Synthesis and Biological Evaluation of a Novel Pentagastrin-Toxin Conjugate Designed for a Targeted Prodrug Monotherapy of Cancer

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Abstract: A novel carbamate prodrug 2 containing a pentagastrin moiety was synthesized. 2 was designed as a detoxified analogue of the highly cytotoxic natural antibiotic duocarmycin SA (1) for the use in a targeted prodrug monotherapy of cancers expressing cholecystokinin (CCK-B)/gastrin receptors. The synthesis of prodrug 2 was performed using a palladium-catalyzed carbonylation of bromide 6, followed by a radical cyclisation to give the pharmacophoric unit 10, coupling of 10 to the DNA-binding subunit 15 and transformation of the resulting seco-drug 3b into the carbamate 2 via addition of a pentagastrin moiety.

Keywords: antibiotics, antitumor agents, pentagastrin, prodrug, prodrug monotherapy

1. Introduction

One of the major problems in the chemotherapy of cancers is the usually low differentiation between normal and malignant cells by the known antiproliferating agents, resulting in severe side effects. Several approaches have been developed to overcome this problem like the antibody-directed enzyme prodrug therapy (ADEPT) [1,2] and the prodrug monotherapy (PMT) [3]. Whereas in ADEPT artificial antibody-enzyme conjugates are needed for targeting tumor cells, in PMT specific
endogenous enzymes or receptors overexpressed in cancerous tissue are addressed to allow a selective killing of tumor cells. In both approaches, a relatively untotoxic prodrug is used, which is then selectively converted into the corresponding cytotoxic drug in the cancer tissue; however, for PMT the prodrug is linked to a ligand which allows a targeting of cancer cells. Among these ligands small peptides play an important role having a low immunogenicity as well as high specificities and affinities to certain receptors which are overexpressed on certain tumour cells [4]. Some of these peptides that are already successfully applied in cancer therapy, belong to the gastrin family. For example, radiolabeled gastrin derivatives have shown a high therapeutic and diagnostic potential in targeting cholecystokinin (CCK-B)/gastrin receptor expressing tumors [5]. In addition, a gastrin derivative was linked to a triazene alkylating agent [6]. However, the observed receptor-mediated cytotoxicity of this conjugate was quite low. Better results were obtained with heptagastrin linked to an ellipticine derivative [7]. A high receptor-mediated cytotoxicity could be achieved with the anthracyclines daunorubicin, doxorubicin and 2-pyrrolinodoxorubicin as well as other cytotoxic agents like melphalan, cisplatin or methotrexate coupled to peptides of the LHRH [4,8], bombesin [4,8a,9], somatostatin [4,8a,10] and neuropeptide Y [4,11] type. Recently, we have developed the pentagastrin toxin conjugate 3a containing a seco-duocarmycin SA derivative (Scheme 1) [12].

Scheme 1. (+)-Duocarmycin SA (1), prodrug 2, seco-drugs 3 and drugs 4; a) enzymatic toxification of the novel carbamate prodrug 2 to the seco-drug 3b inside the tumor cells and b) rapid cyclisation of the seco-drugs 3 in situ to give the cytotoxic drugs 4.

Here, we report the synthesis of a novel pentagastrin conjugate 2 for the use in a targeted tumor therapy, which has the advantage over normal gastrin-toxin conjugates that a prodrug is used instead of a toxic drug (Scheme 1). In this concept, the pentagastrin moiety should serve not only as a targeting ligand for CCK-B/gastrin receptors, but also as a detoxifying unit. Thus, the corresponding
drug 4b, which again is an analogue of the naturally occurring antibiotic (+)-duocarmycin SA (1) with an IC50 value of 10 pM (L1210) [13], should be formed via 3b inside the tumor cells by cleavage of the carbamate by lysosomal enzymes after endocytosis. The antiproliferative effect of 1 and its analogues such as the CBI-drugs 4 derive most probably from a selective alkylation of N-3 of adenine in DNA by nucleophilic attack at the spirocyclopropyl-cyclohexadienone moiety as the pharmacophoric group [14]. Since we have previously shown that the formation of a drug as 4b from a seco-drug as 3b is a very fast process and that the blocking of the phenolic hydroxyl group of 3b allows a very strong reduction of its cytotoxicity, we used the seco-drug 3b as substrate for the conjugation with the pentagastrin moiety, performing the connection to the phenolic hydroxyl group via a carbamate moiety [1b,1c,15].

In our approach we did not employ the whole heptadecapeptide gastrin but the shorter β-alanine modified pentagastrin, because its β-Ala-Trp-Met-Asp-Phe-NH2 sequence representing the C-terminal amide of the natural peptides restores the biological activity of gastrin in a comparable order of magnitude [4e,16]. As a consequence, the seco-duocarmycin moiety 3b had to be attached via the N-terminal amino functionality of pentagastrin using a carbamate. Such a carbamate substructure exists also in KW-2189 (5) (Figure 1) [17], an agent already investigated in clinical trials, and in several other anticancer agents [18].

![Figure 1. KW-2189 (5).](image)

For the formation of the carbamate moiety, we envisaged an addition of an isocyanate to the seco-drug 3b. The TMSE ester moiety was introduced to allow a better comparison with the already prepared pentagastrin-conjugate 3a. Moreover, the handle could be used for the introduction of a fluorescence dye to allow an investigation of the mode of action of such a compound employing a confocal laser scanning microscope.

