Cytotoxic Hybrids Between the Aromatic Alkaloids Bauerine C and Rutaecarpine

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Two hybrids between the alkaloids bauerine C and rutaecarpine were prepared. Screening for cytotoxic activity revealed that introduction of two chlorine substituents to the quinazolinocarboline core of rutaecarpine strongly enhances cytotoxic activity, whereas methylation at the indole nitrogen is detrimental to activity.

Key words: Cytotoxic Activity, Hybrids, Bauerine C, Rutaecarpine, Alkaloids

Introduction

Polycyclic aromatic alkaloids represent a common source of lead structures for the development of new anticancer drugs, mainly based on their ability to interact with DNA by intercalation and/or inhibition of topoisomerases. One of the most recent successful examples is topotecan, a topoisomerase I inhibitor derived from the plant alkaloid camptothecin (**1**) [1]. In the course of our recent investigations in order to develop new anticancer drugs, we worked out the first total synthesis of the 1-oxo-β-carboline alkaloid bauerine C (**2**) [2]. This alkaloid has been isolated from the blue-green alga *Dichotrix baueriana*, and showed very promising cytotoxic activitiy in preliminary screenings [3]. Typical structural elements of this alkaloid are a 1,2-dichlorobenzene ring, an untypical *N*-methylindole moiety, and a pyridone ring. The corresponding 1-oxo-β-carboline lacking the two chloro substituents [4] does not show significant cytotoxicity.

Another relevant example for cytotoxic polycyclic alkaloids is the quinazolinocarboline alkaloid rutaecarpine (**3**), the major alkaloid from *Evodia rutaecarpa* (Rutaceae) (Fig. 1) [5]. This alkaloid has been shown to exhibit moderate cytotoxic activitiy against several tumor cell lines, and its ability to inhibit topoisomerase II has been reported recently [6].

Following our concept of combining typical structural elements of known antimicrobial and cytotoxic compounds [7] in order to find new bioactive compounds we envisaged to prepare hybrids between the cytotoxic alkaloids bauerine C (**2**) and rutaecarpine (**3**) as potential new anticancer agents. These hybrids can be seen as 11,12-dichloro-rutaecarpine (**7**; in case the indole nitrogen is not methylated) and as *N*-methyl-11,12-dichloro-rutaecarpine (**8**). We were primarily interested in exploring the benefit of the chloro substituents and the *N*-methyl group for the cytotoxic activity of the hybrids.

A number of monohalogenated analogues of rutaecarpine (**3**) have already been described as cyclooxygenase inhibitors by Lee *et al*. [8]. 10,11-Methylenedioxyrutaecarpine and 11-methoxyrutaecarpine showed cytotoxic activities superior to the native alkaloid **3** [9], and 10-bromorutaecarpine showed a slight increase (factor 2 – 3) in cytotoxic activity compared to alkaloid **3**, whereas the 10-methoxy analogue (identical to the alkaloid hortiacine from *Hortia arborea*, Rutaceae [10]) was found to be inactive [11]. Baruah *et al*. [12] reported on the cytotoxic activity of 10-chloro derivatives of rutaecarpine (**3**) and the related alkaloid evodiamine, but the results are not clear due to erroneous molecular formulas in the publication.

Results

Chemistry

Several related strategies for the construction of the pentacyclic quinazolinocarboline skeleton of rutaecarpine (**3**) have been published over the years [5b]. Most of these approaches start from anthranilic acid [12], anthranilic esters [13 – 15], or isatoic acid, de-

