Synthesis and Antitumor Activity of Methoxy-indolo[2,1-a]isoquinolines

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Methoxy-indolo[2,1-a]isoquinolines **8a–f** and their dihydroderivatives **7a–f** were synthesized by *Bischler-Napieralski* reaction of the (bromo-methoxyphenyl)-[2-(methoxyphenyl)-ethyl]acetamides **4a–f**, reduction, subsequent cyclization and dehydrogenation. They were tested for cytostatic activity in vitro using P388 D_1 leukemia and MDA MB 231 mammary tumor cells. The trimethoxy-5,6-dihydroindoloisoquinoline **7d** and the tetramethoxyindoloisoquinoline **8f** showed an inhibition of cellproliferation of about 70 % at a concentration of 10^{-5} molar.

Synthese und Antitumoraktivität von Methoxy-indolo[2,1-a]isochinolinen

Die Methoxy-indolo[2,1-a]isochinoline **8a–f** und deren Dihydroderivate **7a–f** wurden durch *Bischler-Napieralski*-Ringschluß der (Brom-methoxyphenyl)-[2-(methoxyphenyl)-ethyl]acetamide **4a–f**, Reduktion, Cyclisierung und Dehydrierung gewonnen. Die cytostatische Wirkung wurde in vitro an der P388 D_1- und der MDA-MB 231-Zellinie getestet. Das Trimethoxy-5,6-dihydroindoloisochinolin **7d** und das Tetramethoxyindoloisochinolin **8f** zeigten eine Hemmung der Zellproliferation von 70 % bei einer Konzentration von 10^{-5} M.

The aim of our investigations is the synthesis of cytostatic compounds with binding affinity for the estrogen receptor, that can be used for the selective treatment of hormone dependent mammary tumors. Suitable structures for this approach are tetracyclic N-heterocycles, which are known to intercalate into the DNA[1] and are able to bind to the estrogen receptor[2].

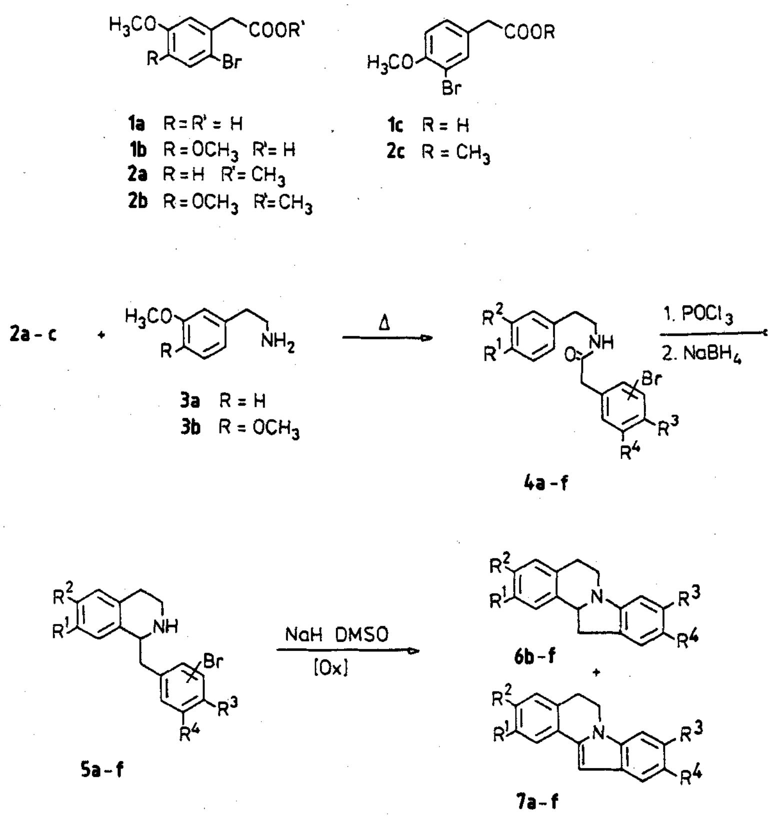
Based on these findings, we synthesized a number of indolo[2,1-a]isoquinolines **8a–f** and their dihydro analogues **7a–f**. Cytostatic activity of these compounds was evaluated in vitro using MDA-MB 231 mammary tumor cells and P388 D_1 leukemia cells.

Chemistry

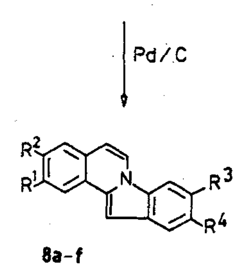
The synthesis was performed as outlined in scheme 1. The starting methyl bromophenylacetates **2a–c** were synthesized by bromination of the corresponding phenylacetic acids and conversion to the methyl esters. The 2-phenylethylamine **3a** was obtained directly by $LiAlH_4$ reduction of the 3-methoxy-β-nitrostyrene[3] or, in better yield, in two steps with $NaBH_4$ followed by $LiAlH_4$. The best method was the hydrogenation of the 3-methoxy-phenylacetonitrile with Rh/C.