2. Results and Discussion

2.1. Synthesis

As starting material for the preparation of 2 we employed the known aminonaphthalin 6 [12]. 6 was converted into TMSE ester 7 in 56% yield by a palladium-catalyzed carbonylation reaction using a CO atmosphere (1 bar) and Mo(CO)6 as additional CO source [19] in a mixture of 2-(trimethylsilyl)-ethanol and DMF (Scheme 2). The moderate yield of 56% of this carbonylation reaction might be due
to the relatively high electron density of 6. Nevertheless, 6 had to be used in the carbonylation reaction as the Curtius rearrangement of the corresponding acid to the protected naphtholamine could not be achieved after the introduction of the TMSE ester moiety. Iodination of 7 employing NIS [20,21] with TsOH·H2O as catalyst followed by N-alkylation of the formed 8 with 1,3-dichloropropene and subsequent radical cyclization [22] using the untotoxic tris-(trimethylsilyl)-silan (TTMSS) [23] as hydride source and AIBN as radical starter provided seco-CBI derivative 10 in 65 % yield over three steps.

Scheme 2. Synthesis of seco-CBI compound 10. a) Mo(CO)6, 1 bar CO, 5 mol% Pd(PPh3)2Br2, 20 mol% dppf, nBu3N, TMSEOH, DMF, 120 °C, 7 h, 56%; b) NIS, TsOH·H2O, THF/MeOH, 50 °C, 1 h, 73%; c) NaH, 1,3-dichloropropene, DMF, 20 °C, 13.5 h, 97%; d) HSi(SiMe3)3, AIBN, benzene, reflux, 2 h, 92%.

In order to connect 10 with the peptide unit, we used the isocyanate 13 containing an ester moiety. This was first reacted with the phenolic hydroxyl group and then bound to the peptide via an amide linkage. The required isocyanate 13 was prepared as follows: first, β-alanine (11) was converted into the corresponding benzyl ester hydrochloride 12 employing TMSCl and benzylic alcohol [24]. Then, the isocyanate moiety was introduced using solid and thus easy to handle triphosgene in refluxing toluene to give 13 in 87 % yield over two steps (Scheme 3).

Scheme 3. Synthesis of isocyanate 13. a) TMSCl, BnOH, 20 °C, 15 h, 89%; b) triphosgene, toluene, reflux, 7.5 h, 98%.

Hence, the synthesis of carbamate prodrug 2 was completed in seven further steps (Scheme 4). After deprotection of the secondary amino functionality in 10 under acidic conditions in an aqueous
HCl/EtOAc mixture with Et$_3$SiH as cation scavenger [25], the obtained hydrochloride salt 14 was directly coupled with the DNA-binding subunit TMI-CO$_2$H (15) to give 16 in 43 % yield over two steps. Then, the benzyl ether moiety in 16 was cleaved by transfer hydrogenolysis with an aqueous ammonium formate solution and palladium on charcoal as the catalyst [26] to yield phenol 3b which was subsequently coupled with isocyanate 13 to afford carbamate 17 in a very good yield of 83 % over two steps. The benzyl ester in 17 was cleaved again by using transfer hydrogenolytic conditions to give 18 in 90 % yield. This reaction had to be carefully monitored by TLC as the carbamate was sensitive to these conditions. Finally, carboxylic acid 18 was treated with HOSu/EDC·HCl and the resulting active ester 19 directly coupled with the fully unprotected tetrapeptide 20 [27] to yield carbamate prodrug 2 in 57 % (83 % based on recovered starting material) over two steps.

**Scheme 4.** Synthesis of carbamate prodrug 2. a) HCl/EtOAc, Et$_3$SiH, CH$_2$Cl$_2$, 20 °C, 7 h; b) TMI-CO$_2$H (15), EDC·HCl, DMF, 20 °C, 1 d, 43% (two steps); c) Pd/C, NH$_4$HCO$_2$, THF/MeOH, 20 °C, 75 min, quant.; d) isocyanate 13, NEt$_3$, CH$_2$Cl$_2$, 0–20 °C, 16 h, 83%; e) Pd/C, NH$_4$HCO$_2$, THF/MeOH, 20 °C, 25 min, 90%; f) HOSu, EDC·HCl, THF/CH$_2$Cl$_2$, 0–20 °C, 15 h; g) tetragastrin (20), NEtPr$_2$, H$_2$O/DMF, 20 °C, 7 h, 57% (two steps).
2.2. In vitro cytotoxicity tests

The in vitro cytotoxicity assays were carried out in duplicate with CCK-B/gastrin-receptor positive cells of the human pancreatic cell line MIA PaCa-2 and CCK-B/gastrin-receptor negative cells of the human bronchial carcinoma cell line A549 as control in six multiwell plates with concentrations of 10^2, 10^3 and 10^4 cells per cavity. Incubation with various concentrations of the seco-drug 3b and the prodrug 2 was performed in ultraculture medium (Table 1).

| Compound | MIA PaCa-2 IC_{50} [nM] | A549 IC_{50} [nM] |
|----------|--------------------------|-----------------|
| 2        | 0.31                     | 0.11            |
| 3b       | 0.31                     | 0.14            |

Prodrug 2 shows the same cytotoxicity as its corresponding seco-drug 3b in the cell culture assays using the CCK-B/gastrin-receptor positive cell line (MIA PaCa) and the CCK-B/gastrin-receptor negative cell line (A549). Thus, the obtained IC_{50}-values are almost identical in these four experiments. This indicates that prodrug 2 seems not to be stable under the used cell culture conditions. In fact, HPLC-MS-measurements revealed a decomposition of prodrug 2 under loss of the targeting pentagastrin moiety thereby forming the corresponding seco-drug 3b.

We suppose that the unstability of the carbamate moiety can be traced back to the hydrogen atom at its nitrogen which in turn is part of the β-alanine moiety of pentagastrin. We therefore plan to replace the hydrogen by a carbon moiety though it is not known whether such a modification of the pentagastrin would interfere with the binding of the conjugate to the corresponding CCK-B/gastrin-receptor.

