Butoxy-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (14d) and 4-hydroxy-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (15d). A mixture of compound **13b**[3] (225 mg, 0.89 mmol), DMF–DEA (0.52 mL, 3.03 mmol) in DMF (3 mL) was warmed at 120 °C, under nitrogen atmosphere for 1 h. After concentration, NH_4Cl (0.5 g, 9.35 mmol) and butanol (75 mL) were added and the mixture was refluxed for 16 h. After concentration, H_2O (50 mL) was added and the mixture was extracted with CH_2Cl_2 (4×100 mL). After drying, the organic layers were concentrated and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH 98:2) to give the tetracyclic compounds.

Compound **14d**. Orange solid (83 mg, 31%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 1.06 (t, 3H, $J = 7.7$ Hz); 1.61 (m, 2H); 1.99 (m, 2H); 4.44 (t, 2H, $J = 6.6$ Hz); 7.74 (dd, 1H, $J = 4.4$ and 8.0 Hz); 8.10 (d, 1H, $J = 5.8$ Hz); 8.70 (s, 1H); 8.92 (d, 1H, $J = 5.8$ Hz); 9.05 (dd, 1H, $J = 4.4$ and 1.6 Hz); 9.19 (dd, 1H, $J = 1.6$ and 8.0 Hz). ^{13}C NMR ($CDCl_3$) 13.82; 19.22; 31.00; 69.72; 115.27; 119.88; 127.64; 130.09; 130.22; 132.87; 133.73; 140.52; 146.68; 148.07; 148.65; 152.66; 152.81; 179.87. IR ($CHCl_3$) 1683 cm^{-1}. MS: m/z 305 (53); 250 (35); 249 (100); 221 (88); 220 (46); 194 (9); 193 (32). t_R is 8.21 min (97.4% purity), using system I (CH_3CN/H_2O/TFA 50:50:0.1), and t_R is 8.84 min (96.9% purity), using system II (isooctane/EtOH 80:20), flow rate 1 mL/min.

Compound **15d**. Red solid (38 mg, 17%), mp > 260 °C. ^{1}H NMR (DMSO-d_6) 7.98 (dd, 1H, $J = 4.4$ and 8.1 Hz); 8.10 (s, 1H); 8.13 (d, 1H, $J = 5.1$ Hz); 8.84 (br s, 1H); 8.96 (d, 1H, 5.1 Hz); 9.10 (dd, 1H, $J = 4.4$ and 1.6 Hz); 9.34 (dd, 1H, $J = 1.6$ and 8.1 Hz). ^{13}C NMR ($CDCl_3$) 116.37; 118.46; 120.09; 126.13; 128.63; 129.97; 132.92; 141.97; 142.78; 144.72; 147.48; 150.09; 151.04; 175.76. MS: m/z 249 (19); 248 (100); 221 (9); 220 (39); 193 (40); 192 (12); 167 (7); 166 (27). t_R is 5.97 min (99.3% purity), using system I (CH_3CN/H_2O/TFA 50:50:0.1), and t_R is 6.57 min (99% purity), using system II (isooctane/EtOH 80:20), flow rate 1 mL/min.

5.1.15. 4-Chloro-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (16d). A solution of compound **15d** (45 mg, 0.18 mmol) in $POCl_3$ (3.5 mL) was refluxed for 2 h. After concentration over vacuum, the reaction media was made alkaline with 1 N $NaHCO_3$ (10 mL). The mixture was extracted with $CHCl_3$/MeOH 95:5 (2×20 mL). The organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 99:1) to give the expected tetracyclic compound as a brown solid (2 mg, 4%) mp > 260 °C. ^{1}H RMN ($CDCl_3$) 7.78 (dd, 1H, $J = 4.4$ and 8.1 Hz); 8.08 (d, 1H, 5.9 Hz); 9.03 (d, 1H, $J = 5.9$ Hz); 9.07 (dd, 1H, $J = 4.4$ and 1.8 Hz); 9.18 (s, 1H); 9.19 (dd, 1H, $J = 1.8$ and 8.1 Hz). ^{13}C NMR ($CDCl_3$) 116.63; 119.80; 128.25; 132.64; 134.05; 137.03; 145.92; 147.56; 147.78 (2C); 148.47; 149.93; 153.31; 180.08. IR ($CHCl_3$) 1692; 1608 cm^{-1}. MS: m/z 269 (34); 267 (100); 232 (60); 204 (29). t_R is 4.32 min (96.3% purity), using system I (CH_3CN/H_2O/TFA 50:50:0.1),

and t_R is 5.91 min (98.1% purity), using system II (isooctane/EtOH 80:20), flow rate 2 mL/min.