The reaction of the bromo-phenylacetates **2a–c** with the 2-phenylethylamines **3a** and **3b** afforded the corresponding amides **4a–f**. Cyclisation to the 3,4-dihydroisoquinolines was accomplished by a modified *Bischler-Napieralski* method using $POCl_3$ in CH_3CN. The crude products were treated with $NaBH_4$ to give the 1,2,3,4-tetrahydro-1-benzylisoquinolines **5a–f**. In the case of **4c** (2-Bromo-4,5-dimethoxyphenyl)-(3,4-dihydro-6-methoxyisoquinolyl-1)-ketone was formed as a byproduct, probably by air oxidation[4]. The correct substitution pattern in the isoquinoline ring was confirmed by ^{1}H-NMR spectroscopy.

The benzylisoquinolines **5a–f** were converted into the tetracyclic indolo[2,1-a]isoquinolines by treatment with NaH in DMSO. This reaction must involve a benzyne intermediate[5] because both bromo compounds **5a** and **5b** led to the same structure. The reaction mixture contained two products



4 - 8	R1	R2	R3	R4
a	H	OCH3	H	OCH3
b	H	OCH3	OCH3	H
c	H	OCH3	OCH3	OCH3
d	OCH3	OCH3	H	OCH3
e	OCH3	OCH3	OCH3	H
f	OCH3	OCH3	OCH3	OCH3



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in a ratio of 1:4 which can be separated by column chromatography (CC). The expected tetrahydroindoloisoquinoline was only the minor product, whereas the main fraction contained the dihydro derivative. ^{1}H-NMR spectroscopy revealed, that under the reaction conditions oxidation occures to the indoles **7a–f**: the double dublett for the hydrogen at C-12a had disappeared and two tripletts for the hydrogen atoms at C-5 and C-6 appeared instead of the complex multiplett in **6b–f**.

This oxidation reaction can also be performed with DDQ or with Pd/C at a temp. just above the melting point of the tetrahydro compound. Heating the dihydro derivative in the presence of Pd/C above its melting point, which is considerably higher than that of the tetrahydro compound, afforded the aromatic indoloisoquinolines **8a–f**.

Cytostatic Activity

Two different cell lines were used for the determination of cytostatic activity. The P388 D_1 cell line derives from a mouse leukemia, the MDA-MB 231 cells are hormone-independent mammary tumor cells of human origin. All of the new indoloisoquinolines were tested for cytostatic effects at a concentration of 10^{-5} molar. The inhibition of cell growth was measured by cell counting and ^{3}H-thymidine labeling. The tetrahydro derivatives **6b–f** were devoid of activity, probably due to their nonplanar structure. The dihydro compounds **7** showed a weak inhibitory effect except compound **7d** that proved to be active against MDA-MB 231 (Table 1). Going to the aromatic indoloisoquinolines **8a–f**, no significant increase of cytostatic activity was observed. The tetramethoxy derivative **8f** was the most active compound in this series (Table 2).

These results showed, that cytostatic activity is not generally associated with a planar structure of the tetracyclic heterocycles. Compounds with a significant inhibition of cell growth possess a dimethoxyisoquinoline fragment **(8d, 8f, 7d)**. This effect was not very marked, since a concentration of 10^{-5} M was required for it. The rather low activity of this type of tetracycles may be due to the lack of a positive charge on the nitrogen.

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Experimental Part

Melting points: Büchi 510 apparatus, uncorrected. – Elemental analyses: Mikroanalytisches Laboratorium, University of Regensburg. – IR-spectra: Beckman Acculab 3; KBr. – ^{1}H-NMR spectra: Varian EM 360 A (60 MHz); TMS as internal standard. – Temp. in °C.

General Procedure for the Synthesis of the Bromo-phenylacetic acids **1a–c**

Bromine (17 ml, 0.23 mol) was added slowly to a solution of methoxyphenylacetic acid (0.24 mol) (Janssen) and 10.7 g (0.27 mol) of NaOH in 400 ml water at 50°. After stirring for 30 min and cooling to room temp., the precipitate was filtered off and washed with water. Recrystallization from aqueous MeOH yielded colorless crystals.

Tab. 1: Effect of **7a–f** on the growth of MDA–MB 231 and P388 D_1 cells

Compound[a)]	P388 D_1 cell no.[b)] % T/C[d)]	P388 D_1 ^{3}H-thymidine incorp.[c)] % T/C[d)]	MDA–MB 231 cell no.[b)] % T/C[d)]	MDA–MB 231 ^{3}H-thymidine incorp.[c)] % T/C[d)]
7a	95	98	90	90
7b	100	97	100	98
7c	93	93	87	90
7d	96	92	32	16
7d[e)]			90	90
7e	96	93	87	62
7f	93	93	93	92

a) Concentration 10^{-5} M.
b) Mean of three tests with six dishes/test tubes.
c) cpm/10^6 cells.
d) % T/C = test compound/control × 100.
e) Concentration 10^{-6} M.

Tab. 2: Effect of **8a–f** on the growth of MDA–MB 231 and P388 D_1 cells

Compound[a)]	P388 D_1 cell no.[b)] % T/C[d)]	P388 D_1 ^{3}H-thymidine incorp.[c)] % T/C[d)]	MDA–MB 231 cell no.[b)] % T/C[d)]	MDA–MB 231 ^{3}H-thymidine incorp.[c)] % T/C[d)]
8a	89	92	82	85
8b	78	84	80	85
8c	88	95	84	90
8d	71	73	48	45
8e	73	78	97	100
8f	30	15	48	21

a, b, c, d) see Tab. 1.