3. Experimental Section

General: All reactions were performed in flame dried glassware under an argon atmosphere. Solvents were dried and purified according to standard procedures and redistilled prior to use. TLC chromatography was performed on precoated aluminium silica gel SIL G/UV254 plates (Macherey-Nagel & Co.) and silica gel 60 (0.040-0.063 mm) (Merck) was used for column chromatography. IR: Bruker Vector 22. UV/VIS: Perkin-Elmer Lambda 2. ^1^H-NMR: Varian Mercury-200, Unity-300 (300 MHz), Unity Inova-600 (600 MHz). ^13^C-NMR: Varian Mercury-200 (50 MHz), Unity-300 (75 MHz), Unity Inova-600 (150 MHz). For ^1^H and ^13^C, CDCl3, [D_6]DMSO and [D_2]DMF were used as solvents. Chemical shifts are reported on a δ scale. Signals are quoted as s (singlet), d (doublet), t
(triplet), q (quartet), m (multiplet), m_c (centered multiplet) and br (broad). MS: Finnigan MAT 95, TSQ 7000, LCQ. HRMS was performed using among others a modified peak matching technique, error ±2 ppm, with a resolution of ca. 10,000. Elemental analysis: Mikroanalytisches Labor des Institutes für Organische und Biomolekulare Chemie der Universität Göttingen.

3-Amino-1-benzyloxy-N-(tert-butoxycarbonyl)-7-[2-(trimethylsilyl)-ethoxycarbonyl]-naphthalene (7): A magnetically stirred and degassed solution of bromide 6 (3.60 g, 8.40 mmol), N(nBu)_3 (6.0 mL, 4.7 g, 25 mmol) and 2-(trimethylsilyl)-ethanol (6.0 mL, 5.0 g, 42 mmol) in DMF (35 mL) was treated with Pd(PPh_3)_2Br_2 (332 mg, 420 µmol), 1,1'-bis-(diphenylphosphino)-ferrocene (931 mg, 1.68 mmol) and Mo(CO)_6 (1.1 g, 4.2 mmol). The reaction mixture was degassed again, set under a carbon monoxide atmosphere (1 bar) and stirred for 7 h at 120 °C (preheated bath). The alcohol and tributylamine were distilled off under reduced pressure, the resulting red oil adsorbed on silica gel and subjected to column chromatography (pentane/EtOAc = 10:1 → 7:1) to afford ester 7 (2.32 g, 56 %) as orange solid. R_f = 0.43 (pentane/EtOAc = 7:1); ¹H NMR (300 MHz, CDCl_3): δ = 0.08 (s, 9 H, Si(CH_3)_3), 1.13–1.18 (m, 2 H, CH_2SiMe_3), 1.55 (s, 9 H, C(CH_3)_3), 4.43–4.49 (m, 2 H, CH_2OC=O), 5.26 (s, 2 H, CH_2Ph), 6.73 (brs, 1 H, NH), 7.08 (d, J = 1.5 Hz, 1 H, 2-H), 7.34–7.64 (m, 6 H, 5 × Ph-H, 4-H), 7.69 (d, J = 8.7 Hz, 1 H, 5-H), 8.02 (dd, J = 8.7, 2.1 Hz, 1 H, 6-H), 8.96 ppm (d, J = 2.1 Hz, 1 H, 8-H); ¹³C NMR (50 MHz, CDCl_3): δ = –1.40 (Si(CH_3)_3), 17.35 (CH_2SiMe_3), 28.32 (C(CH_3)_3), 63.09 (CH_2OC=O), 70.30 (CH_2Ph), 80.97 (C(CH_3)_3), 99.48 (C-3), 106.31 (C-1), 121.59, 125.61 (C-4a, C-6), 125.31, 126.61, 126.87, 127.45, 128.04, 128.61 (C-5, C-7, C-8, 5 × Ph-C), 136.41, 137.13, 138.65 (C-2, C-8a, Ph-C), 152.51, 156.23 (C-4, C=O), 167.11 ppm (C(O)OTMSE); MS (EI, 70 eV): m/z (%) = 493 (18) [M]^+, 437 (10) [M – C_4H_9 + H]^+, 91 (100) [C_7H_7]^+.

