Unexpected Formation of Pyridodiindole Derivatives with Cytotoxic Activity via Double Nenitzescu Reaction

Christine Willemann[a], Reiner Waibel[a], Renate Grünert[b], Patrick Bednarski[b], Reinhard Troschütz[a]*

[a]Department Chemie und Pharmazie der Universität Erlangen-Nürnberg, Lehrstuhl für Pharmazeutische Chemie, Schuhstr. 19, D-91052 Erlangen, Germany
[b]Institut für Pharmazie der Ernst-Moritz-Arndt-Universität, Friedrich-Ludwig-Jahn-Str. 17, D-17487 Greifswald, Germany

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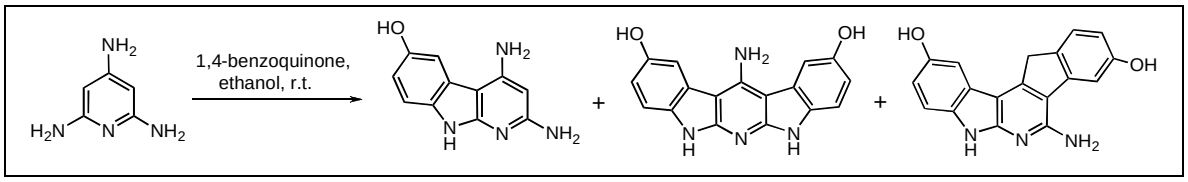

The title compounds **10** and **11** were prepared by a one-step procedure from 1,4-benzoquinone (**4**) and pyridine-2,4,6-triamine (**5**) *via* an extension of the Nenitzescu reaction

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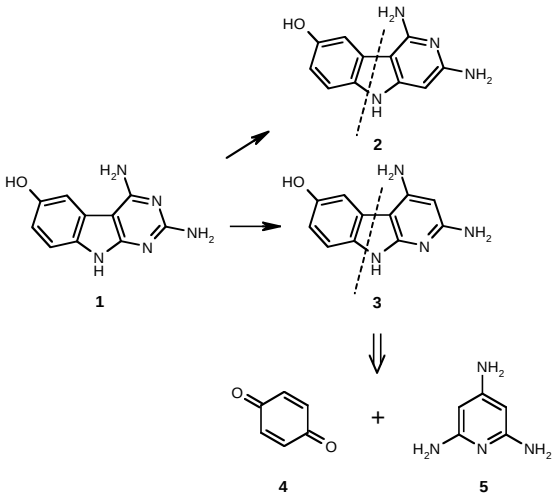
INTRODUCTION

Dotzauer *et al*. synthesized 2,4-diamino-9*H*-pyrimido[4,5-*b*]indol-6-ole (**1**) and *N*-substituted derivatives as a prototype of a series of ellipticine analogues by an extension of the Nenitzescu reaction using 1,4-benzoquinone (**4**) and pyrimidine-2,4,6-triamines. These indole derivatives showed interesting antitumor activity [1-3]. Related to these compounds, we planned pyridine-2,4,6-triamine (**5**) to react as a new type of enamine component with 1,4-benzoquinone (**4**) to achieve desired 6-hydroxy-carbolines **2** and **3** (Scheme 1).

Scheme 1

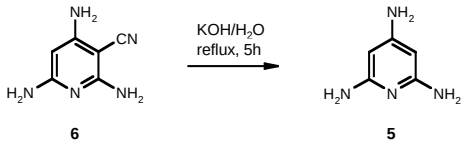


In this paper we are going to present the unexpected synthesis of pyridodiindole derivatives and their cytotoxic activity.

RESULTS AND DISCUSSION

The preparation of pyridine-2,4,6-triamine (**5**) has been reported several times. Meyer *et al*. started their multi step synthesis using 2,6-dichloropyridin-4-amine [4] whereas in a Schering patent a triple Chichibabin reaction using sodium amide and high temperature is described [5,6]. Another exhausting multi step strategy is reported by Coburn *et al*. [7]. For quick access to pyridine-2,4,6-triamine (**5**), we planned to hydrolyze the nitrile group of 2,4,6-triamino-nicotinonitrile (**6**) to gain triamine **5** after decarboxylation of the corresponding nicotinate. Nitrile **6** can be prepared *via* Pinner reaction of malononitrile and following ammonolysis (Scheme 2) [8,9].