2-Bromo-5-methoxy-phenylacetic acid **(1a)**

Yield 90 %; m.p. 115° (lit. 114–115° [6]). – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.8 (s; 5H, $-OCH_3$, $-CH_2-$), 6.67–6.9 (m; 2H, ArH), 7.47 (d; J = 9 Hz, 1H, ArH), 7.96 (s broad; 1H, OH).

2-Bromo-4,5-dimethoxy-phenylacetic acid **(1b)**

Yield 90 %; m.p. 115° (lit. 115–116° [6]). – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.77 (s; 2H, $-CH_2-$), 3.88 (s; 6H, $-OCH_3$), 6.76 (s; 1H, ArH), 7.02 (s; 1H, ArH), 8.16 (s broad; 1H, OH).

3-Bromo-4-methoxy-phenylacetic acid **(1c)**

Yield 80 %; m.p. 115° (lit. 115° [7]). – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.8 (s; 5H, $-OCH_3$, $-CH_2-$), 6.67–6.9 (m; 2H, ArH), 7.47 (d; J = 9 Hz, 1H, ArH), 7.96 (s broad; 1H, OH).

General Procedure for the Synthesis of the Methyl Bromo-methoxyphenylacetates **2a–c**

Bromo-methoxyphenylacetic acid (0.23 mol) in 180 ml absol. MeOH and 6 ml conc. H_2SO_4 was boiled for 17 h. The volume of the solution was reduced to 50 ml. After addition of 100 ml of water, the mixture was extracted with CH_2Cl_2. The org. layer was dried (Na_2SO_4) and the solution was evaporated.

Methyl 2-bromo-5-methoxyphenylacetate **(2a)**

The product was purified by Kugelrohr-distillation: colorless oil. – Yield 90 %; b.p. 77–80°, 0.2 mm. – $C_{10}H_{11}BrO_3$ (259.0) Calc. C 46.4 H 4.25 Found C 47.0 H 4.61. – IR (film): 1740 (CO) cm^{-1}. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.70 (s; 2H, $-CH_2-$), 3.76 (s; 3H, $COOCH_3$), 3.80 (s; 3H, $-OCH_3$), 6.6–6.9 (m; 2H, ArH), 7.43 (d; J = 9 Hz, 1H, ArH).

Methyl 2-bromo-4,5-dimethoxyphenylacetate **(2b)**

The product was recrystallized from aqueous MeOH: colorless crystals. Yield 80 %; m.p. 48°. – $C_{11}H_{13}BrO_4$ (289.1) Calc. C 45.7 H 4.53 Found C 45.4 H 4.68. – IR (KBr): 1740 (CO) cm^{-1}. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.77 (s; 5H, $COOCH_3$, $-CH_2-$), 3.91 (s; 6H, $-OCH_3$), 6.87 (s; 1H, ArH), 7.13 (s; 1H, ArH).

Methyl 3-bromo-4-methoxyphenylacetate **(2c)**

Recrystallization from aqueous MeOH yielded colorless crystals. Yield 90 %; m.p. 31° (lit. 48° [8]). – $C_{10}H_{11}BrO_3$ (259.0) Calc. C 46.4 H 4.25 Found C 46.2 H 4.35. – IR(KBr): 1740 (CO) cm^{-1}. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.6 (s; 2H, $-CH_2-$), 3.73 (s; 3H, $-COOCH_3$), 3.9 (s; 3H, $-OCH_3$), 6.86 (d; J = 9 Hz, 1H, ArH), 7.23 (dd; $J_{1,2}$ = 9/2 Hz, 1H, ArH), 7.5 (d; J = 2 Hz, 1H, ArH).

1-Amino-2-(3-methoxyphenyl)-ethane **(3a)**

1 g Rh/C 10 % was added to a solution of 50 g (0.34 mol) 3-methoxy-phenylacetonitrile (Janssen) dissolved in 110 ml EtOH saturated with NH_3. The mixture was hydrogenated at 30 bar for 7 d. The catalyst was filtered off, washed with EtOH, and the solvent was evaporated. Pure **3a** was obtained as colorless oil. Yield 98 %; b.p. 122–123°, 7 mm (lit. 122–123°, 7 mm[9]). – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.48 (s; 2H, $-NH_2$), 2.82 (mc; 4H, $-CH_2-$), 3.79 (s; 3H, $-OCH_3$), 6.59–7.3 (m; 4H, ArH).

General Procedure for the Synthesis of the Acetamides **4a–f**

A flask containing 0.75 mol methoxy-phenylethylamine and 0.75 mol methyl bromo-phenylacetate was placed into a hot oil bath. The temp. was kept at 150–155° for 10 h. After cooling to 35° 10 ml EtOAc were added with stirring. The product crystallizing at 4° was filtered off and washed with ether. Acetamides, which did not crystallize spontaneously, were purified by CC (SiO_2; ether/$CHCl_3$ 1:1) and crystallized from EtOAc/ether. The crystals of **4a–f** are colorless.

2-(2-Bromo-5-methoxyphenyl)-N-[2-(3-methoxyphenyl-ethyl]-acetamide **(4a)**

4a was synthesized from **2a** and **3a** and recrystallized from EtOH. Yield 50 %; m.p. 91°. – $C_{18}H_{20}BrNO_3$ (378.3) Calc. C 57.2 H 5.35 Found C 57.2 H 5.28. – IR(KBr): 3300 (NH), 1650, 1555 (CO) cm^{-1}. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.80 (t; J = 7 Hz, 2H, $-CH_2-$), 3.53 (t; J = 7 Hz, 2H, $-CH_2-$), 3.67 (s; 2H, $-COCH_2-$), 3.83 (s; 3H, $-OCH_3$), 3.93 (s; 3H, $-OCH_3$), 5.53 (s broad; 1H, -NH), 6.67–7.43 (m; 6H, ArH), 7.77 (s; 1H, ArH).