2-Amino-4-benzyloxy-N-(tert-butoxycarbonyl)-1-iodo-6-[2-(trimethylsilyl)-ethoxycarbonyl]-naphthalene (8): A magnetically stirred solution of 7 (352 mg, 713 µmol) in 1:1 THF/MeOH (10 mL) was treated with a solution of TsOH·H_2O (14 mg, 71 µmol) in THF (1 mL) and N-iodosuccinimide (322 mg, 1.43 mmol). The resulting mixture was warmed to 50 °C and stirred for 1 h. The reaction mixture was quenched with NaHCO_3 (5 mL, saturated solution) and water and extracted with EtOAc (2 × 10 mL). The combined organic phases were washed with Na_2S_2O_3 (1 × 15 mL, saturated solution) and brine (15 mL), dried (MgSO_4) and concentrated under reduced pressure to give an orange oil. This material was adsorbed on silica gel and subjected to column chromatography (pentane/EtOAc = 20:1) to afford iodide 8 (320 mg, 73 %) as colorless foam. R_f = 0.65 (pentane/EtOAc = 10:1); ¹H NMR (300 MHz, CDCl_3): δ = 0.08 (s, 9 H, Si(CH_3)_3), 1.13–1.18 (m, 2 H, CH_2SiMe_3), 1.59 (s, 9 H, C(CH_3)_3), 4.44–4.49 (m, 2 H, CH_2OC=O), 5.31 (s, 2 H, CH_2Ph), 7.33–7.60 (m, 5 H, 5 × Ph-H), 8.04 (d, J = 9.3 Hz, 1 H, 8-H), 8.09 (dd, J = 9.3, 1.8 Hz, 1 H, 7-H), 8.14 (s, 1 H, 3-H), 8.95 ppm (d, J = 1.8 Hz, 1 H, 5-H); ¹³C NMR (50 MHz, CDCl_3): δ = –1.40 (Si(CH_3)_3), 17.34 (CH_2SiMe_3), 28.30 (C(CH_3)_3), 63.29 (CH_2OC=O), 70.55 (CH_2Ph), 79.16 (C(CH_3)_3), 81.53 (C-1), 100.13 (C-3), 122.77, 126.27, 136.16, 137.06 (C-4a, C-6, C-8a, Ph-C), 125.62, 127.90, 128.07, 128.16, 128.58, 131.39 (C-5, C-7, C-8, 5 × Ph-C), 140.53 (C-2), 152.51, 156.20 (C-4, C=O), 167.11 ppm (C(O)OTMSE); MS (EI, 70 eV): m/z (%) = 619 (14) [M]^+, 563 (10) [M – C_4H_9 + H]^+, 535 (18) [M – C_4H_8 – CO]^+, 491 (5) [M – I – H]^+, 91 (100) [C_7H_7]^+, 57 (36) [C_4H_6]^+. 
(E/Z)-2-Amino-4-benzyloxy-N-(tert-butoxycarbonyl)-N-(3-chloro-2-propenyl)-1-iodo-6-[2-(trimethylsilyl)-ethoxycarbonyl]-naphthalene (9): A magnetically stirred solution of 8 (54 mg, 87 µmol) in DMF (1.5 mL) was treated with NaH (5.20 mg, 60 % in oil, 218 µmol). Stirring was continued for 40 min at 20 °C before (E/Z)-1,3-dichloropropene (16.0 µL, 19.0 mg, 174 µmol) was added dropwise, and it was stirred for a further 13 h at 20 °C. The ensuing mixture was then adjusted to pH 5 with NH₄Cl (saturated solution) and extracted with EtOAc (4 × 5 mL). The combined organic phases were washed with water (10 mL), brine (10 mL), dried (MgSO₄) and concentrated under reduced pressure to give an orange oil. Subjection of this material to column chromatography (pentane/EtOAc = 10:1) gave iodide 9 (59 mg, 97 %) as pale yellow foam. 

R_f = 0.43, 0.52 (pentane/EtOAc = 10:1); ¹H NMR (200 MHz, CDCl₃): δ = –0.14/0.10 (2 × s, 9 H, Si(CH₃)₃), 1.14–1.22 (m, 2 H, CH₂SiMe₃) 1.30/1.58 (2 × s, 9 H, C(CH₃)₃), 3.78 (dd, J = 13.8, 6.4 Hz, 1 H, 1’-Ha), 4.19–4.35 (m, 1 H, 1’-Hb), 4.46–4.54 (m, 2 H, CH₂OC=O), 5.31 (brs, 2 H, CH₂Ph), 5.92–6.15 (m, 2 H, 2’-H, 3’-H), 6.65–6.85 (m, 5 H, 5 × Ph-H), 7.30–7.55 (m, 5 H, 5 × Ph-H), 8.15 (d, J = 8.0 Hz, 1 H, 8-H), 8.25 (d, J = 8.0 Hz, 1 H, 7-H), 9.01–9.10 ppm (m, 1 H, 5-H); ¹³C NMR (50 MHz, CDCl₃): δ = –1.40 (Si(CH₃)₃), 17.33 (C(CH₃)₃), 28.19/28.46 (C(C(H₃))₃), 45.76/48.97 (C-1’), 63.53/64.59 (C(CH₂OC)=O), 70.49/70.58 (C(CH₂Ph), 80.88/81.39 (C(CH₃)₃), 94.52 (C-1), 107.93/108.51 (C-3), 120.87/121.96 (C-3’), 124.79, 128.26/128.29, 135.91/136.01, 137.51/137.64 (C-4a, C-6, C-8a, Ph-C), 125.36, 127.00/127.20, 127.90/127.94, 128.17/128.21, 128.45, 128.72/128.76, 133.06 (C-5, C-7, C-8, C-2’, 5 × Ph-C), 144.60/144.98 (C-2), 153.37/153.59 (C-4), 155.99/156.12 (C=O), 166.40/166.43 ppm (C(O)OTMSE); MS (EI, 70 eV): m/z (%) = 694 (16) [M + H]+, 566 (18) [M – I]+, 510 (88) [M – I – C₄H₉ + H]+, 91 (100) [C₇H₇]+, 57 (16) [C₄H₉]+.

(1R/S)-5-Benzoxy-3-(tert-butoxycarbonyl)-1-chloromethyl-2,3-dihydro-1H-benz[e]indole-7-carboxylic acid [2-(trimethylsilyl)-ethyl] ester (10): Through a magnetically stirred solution of iodide 9 (308 mg, 444 µmol) in benzene (13 mL) was bubbled argon for 45 min. The oxygen-free solution was then treated with tris-(trimethylsilyl)-silane (124 µL, 99.0 mg, 400 µmol) and AIBN (17.0 mg, 102 µmol) and stirred for 2 h under reflux. The ensuing mixture was adsorbed on silica gel and subjected to column chromatography (pentane/EtOAc = 10:1) to afford 10 (208 mg, 92 %) as pale yellow solid. 