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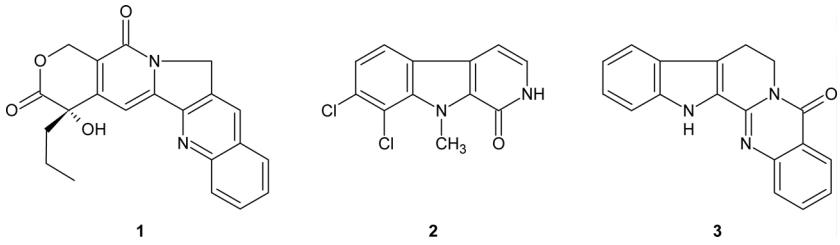
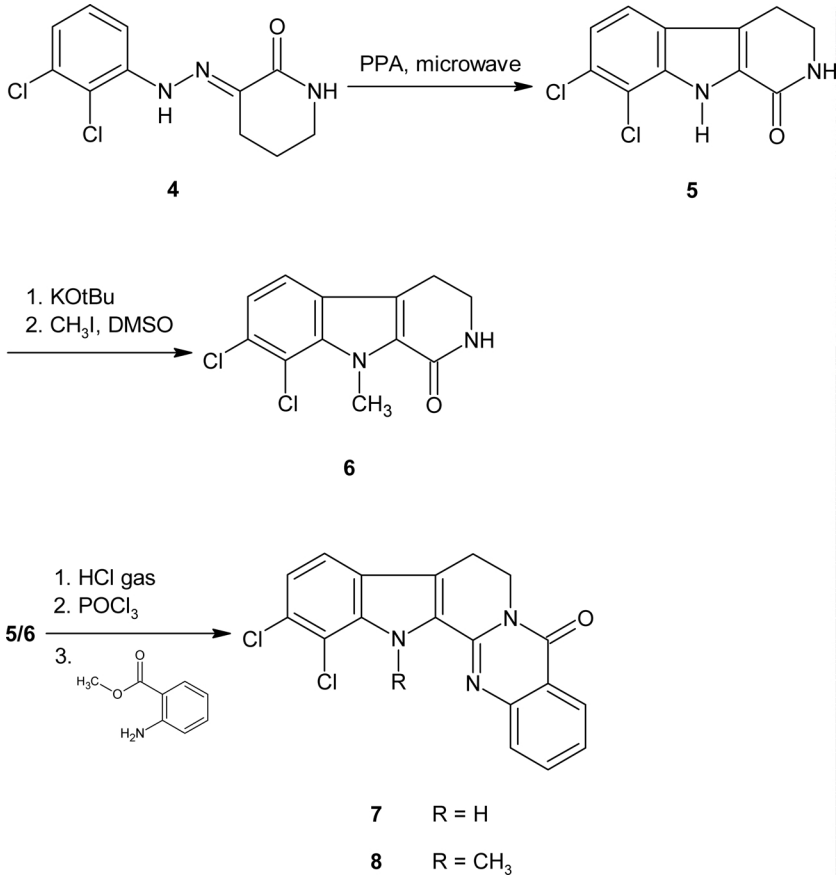


Fig. 1. Polycyclic aromatic alkaloids: Camptothecin (**1**), bauerine C (**2**), rutaecarpine (**3**).



Scheme 1. Synthesis of the hybrids **7** and **8**.

rived from anthranilate [12, 16, 17] as building blocks for the quinazoline partial structure. The indole part is most commonly introduced using tryptamine [12, 15–17] or by Fischer indolization [8]. Moreover, 1-oxo-1,2,3,4-tetrahydro-β-carbolines can be applied as versatile tricyclic building blocks [12–14].

We decided to use the last mentioned strategy, since the dichloro-oxocarbolines **5** and **6** were already in our hands as intermediates of our total synthesis of bauerine C (**2**) [2] (Scheme 1). Compound **5** was conveniently prepared in a Japp-Klingemann reaction by cyclization of arylhydrazone **4** in formic acid at 80 °C for 24 h. Since large amounts of **6** were needed for our present investigations, we re-examined this cyclization and found that the reaction can be dramatically accelerated by microwaves [18]. Irradiation for 15 min gave the desired product in 40 % yield when formic acid was used as the acidic solvent. Reaction in polyphosphoric acid trimethylsilyl ester [19] yielded 41 %, and finally, reaction in polyphosphoric acid 47 % of **5**. *N*-Methylation at the indole nitrogen atom to give **6** was performed as described previously [2]. Both **5** and **6** were converted to the quinazolinocarbolines **7** and **8** using Lee's protocol [14]. Thus the lactams were con-

Compound	IC_{50} [μM]	IC_{70} [μM]
2	11.9	27.7
3	5.4	12.6
5	5.9	13.7
6	31.0	72.1
7	0.15	0.36
8	0.67	1.58

Table 1. Cytotoxic activities of the compounds on HL-60 cells (MTT assay).

verted to their hydrochloride salts with HCl gas in chloroform, and then to the corresponding iminochlorides with $POCl_3$. After removal of excess $POCl_3$ the crude products were reacted with methyl anthranilate to give **7** and **8** in 80 and 76 % yield, respectively. Rutaecarpine (**3**) was prepared for comparison in the same manner starting from easily available 1-oxo-1,2,3,4-tetrahydro-β-carboline [4].