2-(3-Bromo-4-methoxyphenyl)-N-[2-(3-methoxyphenyl)-ethyl]-acetamide **(4b)**

4b was synthesized from **2c** and **3a** and recrystallized from EtOAc/ether (7+3). Yield 55 %; m.p. 63–64°. – $C_{18}H_{20}BrNO_3$ (378.3) Calc. C 57.2 H 5.35 Found C 56.8 H 5.43. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.73 (t; J = 7 Hz, 2H, $-CH_2-$), 3.3–3.53 (m; 4H, $-COCH_2-$, $-CH_2-$), 3.77 (s; 3H, $-OCH_3$), 3.87 (s; 3H, $-OCH_3$), 5.49 (s broad; 1H, -NH), 6.56–6.79 (m; 3H, ArH), 6.86 (s; 1H, ArH), 7.0–7.17 (m; 2H, ArH), 7.34 (d; J = 2 Hz, 1H, ArH).

2-(2-Bromo-4,5-dimethoxyphenyl)-N-[2-(3-methoxyphenyl)-ethyl]-acetamide **(4c)**

4c was synthesized from **2b** and **3a** and recrystallized from EtOH. Yield 60 %; m.p. 145–146.5°. – $C_{19}H_{22}BrNO_4$ (408.3) Calc. C 55.9 H 5.39 Found C 55.8 H 5.40. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.77 (t; J = 7 Hz, 2H, $-CH_2-$), 3.47 (t; J = 7 Hz, 2H, $-CH_2-$), 3.63 (s; 2H, $-COCH_2-$), 3.82 (s; 3H, $-OCH_3$), 3.85 (s; 3H, $-OCH_3$), 3.90 (s; 3H, $-OCH_3$), 5.50 (s broad; 1H, -NH), 6.65–7.33 (m; 6H, ArH).

2-(2-Bromo-5-methoxyphenyl)-N-[2-(3,4-dimethoxyphenyl)-ethyl]-acetamide **(4d)**

4d was synthesized from **2a** and 1-amino-2-(3,4-dimethoxyphenyl)-ethane **(3b)** (Janssen) and recrystallized from EtOH. Yield 55 %; m.p. 128–129°. – $C_{19}H_{22}BrNO_4$ (408.3) Calc. C 55.9 H 5.39 Found C 55.4 H 5.70. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.70 (t; J = 7 Hz, 2H, $-CH_2-$) 3.42 (t; J = 7 Hz, 2 H, $-CH_2-$), 3.60 (s; 2H, $-COCH_2-$), 3.73 (s; 3H, $-OCH_3$), 3.80 (s; 3H, $-OCH_3$), 3.82 (s; 3H, $-OCH_3$), 5.53 (s broad; 1H, -NH), 6.63–6.85 (m; 5H, ArH), 7.43 (d; J = 9 Hz, 1H, ArH).

2-(3-Bromo-4-methoxyphenyl)-N-[2,-(3,4-dimethoxyphenyl)-ethyl]-acetamide **(4e)**

4e was synthesized from **2c** and **3b** and recrystallized from EtOH. Yield 50 %; m.p. 123–124°. – $C_{19}H_{22}BrNO_4$ (408.3) Calc. C 55.9 H 5.39 Found C 55.9 H 5.44. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.73 (t; J = 7 Hz, 2H, $-CH_2-$), 3.42 (t; J = 7 Hz, 2H, $-CH_2-$), 3.47 (s; 2H, $-COCH_2-$), 3.88 (s; 3H, $-OCH_3$), 3.93 (s; 3H, $-OCH_3$), 3.95 (s; 3H, $-OCH_3$), 5.55 (s broad; 1H, -NH), 6.67–7.0 (m; 4H, ArH), 7.18 (dd; $J_{1/2}$ = 9/2 Hz, 1H, ArH), 7.45 (d; J = 2 Hz, 1H, ArH).

2-(2-Bromo-4,5-dimethoxyphenyl)-N-[2-(3,4-dimethoxyphenyl)-ethyl]-acetamide **(4f)**

4f was synthesized from **2b** and **3b** and recrystallized from EtOH. Yield 60 %; m.p. 158.5–159°. – $C_{20}H_{24}BrNO_5$ (438.3) Calc. C 54.8 H 5.52 Found C 54.9 H 5.53. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.73 (t; J = 7 Hz, 2H, $-CH_2-$), 3.47 (t; J = 7 Hz, 2H, $-CH_2-$), 3.63 (s; 2H, $-COCH_2-$), 3.87 (s; 12H, $-OCH_3$), 5.57 (s broad; 1H, -NH), 6.67–7.07 (m; 5H, ArH).