R_f = 0.44 (pentane/EtOAc = 10:1); UV/VIS (CH₃CN): λmax (lg ε) = 217 (4.417), 271 (4.753), 354 nm (4.158); IR (KBr): ν~ = 3388 (NH), 2955, 1700 (C=O), 1621, 1461, 1412, 1366, 1249, 1139, 928, 839 cm –1; ¹H NMR (300 MHz, CDCl₃): δ = 0.08 (s, 9 H, Si(CH₃)₃), 1.12–1.18 (m, 2 H, CH₂SiMe₃), 1.61 (s, 9 H, C(CH₃)₃), 3.45 (t, J = 10.5 Hz, 1 H, 10-Hb), 3.88–4.02 (m, 2 H, 1-H, 10-Ha), 4.14 (dd, J = 11.4, 9.0 Hz, 1 H, 2-Ha), 4.27 (d, J = 11.4 Hz, 1 H, 2-Hb), 4.37 (d, J = 11.5 Hz, 1 H, 2-Hb), 4.44–4.49 (m, 2 H, CH₂OC=O), 5.29 (s, 2 H, CH₂Ph), 7.35–7.48 (m, 3 H, 3 × Ph-H), 7.54–7.59 (m, 2 H, 2 × Ph-H), 7.64 (d, J = 8.7 Hz, 1 H, 9-H), 7.87 (brs, 1 H, 4-H), 8.08 (dd, J = 8.7, 1.5 Hz, 1 H, 8-H), 9.02 ppm (d, J = 1.5 Hz, 1 H, 6-H); ¹³C NMR (125 MHz, CDCl₃): δ = –1.41 (Si(CH₃)₃), 17.33 (C(CH₂SiMe₃), 28.41 (C(CH₃)₃), 41.38 (C-1), 46.36 (C-10), 53.11 (C-2), 63.14 (C(CH₂OC)=O), 70.42 (C(CH₂Ph), 80.43 (C(CH₃)₃), 96.93 (C-4), 114.37, 121.50, 124.92, 132.37, 144.14 (C-3a, C-5a, C-7, C-9a, C-9b), 121.72 (C-9), 126.75 (C-6), 127.21 (C-8), 127.62, 128.07, 128.59 (5 × Ph-C), 136.34 (Ph-C), 152.41 (C-5), 157.25 (C=O), 166.91 ppm (C(O)OTMSE); MS (EI, 70 eV): m/z (%) = 567 (4) [M]+, 511 (7) [M – C₄H₉ + H]+, 91 (90) [C₇H₇]+, 57 (30) [C₄H₉]+; HRMS: calcd for C₃₁H₃₈ClNO₅Si: 567.2208; confirmed.
**β-Alanine benzyl ester hydrochloride (12):** A magnetically stirred suspension of β-alanine (11) (1.00 g, 11.2 mmol) in benzylic alcohol (56.0 mL, 58.0 g, 539 mmol) was treated dropwise over a period of 10 min with trimethylsilylchloride (3.6 mL, 3.0 g, 28 mmol) and stirring continued for a further 15 h at 20 °C. The resulting clear solution was poured into Et₂O (600 mL), the precipitate collected by filtration and washed with Et₂O (100 mL). Drying of this material under reduced pressure gave hydrochloride 12 (2.15 g, 89 %) as white solid; ¹H NMR (200 MHz, [D₆]DMSO): δ = 2.79 (t, J = 7.0 Hz, 2 H, 2-H₂), 3.03 (t, J = 7.0 Hz, 2 H, 3-H₂), 5.13 (s, 2 H, CH₂Ph), 7.32–7.41 (m, 5 H, 5 × Ph-H), 8.23 ppm (brs, 3 H, NH₃⁺); ¹³C NMR (50 MHz, [D₆]DMSO): δ = 31.34 (C-2), 34.47 (C-3), 65.87 (CH₂Ph), 127.95 (2 × Ph-C), 128.01 (Ph-Cₚ), 128.34 (2 × Ph-C), 135.73 (Ph-Cᵢ), 170.05 ppm (C=O); MS (ESI): m/z (%) = 180 (100) [M – Cl]⁺, 359 (100) [2M – Cl – HCl]⁺.

3-Isocyano-propionic acid benzyl ester (13): A magnetically stirred suspension of hydrochloride 12 (2.13 g, 9.88 mmol) in toluene (15 mL) was treated with triphosgene (2.93 g, 9.88 mmol) and heated to reflux for 7.5 h (end of HCl-evolution). The resulting solution was concentrated under reduced pressure to afford isocyanate 13 (1.99 g, 98 %) as yellow liquid which was used for the next reaction without further purification. Rf = 0.21 (pentane/EtOAc = 10:1); IR (film): ν ~ = 3349, 2957, 2277 (NCO), 1736 (C=O), 1498, 1176, 823, 752, 699 cm⁻¹; ¹H NMR (200 MHz, CDCl₃): δ = 2.65 (t, J = 6.4 Hz, 2 H, 2-H₂), 3.61 (t, J = 6.4 Hz, 2 H, 3-H₂), 5.18 (s, 2 H, CH₂Ph), 7.35–7.40 ppm (m, 5 H, 5 × Ph-H).