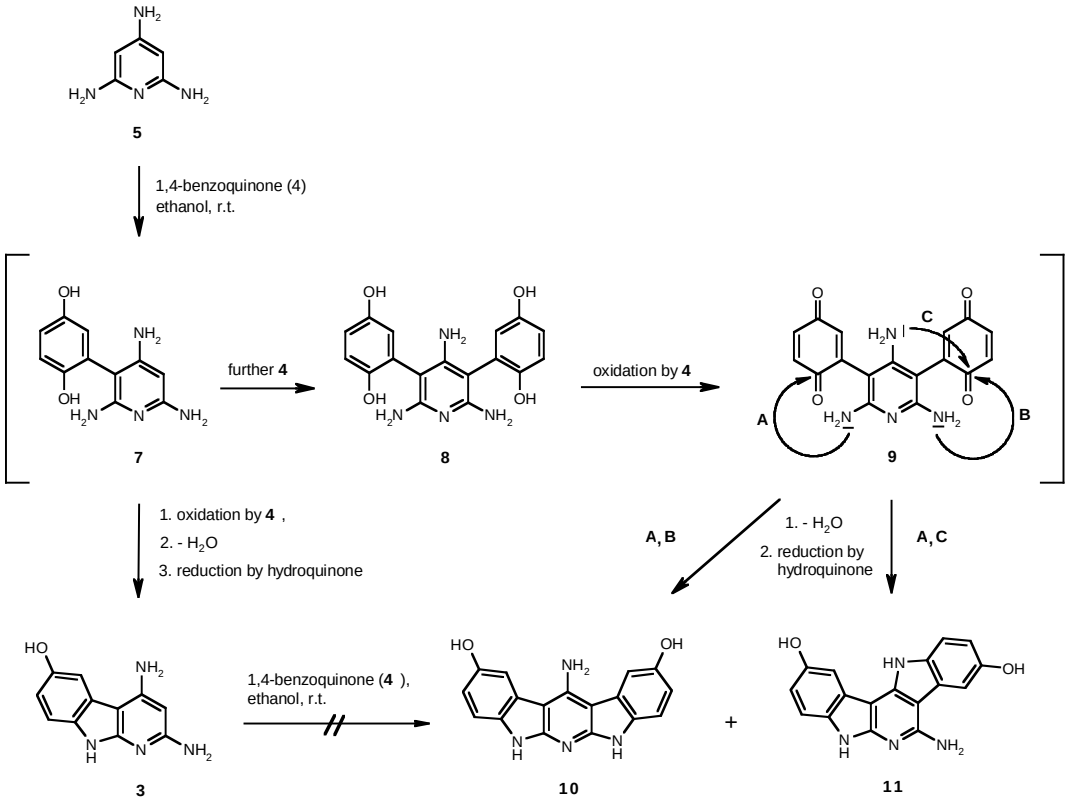
Scheme 2



Alkaline hydrolysis of nitrile **6** and adjacent extraction with diethyl ether led to pure pyridine-2,4,6-triamine (**5**) in 32 % yield. Prior adjustment of the pH to 8-9 with diluted sulfuric acid followed by evaporation of the aqueous phase and extraction of the solid sample by cool ethanol increased the yield to average 70 % even though this time including small amounts of inorganic impurities.

For our Nenitzescu reaction [10-14], pyridine-2,4,6-triamine (**5**) was dissolved in ethanol and 2 eq. of 1,4-benzoquinone (**4**) added carefully [15]. The solution turned to dark brown and after only a few minutes at least three products could be detected by tlc beside quinone **4** and hydroquinone. A first mass spectrometrical analysis

Scheme 3



of the raw sample indicated the new masses m/z 214 and 304.

After isolation of the reaction products by column chromatography, further spectral analysis confirmed our guess that a 1:1 and 2:1 Nenitzescu reaction had occurred. The following reaction sequence seems to be plausible. After a first Michael type addition, resulting adduct **7** runs through the typical Nenitzescu reaction steps: oxidation by 1,4-benzoquinone (**4**) leading to a quinone intermediate and intramolecular quinonimine formation followed by a final reduction by hydroquinone resulting in desired α-carbolinediamine **3**. But compound **7**, while owning a rather nucleophilic position 5, might react with another equivalent of 1,4-benzoquinone (**4**) to form intermediate **8**. Thereafter, oxidation by excess of quinone **4** takes place leading to not isolated intermediate **9**. Then, ring closure can proceed in two different ways: Performing reaction pathway **A** and **B**, linear 12-amino-5,7-dihydropyrido[2,3-*b*:6,5-*b*']diindole-2,10-diole (**10**), and *via* pathway **A** and **C**, 7-amino-5,12-dihydropyrido[2,3-*b*:4,5- *b*']diindole-2,9-diole (**11**) is formed. The diindoles **10** and **11** could not be prepared from carbolinediamine **3** and 1,4-benzoquinone (**4**) (Scheme 3).