General Procedure for the Synthesis of the 1-Benzyl-1,2,3,4-tetrahydroisoquinolines **5a–f**

A mixture of 55 mmol of acetamide, 20 ml of $POCl_3$ and 75 ml of absol. CH_3CN was refluxed for 4 h. With cooling, 150 ml of 20 % NaOH solution were added, the mixture was poured onto ice water and extracted with $CHCl_3$. The $CHCl_3$ solution was extracted with 150 ml 2N HCl. The free base was liberated with 20 % NaOH and extracted with $CHCl_3$. The org. layer was washed with water and saline, and dried (Na_2SO_4). The 3,4-dihydroisoquinolines obtained after evaporation of the solvent were used without further purification. The yields were between 55 and 70 %. Air must be excluded as far as possible during workup.
$NaBH_4$ (0.11 mol) was added slowly to a solution of 13.4 mmol of 3,4-dihydroisoquinoline in 100 ml MeOH and 15 ml water at 0°. The mixture was stirred for 2 h at room temp. After the solvent had been removed, the residue was treated with 100 ml water and extracted with $CHCl_3$. The org. layer was washed with water, and dried (Na_2SO_4). After evaporation of the solvent the residue crystallized with aqueous EtOH or was purified by CC (SiO_2; $CHCl_3$/ether 1:1). Recrystallization from aqueous EtOH afforded colorless crystals. The yields were 58–80 %.

1-(2-Bromo-5-methoxybenzyl)-1,2,3,4-tetrahydro-6-methoxyisoquinoline **(5a)**

Yield 50 %; m.p. 72–74°. – $C_{18}H_{20}BrNO_2$ (362.3) Calc. C 59.7 H 5.56 Found C 59.3 H 5.54. – IR (KBr): 3350 (NH) cm^{-1}. – ^1H-NMR ($CDCl_3$): δ (ppm) = 1.79 (s broad; 1H, -NH), 2.67–3.53 (m; 6H, $-CH_2-$), 3.80 (s; 6H, $-OCH_3$), 4.31 (dd; $J_{1/2}$ = 10/4 Hz, 1H, -CH-N), 6.58–6.87 (m; 4H, ArH), 7.26 (d; J = 9 Hz, 1H, ArH), 7.48 (d; J = 9 Hz, 1H, ArH).

1-(3-Bromo-4-methoxybenzyl)-1,2,3,4-tetrahydro-6-methoxyisoquinoline **(5b)**

Yield 70 %; m.p. 97–98°. – $C_{18}H_{20}BrNO_2$ (362.3) Calc. C 59.7 H 5.56 Found C 59.0 H 5.68. – ^1H-NMR ($CDCl_3$): δ (ppm) = 2.57 (s, broad; 1H, -NH), 2.73–3.30 (m; 6H, $-CH_2-$), 3.77 (s; 3H, $-OCH_3$), 3.87 (s; 3H, $-OCH_3$), 4.15 (dd; $J_{1/2}$ = 10/4 Hz, 1H, -CH-N), 6.59–6.82 (m; 3H, ArH), 6.88 (s; 1H, ArH), 7.1–7.28 (m; 2H, ArH), 7.47 (d; J = 2 Hz, 1H, ArH).

1-(2-Bromo-4,5-dimethoxybenzyl)-1,2,3,4-tetrahydro-6-methoxyisoquinoline **(5c)**

Yield 60 %; m.p. 114°. – $C_{19}H_{22}BrNO_3$ (392.3) Calc. C 58.2 H 5.65 Found C 57.7 H 5.51. – ^1H-NMR ($CDCl_3$): δ (ppm) = 2.0 (s broad; 1H, -NH), 2.70–3.40 (m; 6H, $-CH_2-$), 3.83 (s; 3H,- OCH_3), 3.87 (s; 3H, $-OCH_3$), 3.92 (s; 3H, $-OCH_3$), 4.33 (dd; $J_{1/2}$ = 10/4 Hz, 1H, -CH-N), 6.70–6.90 (m; 3H, ArH), 7.15 (s; 1H, ArH), 7.30 (d; J = 9 Hz, 1H, ArH).

(2-Bromo-4,5-dimethoxyphenyl)-(3,4-dihydro-6-methoxyisoquinolyl-1-)-ketone

This compound was formed as a byproduct of the cyclization of **4c**. It was purified by CC (SiO_2; CH_2Cl_2) to afford colorless crystals. Yield 20 %; m.p. 180–181°. – $C_{19}H_{18}BrNO_4$ (404.3) Calc. C 56.4 H 4.46 Found C 56.0 H 4.62. – IR(KBr): 1670 (CO) cm^{-1}. – ^1H-NMR ($CDCl_3$): δ (ppm) = 2.80 (t: J = 7 Hz, 2H, $-CH_2-$), 3.80 (t; J = 7 Hz, 2H, $-CH_2-$), 3.90 (s; 3H, $-OCH_3$), 3.95 (s; 6H, $-OCH_3$), 6.80–6.97 (m; 2H, ArH), 7.27 (s; 1H, ArH), 7.63 (d; J = 9 Hz, 1H, ArH).

1-(2-Bromo-5-methoxybenzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline **(5d)**

Yield 65 %; m.p. 117°. – $C_{19}H_{22}BrNO_3$ (392.3) Calc. C 58.2 H 5.65 Found C 58.0 H 5.55. – ^1H-NMR ($CDCl_3$): δ (ppm) = 1.90 (s broad; 1H, -NH), 2.67–3.50 (m; 6H, $-CH_2-$), 3.73 (s; 3H, $-OCH_3$), 3.77 (s; 3H, $-OCH_3$), 3.82 (s; 3H, $-OCH_3$), 4.33 (dd; $J_{1/2}$ = 10/4 Hz, 1H, -CH-N), 6.57 (s; 1H, ArH), 6.58–6.83 (m; 2H, ArH), 6.72 (s; 1H, ArH), 7.47 (d; J = 9 Hz, 1H, ArH).