(1R/S)-5-Benzxylo-1-chloromethyl-3-(5,6,7-trimethoxyindole-2-carbonyl)-2,3-dihydro-1H-benz[e]indole-7-carboxylic acid [2-(trimethylsilyl)-ethyl] ester (16): A solution of the protected amine 10 (369 mg, 650 μmol) in CH₂Cl₂ (5 mL) was treated with HCl (18 mL of a 4 M solution in EtOAc) and Et₃SiH (105 μL, 76.0 mg, 650 μmol) and stirred for 7 h at 20 °C. The solvent was removed under reduced pressure and the ensuing residue treated with toluene (2 × 10 mL) and again concentrated under reduced pressure. The resulting crude hydrochloride 14 was dried under reduced pressure and then treated with 5,6,7-trimethoxyindole-2-carboxylic acid (15) (180 mg, 715 μmol), EDC·HCl (374 mg, 1.95 mmol) and DMF (16 mL) and stirred for 1 d at 20 °C. The reaction mixture was adjusted to pH 2 with HCl (2 N) and extracted with EtOAc (4 × 20 mL). The combined organic phases were washed with water (3 × 20 mL), brine (20 mL), dried (MgSO₄) and concentrated under reduced pressure to give a brown solid. This material was adsorbed on silica gel and subjected to column chromatography (pentane/EtOAc = 3:1) to yield 16 (196 mg, 43 % over two steps) as green solid. Rₗ = 0.57 (pentane/EtOAc = 2:1); UV/VIS (CH₃CN): λ max (lg ε) = 209 (4.758), 271 (4.470), 315 (4.465), 363 nm (4.524); IR (KBr): ν = 3461 (NH), 2951, 1711 (C=O), 1624, 1527, 1459, 1408, 1309, 1107, 837, 747 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ = 0.09 (s, 9 H, Si(CH₃)₃), 1.13–1.19 (m, 2 H, CH₂SiMe₃), 3.42 (dd, J = 10.8, 10.2 Hz, 1 H, 1-H₆), 3.90–4.05 (m, 11 H, 3 × OCH₃, 1-H, 10-H₆), 4.43–4.48 (m, 2 H, CH₂OC=O), 4.56 (dd, J = 11.1, 8.7 Hz, 1 H, 1-H₂), 4.71 (dd, J = 11.1, 1.8 Hz, 1 H, 2-H₆), 5.25–5.31 (m, 2 H, CH₂Ph), 6.85 (s, 1 H, 4'-H), 6.96 (d, J = 2.4 Hz, 1 H, 3'-H), 7.31–7.55 (m, 5 H, 5 × Ph-H), 7.62 (d, J = 9.0 Hz, 1 H, 9-H), 8.07 (dd, J = 9.0, 1.8 Hz, 1 H, 8-H), 8.21 (s, 1 H, 4-H), 9.03 (d, J = 1.8 Hz, 1 H, 6-H), 9.75 ppm (d, J = 2.4 Hz, 1 H, indole-NH); ¹³C NMR (75 MHz, CDCl₃): δ = −1.47 (Si(CH₃)₃), 17.26 (CH₂SiMe₃), 42.72 (C-1), 45.88 (C-10), 55.11 (C-2), 56.11, 61.00, 61.36 (3 × OCH₃), 63.18 (CH₂OC=O), 70.30 (CH₂Ph), 97.54 (C-4'), 98.87 (C-4), 106.75 (C-3'), 115.94,
123.45, 125.65, 131.71, 144.30 (C-3a, C-5a, C-7, C-9a, C-9b), 121.99 (C-9), 122.48, 125.61, 129.48, 138.72, 140.55 (C-2’, C-3’a’, C-6’, C-7’, C-7’a’), 126.47 (C-6), 127.12 (C-8), 127.44, 127.97, 128.49 (5 × Ph-C), 136.22 (Ph-Ci), 150.08 (C-5’a’), 156.65 (C-5), 160.51 (C=O), 166.62 ppm (C(O)OTMSE); MS (ESI): m/z (%) = 723 (100) [M + Na]+, 1423 (75) [2M + Na]+, 699 (100) [M – H]–; HRMS: calcd for C38H41ClN2O7Si: 701.2444 [M + H]+; found: 701.2441.

\((1R/S)-1\text{-Chloromethyl-5-hydroxy-3-(5,6,7-trimethoxyindole-2-carbonyl)-2,3-dihydro-1H-benz[e]indole-7-carboxylic acid [2-(trimethylsilyl)-ethyl] ester (3b)}\): A magnetically stirred solution of benzyl ether 16 (152 mg, 217 μmol) in 3:1 THF/MeOH (6 mL) was treated with 10 % Pd/C (62 mg) and dropwise with NH₄HCO₂ (572 μL of a 25 % solution in water, 2.26 mmol) and stirring continued for 75 min at 20 °C. The reaction mixture was filtered through a pad of Celite® and it was thoroughly washed with MeOH and THF. The filtrate was dried (MgSO₄) and concentrated under reduced pressure to give 3b (133 mg, quant.) as yellow solid which was used for the next reaction without further purification. Rf = 0.25 (pentane/ EtOAc = 2:1); 1H NMR (300 MHz, [D₆]DMSO): δ = 0.09 (s, 9 H, Si(CH₃)₃), 1.12–1.17 (m, 2 H, CH₂SiMe₃), 3.81–3.88 (m, 7 H, 2 × OCH₃, 10-H b), 3.95 (s, 3 H, OCH₃), 4.02 (dd, J = 11.1, 9.1 Hz, 1 H, 2-Ha), 6.97 (s, 1 H, 4-H'), 7.07 (d, J = 2.0 Hz, 1 H, 3'-H), 7.91–7.98 (m, 3 H, 4-H, 8-H, 9-H), 8.82 (d, J = 1.0 Hz, 1 H, 6-H), 10.83 (s, 1 H, OH), 11.42 ppm (d, J = 2.0 Hz, 1 H, indole-NH); 13C NMR (75 MHz, [D₆]DMSO): δ = –1.45 (Si(CH₃)₃), 16.87 (C₆H₂SiMe₃), 40.72 (C-1), 47.36 (C-10), 55.09 (C-2), 55.93, 60.84, 61.00 (3 × OCH₃), 62.56 (C₆H₂OC=O), 98.07 (C-4'), 100.82 (C-4), 106.32 (C-3'), 115.12, 123.06, 125.45, 131.92, 144.76 (C-3'a, C-5'a, C-7, C-9a, C-9b), 120.95, 123.12, 130.69, 139.00, 139.94 (C-2’, C-3’a’, C-6’, C-7’, C-7’a’), 123.94 (C-9), 125.89 (C-8), 126.02 (C-6), 149.19 (C-5’), 155.57 (C-5), 160.39 (C=O TMI), 165.89 ppm (C(O)OTMSE); MS (ESI): m/z (%) = 633 (100) [M + Na]⁺, 1243 (29) [2M + Na]⁺, 573 (100) [M – H – HCl]–, 609 (22) [M – H]–; HRMS: calcd for C₃₁H₃₅ClN₂O₇Si: 611.1975 [M + H]⁺; found: 611.1975.