To accomplish better yields of α-carbolinediamine **3**, we carried out the same reaction with modified amounts of 1,4-benzoquinone (**4**). The best yields of compound **3** were performed using same molar ratios of quinone **4** and pyridine-2,4,6-triamine (**5**). The structure as an α-carboline **3** is proved by Heteronuclear Multiple Bond Correlation (HMBC). In this sample, pyridodiindoles **10** and **11** have only been isolated in very little amounts whereas pyridine-2,4,6-triamine **5** was indicated in considerable amounts by tlc but could not be quantified due to its instability. γ-Carboline **2** could not be detected in any case.

To evaluate the *in vitro* cytotoxic activity of our indole derivatives **3**, **10** and **11**, an established microtiter assay, based on cell staining with crystal violet, was used to determine the inhibition of cell growth caused by the test compounds. In these studies, different human cancer cell lines from solid tumors were used. Surprisingly, α-carboline derivative **3** did not show any activity while IC_{50} values from 3.1 to 10.5 μM were found for pyridodiindoles **10** and **11** on cell lines 5637, RT-4 and A-427 [16].

EXPERIMENTAL

Starting materials were obtained from commercial sources and were used without further purification. Reaction progress was observed by thin-layer chromatography (tlc) making use of commercial silica gel plates (Merck, silica gel F254 on

aluminum sheets). Column chromatography was done on silica gel 60 and LiChroprep RP-18 (Merck). Melting points were determined in open capillary tubes on a Buechi 510 melting point apparatus and are uncorrected. Elemental analyses were performed by the Institut für Organische Chemie (Universität Erlangen-Nürnberg) using Carlo Erba Elemental Analyzer 1108. ^{1}H nuclear magnetic resonance (^{1}H nmr) spectra were determined with a Bruker AM 360 (360 MHz) spectrometer in appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethylsilane (internal standard). In nmr data, all NH, OH and NH_2 signals were exchangeable with D_2O. Mass spectra (ms, hrms) were taken with a Finnigan MAT TSQ 700 mass spectrometer in the electron impact mode (70 eV). Significant infrared (ir) spectra were obtained on a Jasco FT/IR 410 spectrometer. HRMS on an Jeol GC-Mate II.

Pyridine-2,4,6-triamine (5). 2,4,6-Triaminonicotinonitrile (**6**) (3.60 g, 24.1 mmol) was heated to reflux for 5 h in a KOH solution (26.0 g KOH and 13.0 g H_2O). After cooling to r.t. pH 8-9 was adjusted with diluted H_2SO_4.

a) The aqueous solution was extracted five times with diethyl ether, the organic phase dried over Na_2SO_4 and evaporated to dryness. 0.98 g (32.8 %) of a beige powder was obtained, mp 185 °C. (Lit. 185 °C. [4]); ir: NH 3380, NH 3324, C=C 1604 cm^{-1}; ^{1}H nmr (DMSO-d6): δ 5.07 (s, 2H, NH_2), 5.01 (s, 2H, 3-H, 5-H), 4.84 (s, 4H, NH_2), ^{13}C nmr (DMSO-d6): δ 159.0 (2-C, 6-C), 157.1 (4-C), 83.1 (3-C, 5-C); ms: m/z 124 (M+); hrms calcd. for $C_5H_8N_4$: 124.0749. Found: 124.0750.

b) The aqueous solution was evaporated to dryness. The residue was pulverized and than washed five times with cold ethanol. After filtration the alcoholic solution was evaporated to dryness. Resulting colourless solid gave the same spectroscopical analysis as mentioned above but contained a small amount of inorganic impurities. 2.10 g (70.2 %) was obtained.