1-(3-Bromo-4-methoxybenzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline **(5e)**

Yield 55 %; m.p. 107–109°. – $C_{19}H_{22}BrNO_3$ (392.3) Calc. C 58.2 H 5.65 Found C 57.8 H 5.62. – ^1H-NMR ($CDCl_3$): δ (ppm) = 1.80 (s broad; 1H, -NH), 2.67–3.50 (m; 6H, $-CH_2-$), 3.88 (s; 3H, $-OCH_3$), 3.92 (s; 3H, $-OCH_3$), 3.95 (s; 3H, $-OCH_3$), 4.17 (dd; $J_{1/2}$ = 10/4 Hz, 1H, -CH-N), 6.70 (s; 2H, ArH), 6.90 (d; J = 9 Hz, 1H, ArH), 7.27 (dd; $J_{1/2}$ = 9/2 Hz, 1H, ArH), 7.57 (d; J = 2 Hz, 1H, ArH).

1-(2-Bromo-4,5-dimethoxybenzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline **(5f)**

Yield 80 %; m.p. 107–109° (lit. 111° [10]).

General Procedure for the Ring Closure of the Bromo-tetrahydrobenzylisoquinolines to the Tetrahydro- and Dihydro-indolo[2,1-a]isoquinolines **6b–f** *and* **7a–f**

A solution of bromo-tetrahydro-benzylisoquinoline (10 mmol) in 40 ml DMSO was added to a solution of sodium methylsulfinylmethanide (prepared from 2.1 g (70 mmol) NaH (80 % in oil dispersion) and 40 ml DMSO). After stirring had been continued for 15 h, the mixture was poured into 400 ml water containing excess NH_4Cl and extracted with $CHCl_3$. The org. layer was washed with water and saline. After drying (Na_2SO_4), evaporation of the solvent afforded a brownish oil, which was chromatographed (SiO_2; CH_2Cl_2). The first fraction (Rf 0.7) contained the dihydroindoloisoquinolines as main product. The tetrahydro-indoloisoquinolines were isolated as second fraction (Rf 0.3). Both products were recrystallized from EtOH to afford colorless crystals. Their yields ranged from 40 to 70 %. – In the case of **5a** no tetrahydro product was isolated.

5,6,12,12a-Tetrahydro-3,9-dimethoxy-indolo[2,1-a]isoquinoline **(6b)**

M.p. 53–54°. – $C_{18}H_{19}NO_2$ (281.3) Calc. C 76.8 H 6.81 Found C 76.6 H 6.77. – ^1H-NMR ($CDCl_3$): δ (ppm) = 2.53–3.66 (m; 6H, $-CH_2-$), 3.76 (s; 3H, $-OCH_3$), 3.79 (s; 3H, $-OCH_3$), 4.69 (dd; $J_{1/2}$ = 8/3 Hz, 1H, -CH-N), 6.1–7.2 (m; 6H, ArH).

5,6,12,12a-Tetrahydro-3,9,10-trimethoxy-indolo[2,1-a]isoquinoline **(6c)**

M.p. 101°. – $C_{19}H_{21}NO_3$ (311.4) Calc. C 73.3 H 6.80 Found C 73.0 H 6.89. – ^1H-NMR ($CDCl_3$): δ (ppm) = 2.43–3.67 (m; 6H, $-CH_2-$), 3.82 (s; 6H, $-OCH_3$), 3.93 (s; 3H, $-OCH_3$), 4.92 (dd; $J_{1/2}$ = 8/3 Hz, 1H, -CH-N), 6.40 (s; 1H, ArH), 6.65–7.0 (m; 3H, ArH), 7.27 (d; J = 9 Hz, 1H, ArH).

5,6,12,12a-Tetrahydro-2,3,10-trimethoxy-indolo[2,1-a]isoquinoline **(6d)**

M.p. 128–129°. – $C_{19}H_{21}NO_3$ (311.4) Calc. C 73.3 H 6.80 Found C 73.2 H 6.80. – ^1H-NMR ($CDCl_3$): δ (ppm) = 2.33–3.80 (m; 6H, $-CH_2-$), 3.67 (s; 3H, $-OCH_3$), 3.77 (s; 3H, $-OCH_3$), 3.83 (s; 3H, $-OCH_3$), 4.82 (dd; $J_{1/2}$ = 8/3 Hz, 1H, -CH-N), 6.45 (s; 1H, ArH), 6.55–6.70 (m; 4H, ArH).

5,6,12,12a-Tetrahydro-2,3,9-trimethoxy-indolo[2,1a]isoquinoline **(6e)**

M.p. 120–121°. – $C_{19}H_{21}NO_3$ (311.4). – Calc. C 73.3 H 6.80 Found C 73.2 H 6.79. – ^1H-NMR ($CDCl_3$): δ (ppm) = 2.50–3.77 (m; 6H, $-CH_2-$), 3.77 (s; 3H, $-OCH_3$), 3.83 (s; 3H, $-OCH_3$), 3.90 (s; 3H, $-OCH_3$), 4.87 (dd; $J_{1/2}$ = 8/3 Hz, 1H, -CH-N), 6.10–6.27 (m; 2H, ArH), 6.50 (s; 1H, ArH), 6.67 (s; 1H, ArH), 6.95 (d; J = 9 Hz, 1H, ArH).