\((1R/S)-5\text{-}{(2\text{-Benzyloxy carbonyl-ethyl carbamoyloxy)-1-chloromethyl-3-(5,6,7-trimethoxyindole-2-carbonyl)-2,3-dihydro-1H-benz[e]indole-7-carboxylic acid [2-(trimethylsilyl)-ethyl] ester (17)}}\): A magnetically stirred solution of phenol 3b (198 μmol, 121 mg) in CH₂Cl₂ (15 mL) at 0 °C was treated dropwise with 13 (203 μL, 203 mg; 990 μmol) and then triethylamine (139 μL, 100 mg, 990 μmol). The reaction mixture was warmed to 20 °C and stirred for a further 16 h. The ensuing solution was cooled to 0 °C, adjusted to pH 2 with HCl (2 N) and extracted with EtOAc (4 × 10 mL). The combined organic phases were washed with brine (10 mL), dried (MgSO₄) and concentrated under reduced pressure to give a yellow solid. This material was adsorbed on silica gel and subjected to column chromatography (pentane/EtOAc = 1:1) to afford carbamate 17 (135 mg, 83 %) as yellow solid. Rf = 0.29 (pentane/EtOAc = 2:1); 1H NMR (300 MHz, [D₆]DMSO, 100 °C): δ = 0.10 (s, 9 H, Si(CH₃)₃), 1.13–1.19 (m, 2 H, CH₂SiMe₃), 2.71 (t, J = 7.1 Hz, 2 H, 2”-H₂), 3.47–3.53 (m, 2 H, 1’”-H₂), 3.85 (s, 6 H, 2 × OCH₃), 3.98 (dd, J = 11.2, 6.8 Hz, 1 H, 10-Hb), 4.00 (s, 3 H, OCH₃), 4.09 (dd, J = 11.2, 3.4 Hz, 1 H, 10-Ha), 4.39 (m, 1 H, 1-H), 4.45–4.50 (m, 2 H, CH₂OC=O), 4.58 (dd, J = 11.1, 2.6 Hz, 1 H, 2-Hb), 4.80 (dd, J = 11.1, 9.2 Hz, 1 H, 2’-Hₐ), 5.17 (s, 2 H, CH₂Ph), 6.99 (s, 1 H, 4’-H), 7.09 (d, J = 2.2 Hz, 1 H, 3’-H), 7.30–7.41 (m, 5 H, 5 × Ph-H), 8.04 (dd, J = 8.7, 1.4 Hz, 1 H, 8-H), 8.09 (d, J = 8.7 Hz, 1 H, 9-H), 8.24 (s, 1 H, 4-H), 8.62 (d, J = 1.4 Hz, 1 H, 6-H), 11.02 ppm (brs, 1 H, OH).
indole-NH); $^{13}$C NMR (75 MHz, [D$_6$]DMSO, 100 °C): $\delta = -0.95$ (Si(CH$_3$)$_3$), 17.61 (CH$_2$SiMe$_3$), 34.62/34.68 (C-2''), 37.58/37.60 (C-1''), 41.76 (C-1), 47.79/47.84 (C-10), 55.62 (OCH$_3$), 61.38 (2 × OCH$_3$), 63.36 (2 × OCH$_2$OC=O), 66.15 (CH$_2$Ph), 99.48 (C-4'), 107.05 (C-3'), 111.34 (C-4), 122.42, 124.17, 126.73, 132.19, 144.43 (C-3a, C-5a, C-7, C-9a, C-9b), 123.78, 126.36, 131.09, 139.56, 140.95 (C-2', C-3a', C-6', C-7', C-7a'), 124.29 (C-9), 125.41 (C-6), 126.78 (C-8), 128.23, 124.21, 126.85, 131.42, 143.39 (C-3a, C-5a, C-7, C-9a, C-9b), 122.50 (C-9), 123.56, 125.86, 129.29, 140.83 (C-2', C-3a', C-6', C-7', C-7a'), 125.93 (C-6), 149.00 (C-5'), 150.10 (OC(O)N), 156.16/154.68 (C-5), 161.23 (C=O TMI), 166.19 (C(O)OTMSE), 171.23 ppm (C(O)OBn); MS (ESI): m/z (%) = 611 (100) [M – C(O)NH-$\beta$-Ala-OBn + H]$^+$, 838 (18) [M + Na]$^+$, 1653 (14) [2M + Na]$^+$, 573 (100) [M – C(O)NH-$\beta$-Ala-OBn – HCl]$^-$, 609 (55) [M – C(O)NH-$\beta$-Ala-OBn – H]$^+$. 