5.1.16. 3-Acetoxymethyl-7*H*-pyrido[4,3,2-*de*][1,10]phenanthrolin-7-one (18c). A mixture of compound **17a** (100 mg, 0.45 mmol) and DMF–DEA (0.29 mL, 1.69 mmol) in DMF (1 mL) was warmed at 120 °C, under nitrogen atmosphere for 30 min. After concentration, the crude product was solubilized in DMF (14 mL), Eschenmoser's salt (142 mg, 0.765 mmol) was added and the reaction was warmed at 115 °C under nitrogen atmosphere for 30 min. NH_4Cl (660 mg, 12.34 mmol) and acetic acid (60 mL) were added and heating was continued for an additional 30 min. After cooling down, the mixture was poured into 10% KOH (100 mL) and extracted with $CHCl_3$ (3×200 mL). The organic layers were dried over $MgSO_4$ and concentrated over vacuum. Purification by flash chromatography (CH_2Cl_2/MeOH 98:2) gave the expected tetracyclic compound as a brown solid (10 mg, 7%), mp > 260 °C. ^1H NMR ($CDCl_3$) 2.14 (s, 3H); 5.62 (s, 2H); 7.69 (dd, 1H, $J = 8.1$ and 4.5 Hz); 8.19 (d, 1H, $J = 5.5$ Hz); 8.77 (dd, 1H, $J = 8.1$ and 1.5 Hz); 9.12 (s, 1H); 9.17 (dd, 1H, $J = 4.5$ and 1.5 Hz); 9.25 (d, 1H, $J = 5.5$ Hz). ^{13}C NMR ($CDCl_3$) 20.81; 60.87; 120.36; 120.84; 126.00; 126.21; 128.94; 136.47; 137.99; 147.45; 148.58; 149.06; 151.02; 151.51; 155.56; 170.41; 181.54. IR ($CHCl_3$) 1743, 1684 cm^{-1}. MS: m/z 305 (50); 262 (24); 245 (43). t_R is 5.15 min (95.4% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 12.38 min (99.7% purity), using system II (isooctane/EtOH 80:20), flow rate 2 mL/min.

5.1.17. 3-Acetoxymethyl-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (18d). A mixture of compound **17b** (100 mg, 0.45 mmol) and DMF–DEA (0.29 mL, 1.69 mmol) in DMF (1 mL) was warmed at 120 °C, under nitrogen atmosphere for 30 min. After concentration the crude product was solubilized in DMF (14 mL), Eschenmoser's salt (142 mg, 0.765 mmol) was added and the reaction was warmed at 115 °C under nitrogen atmosphere for 30 min. NH_4Cl (660 mg, 12.34 mmol) and acetic acid (60 mL) were added and heating was continued for an additional 30 min. After cooling down, the mixture was poured into 10% KOH (100 mL) and extracted with $CHCl_3$ (3×200 mL). The organic layers were dried over $MgSO_4$ and concentrated over vacuum. Purification by flash chromatography (CH_2Cl_2/MeOH 98:2) gave the expected tetracyclic compound as a brown solid (3 mg, 2%), which decomposes before melting. ^1H NMR ($CDCl_3$) 2.14 (s, 3H); 5.62 (s, 2H); 7.69 (dd, 1H, $J = 8.0$ and 4.7 Hz); 8.20 (d, 1H, $J = 5.6$ Hz); 8.78 (dd, 1H, $J = 8.0$ and 1.8 Hz); 9.13 (s, 1H); 9.18 (dd, 1H, $J = 4.7$ and 1.8 Hz); 9.25 (d, 1H, $J = 5.6$ Hz). ^{13}C NMR ($CDCl_3$) 20.81; 60.87; 120.31; 120.84; 126.01; 126.22; 128.95; 136.49; 137.99; 147.46; 148.59; 149.06; 151.00; 151.49; 155.56; 170.45; 181.55. IR ($CHCl_3$) 1743, 1684 cm^{-1}. MS: m/z 305 (50%); 262 (23%); 245 (42%). t_R is 11.15 min (100% purity), using system I (CH_3CN/H_2O/TFA 20:80:0.1), and t_R is 12.93 min (100% purity), using system II (isooctane/EtOH 80:20), flow rate 2 mL/min.