5,6,12,12a-Tetrahydro-2,3,9,10-tetramethoxy-indolo[2,1a]isoquinoline **(6f)**

M.p. 105–107° (lit. 105–107° [11]).

5,6-Dihydro-3,10-dimethoxy-indolo[2,1-a]isoquinoline **(7a)**

M.p. 208°. – $C_{18}H_{17}NO_2$ (279.3) Calc. C 77.4 H 6.13 Found C 76.9 H 6.05. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.16 (t; J = 7 Hz, 2H, -CH_2-), 3.83 (s; 6H, -OCH_3), 4.19 (t; J = 7 Hz, 2H, -CH_2-), 6.65–7.10 (m; 6H, ArH, vinyl-H), 7.61 (d; J = 9 Hz, 1H, ArH).

5,6-Dihydro-3,9-dimethoxy-indolo[2,1-a]isoquinoline **(7b)**

M.p. 176°. – $C_{18}H_{17}NO_2$ (279.3) Calc. C 77.4 H 6.13 Found C 77.6 H 6.30. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.1 (t; J = 7 Hz, 2H, -CH_2-), 3.77 (s; 3H, -OCH_3), 3.85 (s; 3H, -OCH_3), 4.13 (t; J = 7 Hz, 2H, -CH_2-), 6.48–6.86 (m; 5H, ArH, vinyl-H), 7.48 (d; J = 9 Hz, 1H, ArH), 7.66 (d; J = 9 Hz, 1H, ArH).

5,6-Dihydro-3,9,10-trimethoxy-indolo[2,1-a]isoquinoline **(7c)**

M.p. 212°. – $C_{19}H_{19}NO_3$ (309.4) Calc. C 73.8 H 6.19 Found C 73.9 H 6.15. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.18 (t; J = 7 Hz, 2H, -CH_2-), 3.87 (s; 3H, -OCH_3), 3.90 (s; 3H, -OCH_3), 3.97 (s; 3H, -OCH_3), 4.20 (t; J = 7 Hz, 2H, -CH_2-), 6.70 (s; 1H, vinyl-H), 6.83–7.02 (m; 3H, ArH), 7.12 (s; 1H, ArH), 7.70 (d; J = 9 Hz, 1H, ArH).

5,6-Dihydro-2,3,10-trimethoxy-indolo[2,1-a]isoquinoline **(7d)**

M.p. 217°. – $C_{19}H_{19}NO_3$ (309.4) Calc. C 73.8 H 6.19 Found C 73.6 H 6.09. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.15 (t; J = 7 Hz, 2H, -CH_2-), 3.90 (s; 3H, -OCH_3), 3.95 (s; 3H, -OCH_3), 4.00 (s; 3H, -OCH_3), 4.23 (t; J = 7 Hz, 2H, -CH_2-), 6.73 (s; 1H, vinyl-H), 6.83 (s; 1H, ArH), 6.93–7.37 (m; 4H, ArH).

5,6-Dihydro-2,3,9-trimethoxy-indolo[2,1-a]isoquinoline **(7e)**

M.p. 198°. – $C_{19}H_{19}NO_3$ (309.4) Calc. C 73.8 H 6.19 Found C 73.5 H 6.15. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.10 (t; J = 7 Hz, 2H, -CH_2-), 3.88 (s; 3H, -OCH_3), 3.90 (s; 3H, -OCH_3), 3.95 (s; 3H, -OCH_3), 4.17 (t; J = 7 Hz, 2H, -CH_2-), 6.73–6.83 (m; 4H, ArH, vinyl-H), 7.20 (s; 1H, ArH), 7.52 (d; J = 9 Hz, 1H, ArH).

5,6-Dihydro-2,3,9,10-tetramethoxy-indolo[2,1-a]isoquinoline **(7f)**

M.p. 209–210° (lit. 209–210° [11]).

General Procedure for the Dehydrogenation of the Dihydroindoloisoquinolines to the Indolo[2,1-a]isoquinolines **8a–f** *with Pd/C*

Dihydro-indolo[2,1-a]isoquinoline (1.3 mmol) and Pd/C 10 % (150 mg) were mixed thoroughly in an agate mortar. This and all of the following operations were carried out under N_2. A flask containing the mixture was placed in an oil bath of a temp. which was kept 10–15° above the melting point of the dihydro-indoloisoquinoline. After 30 min, the mixture was stirred with a spatula. Heating was continued for 30 min. After cooling, the mixture was dissolved in CH_2Cl_2 and filtrated. The solvent was evaporated and the residue was chromatographed (SiO_2; CH_2Cl_2). Recrystallization from EtOH afforded colorless crystals. The yields were 80–90 %.

3,10-Dimethoxy-indolo[2,1-a]isoquinoline **(8a)**

Yield 80 %; m.p. 255–256°. – $C_{18}H_{15}NO_2$ × 1/4 H_2O (281.4) Calc. C 76.8 H 5.55 Found C 76.8 H 5.53. – IR (KBr): 3420 (H_2O) cm^{-1}. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.93 (s; 6H, -OCH_3), 6.62, 7.67 (AB; J = 9 Hz, 2H, ArH), 6.87–7.24 (m; 5H, ArH), 7.93–8.13 (m; 2H, ArH).