(1R/S)-5-(2-Carboxy-ethylcarbamoyloxy)-1-chloromethyl-3-(5,6,7-trimethoxyindole-2-carbonyl)-2,3-dihydro-1H-benz[e]indole-7-carboxylic acid [2-(trimethylsilyl)-ethyl] ester (18): A magnetically stirred solution of benzyl ester 17 (203 mg, 249 $\mu$mol) in 3:1 THF/MeOH (10 mL) was treated with 10 % Pd/C (83 mg) and dropwise with NH$_4$HCO$_2$ (653 $\mu$L of a 25 % solution in water, 2.59 mmol). Stirring was continued for 25 min (TLC-monitoring necessary as the alanyl residue is easily cleaved off) at 20 °C. The reaction mixture was filtered through a pad of Celite®, which was thoroughly washed with MeOH and CH$_2$Cl$_2$. The combined filtrates were concentrated under reduced pressure, the residue dissolved in CH$_2$Cl$_2$, dried (MgSO$_4$) and concentrated under reduced pressure again to give a yellow oil. This material was adsorbed on silica gel and subjected to column chromatography (CH$_2$Cl$_2$/MeOH = 20:1) to afford acid 18 (163 mg, 90 %) as pale yellow solid. $R_f = 0.44$ (CH$_2$Cl$_2$/MeOH = 10:1); $^1$H NMR (300 MHz, CDCl$_3$): $\delta = 0.06$ (s, 9 H, Si(CH$_3$)$_3$), 1.12 (mc, 2 H, CH$_2$SiMe$_3$), 2.74 (mc, 2 H, 2''-H$_2$), 3.41 (mc, 1 H, 10-H$_b$), 3.65 (mc, 2 H, 1''-H$_2$), 3.83–4.07 (m, 11 H, 1-H, 10-H$_a$, 3 × OCH$_3$), 4.42 (mc, 2 H, CH$_2$OC=O), 4.57–4.64 (m, 1 H, 1-H, 2-H$_b$), 4.71–4.74 (m, 1 H, 2-H$_a$), 6.39 (brs, 1 H, NH), 6.87 (brs, 1 H, 4'-H), 6.99 (brs, 1 H, 3'-H), 7.62 (mc, 1 H, 9-H), 8.01 (mc, 1 H, 8-H), 8.41 (brs, 1 H, 4-H), 8.56 (brs, 1 H, 6-H), 9.99 ppm (brs, 1 H, indole-NH); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = -1.46$ (Si(CH$_3$)$_3$), 17.34 (CH$_2$SiMe$_3$), 34.17 (C-2''), 36.99 (C-1''), 43.01 (C-1), 45.75 (C-10), 55.17 (C-2'), 56.22, 61.31, 61.49 (3 × OCH$_3$), 63.65 (CH$_2$OC=O), 97.78 (C-4'), 107.11 (C-3'), 111.78 (C-4'), 121.31, 124.21, 126.85, 131.42, 143.39 (C-3a, C-5a, C-7, C-9a, C-9b), 122.50 (C-9), 123.56, 125.86, 129.29, 138.68, 140.83 (C-2', C-3a', C-6', C-7', C-7a'), 125.93 (C-6), 126.52 (C-8), 149.00 (C-5'), 150.10 (OC(O)N), 154.39 (C-5), 160.58 (C=O TMI), 166.61 (C(O)OTMSE), 171.50 ppm (C(O)OH); MS (ESI): $m/z$ (%) = 748 (90) [M + Na]$^+$, 1473 (100) [2M + Na]$^+$, 573 (96) [M – C(O)NH-$\beta$-Ala-OBn – H][Cl]$^-$, 1449 (100) [2M – H]$^+$. 

(1R/S)-1-Chloromethyl-5-[2-(N-succinimidyloxycarbonyl)-ethyl-carbamoyloxy]-3-(5,6,7-trimethoxyindole-2-carbonyl)-2,3-dihydro-1H-benz[e]indole-7-carboxylic acid [2-(trimethylsilyl)-ethyl] ester (19): A magnetically stirred solution of acid 18 (110 mg, 152 $\mu$mol) and N-hydroxysuccinimide (26.0 mg, 228 $\mu$mol) in 1:1 THF/CH$_2$Cl$_2$ (10 mL) at 0 °C was treated with EDC-HCl (44.0 mg, 228 $\mu$mol). The reaction mixture was warmed to 20 °C and stirring continued for 15 h. The ensuing solution was then adjusted to pH 2 with HCl (2 N) and extracted with EtOAc (4 × 10 mL). The combined organic phases were washed with brine (10 mL), dried (MgSO$_4$) and concentrated under reduced pressure to afford 19 as yellow solid which was used for the next reaction
without further purification. $R_f = 0.83$ (CH$_2$Cl$_2$/MeOH = 10:1); MS (ESI): $m/z$ (%) = 845 (90) [M + Na]$^+$, 1667 (100) [2M + Na]$^+$, 573 (100) [M – C(O)NH-$\beta$-Ala-OSu – HCl$^-$].

**Cell culture**: Human bronchial carcinoma cells of line A549 (ATCC CCL 185) were kindly provided by the Institut für Zellbiologie, Universität Essen, and human pancreatic carcinoma cells Mia
PaCa-2 by the Universitätsklinikum Göttingen, Abteilung Hämatologie und Onkologie. Cell lines were maintained as exponentially growing cultures at 37 °C and 7.5% CO₂ in air in culture medium (DMEM (Biochrom) supplemented with 10 % fetal calf serum, 44 mM NaHCO₃ (Biochrom) and 4 mM L-Glutamine (Invitrogen)).

**In vitro cytotoxicity assays:** Cells of line A549 or MIA PaCa-2 were seeded in duplicates in 6 multiwell plates at concentrations of 10², 10³ and 10⁴ cells per well. After cells were allowed to adhere, cells were washed in a serum-free incubation medium (Ultraculture medium, Lonza). Incubation with compounds 2 and 3b was then performed in Ultraculture medium at various concentrations for 24 h. All substances were used as freshly prepared solutions in DMSO (Merck) diluted with incubation medium to a final concentration of DMSO of 1% in the wells. After exposure the test substance was removed and cells were washed with fresh medium. Cultivation in normal growth medium was done for 10 days. The medium was removed, the clones were dried and stained with Löeffler’s methylene blue (Merck) and then counted macroscopically.

The IC₅₀ values are based on the relative colony-forming rate, which was determined according to the following formula: relative colony-forming rate [%] = 100 × (number of clones counted after exposure) / (number of clones counted in the control).

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27. Tetragastrin (20) was purchased from Bachem.

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