5.1.18. 2-Phenyl-7*H*-pyrido[4,3,2-*de*][1,10]phenanthrolin-7-one (19c). A suspension of compound **17a** (110 mg, 0.49 mmol) and NH_4Cl (860 mg, 16.07 mmol) in acetic acid (60 mL) was warmed at 70 °C under nitrogen atmosphere for 30 min. A solution of benzaldehyde (0.4 mL, 3.93 mmol) in acetic acid (10 mL) was added dropwise, and the mixture was refluxed for 15 h. After cooling down, the mixture was neutralized with NH_4OH (90 mL) and extracted with CH_2Cl_2 (5×200 mL). The organic layers were dried over $MgSO_4$ and concentrated over vacuum. The crude product was purified by flash chromatography (AcOEt/pentane 65:35) to give the tetracyclic compound as a clear-brown solid (27 mg, 18%), mp > 260 °C. ^1H NMR ($CDCl_3$) 7.52 (d, 1H, $J = 7.3$ Hz); 7.58 (dd, 2H, $J = 8.6$ and 7.3 Hz); 7.69 (dd, 1H, $J = 8.0$ and 4.8 Hz); 8.05 (d, 1H, $J = 5.5$ Hz); 8.23 (s, 1H); 8.31 (d, 2H, $J = 8.6$ Hz); 8.80 (dd, 1H, $J = 1.8$ and 8.0 Hz); 9.16 (d, 1H, $J = 5.5$ Hz); 9.22 (dd, 1H, $J = 1.8$ and 4.8 Hz). ^{13}C NMR ($CDCl_3$) 116.13; 119.87; 124.29; 125.66; 127.82 (2C); 129.10 (2C); 129.33; 130.02; 136.49; 138.14; 139.88; 147.19; 148.68; 150.31; 151.87; 155.49; 156.64; 181.97. IR ($CHCl_3$) 1682, 1615, 1582 cm^{-1}. MS: m/z 309 (100); 280 (33). t_R is 6.19 min (95% purity), using system I (CH_3CN/H_2O/TFA 50:50:0.1), and t_R is 10.96 min (99% purity), using system II (isooctane/EtOH 80:20), flow rate 1 mL/min.

5.1.19. 2-Styryl-7*H*-pyrido[4,3,2-*de*][1,10]phenanthrolin-7-one (20c). A suspension of compound **17a** (60 mg, 0.267 mmol) and NH_4Cl (470 mg, 8.79 mmol) in acetic acid (23 mL) was warmed at 70 °C under nitrogen atmosphere for 40 min. A solution of cinnamaldehyde (0.17 mL, 1.34 mmol) in acetic acid (5 mL) was added dropwise, and the mixture was refluxed for 4 h. After cooling down, the mixture was neutralized with a saturated solution of $NaHCO_3$ (30 mL) and extracted with CH_2Cl_2 (4×60 mL). The organic layers were dried over $MgSO_4$ and concentrated over vacuum. The crude product was purified by flash chromatography ($CHCl_3$) to give the tetracyclic compound as a brown solid (21 mg, 23%), mp > 260 °C. ^1H NMR ($CDCl_3$) 7.34–7.38 (m, 1H); 7.41–7.45 (m, 2H); 7.55 (d, 1H, $J = 16.1$ Hz); 7.68–7.71 (m, 3H); 7.90 (s, 1H); 7.96 (d, 1H, $J = 5.6$ Hz); 8.02 (d, 1H, $J = 16.1$ Hz); 8.8 (dd, 1H, $J = 7.7$ and 1.8 Hz); 9.12 (d, 1H, $J = 5.6$ Hz); 9.22 (dd, 1H, $J = 4.4$ and 1.8 Hz). ^{13}C NMR ($CDCl_3$) 116.85; 119.97; 123.94; 125.73; 127.13; 127.56 (2C); 128.86 (2C); 129.03; 129.29; 136.15 (2C); 136.57; 139.81; 147.13; 148.76; 150.22; 151.74; 155.02; 155.43; 181.85. IR ($CHCl_3$) 1682, 1610, 1582 cm^{-1}. MS: m/z 335 (15); 334 (100); 333 (20). t_R is 8.53 min (95% purity), using system I (CH_3CN/H_2O/TFA 50:50:0.1), and t_R is 10.73 min (99.8% purity), using system II (isooctane/EtOH 80:20), flow rate 1 mL/min.

6. Pharmacology

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

Twelve human tumor cell lines were obtained from the American Type Culture Collection (ATCC, Manassas,

VA). These included 3 glioblastomas (A-172, U-373 MG and U-87 MG), 2 colon (HCT-15 and LoVo), 2 non-small-cell-lung (A549 and A-427), 2 bladder (J82 and T24), 1 prostate (PC-3) and 2 breast (T-47D and MCF7) cancer models. The ATCC numbers of these cell lines are CRL1620 (A-172), HTB 14 (U-87 MG), HTB 17 (U-373 MG), CCL225 (HCT-15), CCL229 (LoVo), CCL 185 (A549), HBT 53 (A-427), HTB1 (J82), HTB4 (T24), HTB133 (T-47D), HTB22 (MCF7) and CRL1435 (PC-3). 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 12 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.[8,9] 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) 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. We validated the MTT-related data using two alternative techniques, namely direct cell counting and the genomic incorporation of tritiated thymidine (data not shown).

Six concentrations ranging from 10^{-5} to 10^{-9} M were assayed for each of the compounds under study.

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