Cytotoxic activity

Quinazolinocarbolines **7** and **8**, alkaloids rutaecarpine (**3**) and bauerine C (**2**), and both building blocks **5** and **6**, related to the alkaloid bauerine C, were tested for cytotoxic activity in a standard MTT assay on HL-60 cells [20]. The results are presented in Table 1.

Discussion

A convenient approach to rutaecarpine-bauerine C hybrids **7** and **8** has been worked out. The cytotoxic activities of these compounds were determined and compared with those of the native alkaloids.

Alkaloids rutaecarpine (**3**) and bauerine C (**2**), as well as the chlorinated oxo-β-carbolines **5** and **6** showed only poor cytotoxic activities (IC_{50} values between 5 and 31 μM) against HL-60 cells. In contrast, the two hybrids 11,12-dichloro-rutaecarpine (**7**; IC_{50} = 0.15 μM) and *N*-methyl-11,12-dichloro-rutaecarpine (**8**; IC_{50} = 0.67 μM) were found to exhibit high cytotoxic activities. Introduction of the chloro substituents at 11- and 12-position obviously leads to a significant increase in cytotoxic activity, whereas additional methylation at the indole nitrogen atoms turns out to be detrimental.

Work is in progress to screen the active hybrids **7** and **8** on a broad panel of tumor cell lines.

Experimental Section

Melting points were determined on a Büchi Melting Point B-540 apparatus (Büchi, Flawil, Switzerland) and are uncorrected. NMR spectra were recorded on a Jeol JNMR-GX400 instrument (Jeol, Peabody, MA, USA). Mass spectra data were acquired on a Hewlett Packard 5989 A mass spectrometer, electronic ionization (EI) 70 eV, chemical ionization (CI) with CH_4 (300 eV), (Agilent Technologies, Palo Alto, CA, USA). IR spectra were measured on a Jasco FT/IR-410 spectrometer (Jasco Inc., Easton, MD, USA). Elemental analysis was performed on a CHN-Rapid instrument (Heraeus Holding GmbH, Hanau, Germany). All microwave experiments were performed using a CEM Discover apparatus (CEM Corporation, Matthews, NC, USA).

MTT assay: This assay was performed on HL-60 cells as described in ref. [20]. The experiments were carried out in triplicate with each compound.

*7,8-Dichloro-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-one (**5**)*

0.50 g (1.84 mmol) 3-(2-(2,3-dichlorophenyl)hydrazono)-piperidin-2-one (**4**) [2] and 1.0 g polyphosphoric acid were stirred at 80 °C for 15 min using microwave irradiation (20 W). After cooling the mixture was poured into ice water (50 mL) and stirred at r. t. for 30 min. The suspension was filtered and the residue recrystallized from EtOH to give 220 mg of **5** (47 %). The spectroscopic data were in close agreement with literature values [2].

*11,12-Dichloro-8,13-dihydro-7H-indolo[2′,3′ : 3,4]-pyrido[2,1-b]quinazolin-5-one (**7**)*

Dry HCl gas was passed into a solution of 820 mg (3.21 mmol) 7,8-dichloro-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-one (**5**) in 100 mL of $CHCl_3$ until no further precipitation was observed. The lactam hydrochloride was collected by filtration, suspended in 30 mL of $POCl_3$ and stirred at 50 °C for 2 h. Excess $POCl_3$ was removed *in vacuo* and the residue dissolved in 100 mL of anhydrous THF. 900 mg (5.95 mmol) methyl anthranilate was added and the mixture was stirred at r. t. for 12 h. Then it was poured into 80 mL of water, 100 mL of aqueous ammonia (30 %) were added, followed by extraction with toluene/ethyl acetate (1 : 1; 3 × 100 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. Recrystallization from toluene/ethanol afforded 916 mg (80 %) of **7** as pale orange needles. M. p. 267 °C. – IR (KBr): ν = 3377, 2929, 1655 (CO–NR_2), 1599, 1560, 1471, 1306, 766 cm^{-1}. – ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 3.18 (t, J = 6.8 Hz, 2 H, 8-H), 4.46 (t, J = 6.8 Hz, 2 H, 7-H), 7.30 (d, ^3J = 8.5 Hz, 1 H, 10-H), 7.50 (ddd, ^3J = 8.0 Hz, 8.0 Hz, ^4J = 1.2 Hz, 1 H, 3-H), 7.67 (d, ^3J = 8.5 Hz, 1 H, 9-H), 7.76 (ddd, ^3J = 8.0 Hz, ^4J = 1.2 Hz, ^5J = 0.5 Hz, 1 H, 1-H), 7.83 (ddd, ^3J = 8.0 Hz, 8.0 Hz, ^4J = 1.5 Hz, 1 H, 2-H), 8.17 (ddd, ^3J = 8.0 Hz, ^4J = 1.5 Hz, ^5J = 0.5 Hz, 1 H, 4-H), 12.28 (br. s, 1 H, N–H). – ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 18.7 (C-8), 40.4 (C-7), 115.1 (C-12), 119.3 (C-8a), 119.7 (C-9), 120.8 (C-4a), 121.6 (C-10), 125.3 (C-8b), 126.3 (C-3), 126.4