3,9-Dimethoxy-indolo[2,1-a]isoquinoline **(8b)**

Yield 85 %; m.p. 217°. – $C_{18}H_{15}NO_2$ × 1/4 H_2O (281.4) Calc. C 76.8 H 5.55 Found 76.7 H 5.69. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.90 (s; 3H, -OCH_3), 3.93 (s; 3H, -OCH_3), 6.5–7.23 (m; 6H, ArH), 7.58–8.07 (m; 3H, ArH).

3,9,10-Trimethoxy-indolo[2,1-a]isoquinoline **(8c)**

Yield 80 %; m.p. 230°. – $C_{19}H_{17}NO_3$ × 1/4 H_2O (311.4) Calc. C 73.2 H 5.66 Found C 73.2 H 5.85. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.92 (s; 3H, -OCH_3), 3.97 (s; 3H, OCH_3), 4.01 (s; 3H, -OCH_3), 6.58, 7.93 (AB; J = 9 Hz, 2H, ArH), 6.93 (s; 1H, ArH), 6.98 (s; 1H, ArH), 7.15–7.26 (m; 3H, ArH), 7.98 (d; J = 9 Hz, 1H, ArH).

2,3,10-Trimethoxy-indolo[2,1-a]isoquinoline **(8d)**

Yield 55 %; m.p. 217–218°. – $C_{19}H_{17}NO_3$ × 1/4 H_2O (311.4) Calc. C 73.2 H 5.66 Found C 73.3 H 5.75. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.97 (s; 3H, -OCH_3), 4.05 (s; 3H, -OCH_3), 4.12 (s; 3H, -OCH_3), 6.72 (d; J = 9 Hz, 1H, ArH), 6.97–7.37 (m; 4H, ArH), 7.60 (s; 1H, ArH), 7.80 (d; J = 9 Hz, 1H, ArH), 8.10 (d; J = 9 Hz, 1H, ArH).

2,3,9-Trimethoxy-indolo[2,1-a]isoquinoline **(8e)**

Yield 60 %; m.p. 217°. – $C_{19}H_{17}NO_3$ × 1/4 H_2O (311.4) Calc. C 73.2 H 5.66 Found C 73.0 H 5.66. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.98 (s; 3H, -OCH_3), 4.02 (s; 3H, -OCH_3), 4.08 (s; 3H, -OCH_3), 6.63, 7.93 (AB; J = 9 Hz, 2H, ArH), 6.97–7.30 (m; 4H, ArH), 7.53 (s; 1H, ArH), 7.73 (d; J = 9 Hz, 1H, ArH).

2,3,9,10-Tetramethoxy-indolo[2,1-a]isoquinoline **(8f)**

Yield 75 %; m.p. 210°. – $C_{20}H_{19}NO_4$ × 1/4 H_2O (341.4) Calc. C 70.3 H 5.62 Found C 70.3 H 5.68. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.98 (s; 3H, -OCH_3), 4.02 (s; 6H, -OCH_3), 4.07 (s; 3H, -OCH_3), 6.63, 7.95 (AB; J = 9 Hz, 2H, ArH), 6.92 (s; 1H, ArH), 7.0 (s; 1H, ArH), 7.20 (s; 1H, ArH), 7.28 (s; 1H, ArH), 7.48 (s; 1H, ArH).

Biological methods

P388 D_1 Leukemia Cells [12]

Murine P388 D_1 leukemia cells were cultured in RMPI 1640 medium (Biochrom, Berlin) supplemented with 10 mM HEPES* buffer, 10 % desactivated horse serum (Biochrom), 2 mM glutamine and 0.085 % $NaHCO_3$. Cells were grown in an incubator in 5 % CO_2 at 37°. Aliquots of 2 ml of the cell suspension containing 7–8 × 10^4 cells were plated in test tubes. Substances dissolved in 2µl of DMSO were added. The medium of control wells contained an equal volume of DMSO. After two days of incubation, cells were labeled for 2 h with 0.3 µCi ^{3}H-thymidine (NEN) per well. 1 ml was used for determination of cell number (Coulter counter ZM). From the remaining part cells were harvested by centrifugation, washed with PBS and sonicated (Branson). After addition of 4 ml of 10 % trichloroacetic acid, the acid-insoluble fraction was collected on a 0.4 µm filter (Sartorius) and counted after addition of 10 ml scintillation liquid (Quickszint 212, Zinnser) in a LS 1801 scintillation counter (Beckman).

* HEPES: 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid

MDA-MB 231 Human Breast Cancer Cells [13]

Cells were grown in *McCoy* 5a medium (Boehringer, Mannheim) supplemented with 10 % newborn calf serum (NCS) (Gibco) and gentamycine (40 µg/ml). Cells were grown in a humidified incubator in 5 % CO_2 at 37°. Cells were harvested with 0.05 % trypsine-0.02 % EDTA in 0.15 M NaCl and approximately 2 × 10^4 cells in 2 ml were plated in six-well dishes (Linbro). Two days later cells were switched to a medium containing 5 % NCS and the substances, dissolved in 2 µl DMSO. The medium of control wells contained an equal volume of DMSO. Two days later, cells were labeled for 2 h with 0.3 µCi ^{3}H-thymidine per well. 1 ml was used for determination of cell number. The remaining cells were harvested by centrifugation, washed with PBS and sonicated. After addition of 4 ml of 10 % trichloroacetic acid, the acid insoluble fraction was collected on a 0.4 µm filter and counted after addition of 10 ml scintillation liquid in a scintillation counter.

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