2,4-Diamino-9*H*-pyrido[2,3-*b*]indol-6-ole (3). A solution of pyridine-2,4,6-triamine (**5**) (310 mg, 2.50 mmol) in 10 mL ethanol was reacted with 1,4-benzoquinone (**4**) (323 mg, 3.00 mmol) for 2 h at r.t. Purification was performed using two subsequent column chromatography runs (first by MPLC on LiChroprep RP-18 with a methanol/H_2O gradient, followed by flash chromatography on silica gel with a cyclohexane/ethyl acetate/methanol gradient). 90 mg (17.0 %) of a grey powder was obtained, mp 247-248 °C; ir: OH 3491, NH 3442, NH 3359, C=C 1604 cm^{-1}; ^{1}H nmr (DMSO-d6): δ 10.45 (s, 1H, NH), 8.54 (s, 1H, OH), 7.29 (d, 1H, J = 2.2 Hz, 5-H), 7.01 (d, 1H, J = 8.4 Hz, 8-H), 6.70 (dd, 1H, J_1 = 2.2 Hz, J_2 = 8.4 Hz, 7-H), 5.83 (s, 2H, 4-NH_2); 5.48 (s, 1H, 3-H), 5.42 (s, 2H, 2-NH_2); ^{13}C nmr (DMSO-d6): δ 158.6 (2-C), 153.4 (9a-C), 151.1 (6-C), 150.3 (4-C), 129.7 (8a-C), 122.8 (4b-C), 109.8 (7-C), 109.6 (8-C), 105.4 (5-C), 94.1 (4a-C), 84.0 (3-C); ms: m/z 214 (M+); hrms calcd. for $C_{11}H_{10}N_4O$: 214.0855. Found: 214.0855.

12-Amino-5,7-dihydropyrido[2,3-*b*:6,5-*b*']diindole-2,10-diole (10). A solution of pyridine-2,4,6-triamine (**5**) (347 mg, 2.80 mmol) in 10 mL ethanol was reacted with 1,4-benzoquinone (**4**) (605 mg, 5.60 mmol) for 2 h at r.t. Purification was performed using two subsequent column chromatography runs (first by MPLC on LiChroprep RP-18 with a methanol/H_2O gradient, followed by flash chromatography on silica gel with a chloroform/methanol gradient). 19 mg (2.2 %) colourless solid was obtained, mp 270 °C. (dec.); ir: OH 3561, NH 3392, NH 3245, C=C 1698 cm^{-1}; ^{1}H nmr (DMSO-d6): δ 10.86 (s, 2H, NH), 8.71 (s, 2H, OH), 7.62 (d, 2H, J = 1.9 Hz, 1-H, 11-H), 7.13 (d, 2H, J = 8.3 Hz, 4-H, 8-H), 6.74 (dd, 2H, J_1 = 1.9 Hz, J_2 = 8.3 Hz, 3-H, 9-H), 6.29 (s, 2H, NH_2), ^{13}C nmr (DMSO-d6): δ 153.5 (5a-C, 6a-C), 150.4 (2-C, 10-C), 145.4 (12-C), 130.4 (4a-C, 7a-C), 122.6 (11a-C, 12b-C), 110.6 (3-C, 9-C), 109.7 (4-C, 8-C), 106.2 (1-C, 11-C), 95.3 (11b-C, 12a-C); ms: m/z 304 (M+). Anal. Calcd. for $C_{17}H_{12}N_4O_2$ (304.31): C, 67.10; H, 3.97; N, 18.41. Found: C, 67.50; H, 3.69; N, 18.22.

7-Amino-5,12-dihydropyrido[2,3-*b*:4,5-*b*']diindole-2,9-diole (11). Preparation following the same method as indicated for compound **10**; 73 mg (8.6 %) of a grey powder, mp 270 °C. (dec.); ir: OH 3625, NH 3376, C=C 1614 cm^{-1}, ^{1}H nmr (DMSO-d6): δ 11.51 (s, 1H, 12-NH), 10.85 (s, 1H, 5-NH), 8.83 (s, 1H, 2-OH), 8.69 (s, 1H, 9-OH), 7.67 (d, 1H, J = 1.9 Hz, 1-H), 7.50 (d, 1H, J = 1.9 Hz, 8-H), 7.33 (d, 1H, J = 8.7 Hz, 4-H), 7.15 (d, 1H, J = 8.7 Hz, 11-H), 6.80 (dd, 1H, J_1 = 1.9 Hz, J_2 = 8.7 Hz, 3-H), 6.73 (dd, 1H, J_1 = 1.9 Hz, J_2 = 8.7 Hz, 10-H), 6.07 (s, 2H, NH_2), ^{13}C nmr (DMSO-d6): δ 153.5 (5a-C), 151.4 (2-C), 151.3 (9-C), 150.7 (12a-C), 140.9 (7-C), 132.4 (11a-C), 129.8 (4a-C), 123.6 (7b-C), 121.9 (12c-C), 111.1 (3-C), 110.6 (4-C, 10-C), 110.3 (11-C), 105.7 (8-C), 105.3 (1-C), 97.8 (7a-C), 91.8 (12b-C); ms: m/z 304 (M+). Anal. Calcd. for $C_{17}H_{12}N_4O_2$ (304.31): C, 67.10; H, 3.97; N, 18.41. Found: C, 66.86; H, 3.65; N, 18.03.

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