(C-4), 126.6 (C-1), 127.1 (C-11), 129.4 (C-13a), 134.4 (C-2), 136.3 (C-12a), 144.4 (C-13b), 147.0 (C-14a), 160.4 (C=O). – MS (CI): *m/z* (%) = 360 (10) $[M+5]^+$, 358 (57) $[M+3]^+$, 356 (100) $[M+1]^+$. – MS (EI, 70 eV): *m/z* (%) = 359 (10) $[M+4]^+$, 357 (62) $[M+2]^+$, 355 (100) M^+. – $C_{18}H_{11}Cl_2N_3O$ (356.2): calcd. C 60.69, H 3.11, N 11.80; found C 60.48, H 3.14, N 11.64.

*11,12-Dichloro-8,13-dihydro-13-methyl-7H-indolo-[2′,3′ : 3,4]pyrido[2,1-b]quinazolin-5-one (**8**)*

This compound was prepared in the same manner as described for **7** starting from 670 mg (2.51 mmol) 7,8-dichloro-9-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*] indol-1-one (**6**) and 605 mg (4.00 mmol) methyl anthranilate. Yield: 714 mg (77 %), as pale yellow needles after recrystallization from toluene. M. p. 261 °C. – IR (KBr): ν = 3055, 2989, 2952, 2898, 2850, 1674 ($CO–NR_2$), 1585, 1468, 1155, 766 cm^{-1}. – ^1H NMR (400 MHz, CF_3COOD): δ = 3.27 (t, J = 6.3 Hz, 2 H, 8-H), 4.22 (s, 3 H, $N–CH_3$), 4.65 (t, J = 6.3 Hz, 2 H, 7-H), 7.35 (d, ^3J = 8.6 Hz, 1 H, 10-H), 7.51 (d, ^3J = 8.6 Hz, 1 H, 9-H), 7.70 (t, J = 8.0 Hz, 1 H, 3-H), 7.80 (d, ^3J = 8.0 Hz, 1 H, 1-H), 7.96 (t, J = 8.0 Hz, 1 H, 2-H), 8.35 (d, ^3J = 8.0 Hz, 1 H, 4-H). – ^{13}C NMR (100 MHz, CF_3COOD): δ = 21.5 (C-8), 38.1 ($N–CH_3$), 43.7 (C-7), 119.5 (C-4a), 120.6 (C-12), 121.0 (C-1), 122.6 (C-9), 126.9 (C-13a), 127.7 (C-8b), 128.3 (C-10), 130.7 (C-4), 132.2 (C-3), 136.9 (C-8a), 138.4 (C-14a), 139.5 (C-11), 140.2 (C-2), 146.1 (C-12a), 148.2 (C-13b), 162.5 (C=O). – MS (CI): *m/z* (%) = 374 (11) $[M+5]^+$, 372 (61) $[M+3]^+$, 370 (100) $[M+1]^+$. – MS (EI, 70 eV): *m/z* (%) = 372 (17) $[M+3]^+$, 370 (74) $[M+1]^+$, 368 (100) $[M–1]^+$. – $C_{19}H_{13}Cl_2N_3O$ (370.2): calcd. C 61.64, H 3.54, N 11.35; found C 61.52, H 3.34, N 11.30.

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