Full paper

Applied Organometallic Chemistry

Received: 12 November 2015 Revised: 9 January 2016 Accepted: 15 January 2016 Published online in Wiley Online Library: 6 March 2016

(wileyonlinelibrary.com) DOI 10.1002/aoc.3452

Ferrocene-substituted 3,3′-diindolylmethanes with improved anticancer activity

Julienne K. Muenzner[a], Aamir Ahmad[b], Matthias Rothemund[a], Sebastian Schrüfer[a], Subhash Padhye[c], Fazlul H. Sarkar[b], Rainer Schobert[a] and Bernhard Biersack[a]*

A series of ferrocene-substituted derivatives (2a–g) of the known drug 3,3′-diindolylmethane (DIM) were prepared and tested for their *in vitro* antitumor activity. The derivatives 2a (featuring indole moiety), 2b (featuring 2-methylindole moiety) and 2f (featuring 5-nitroindole moiety) were growth-inhibiting *in vitro* at lower concentrations than DIM in various tumor cells including pancreas cancer (BcPC-3), three DIM-resistant cancer cell lines (518 A2, KB-V1/Vbl, HT-29), triple-negative breast cancer (MDA-MB-231) and prostate cancer (PC-3). Derivatives 2a, 2b and 2f were the most active compounds of this series, qualifying as drug candidates for various cancer diseases. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: Indole; ferrocene; DIM; anticancer drugs; drug resistance

Introduction

Natural indoles like indole-3-carbinol (I3C; Fig. 1), which occurs in edible brassica plants like broccoli, cabbage, sprouts and cauliflower, as well as its major condensation product 3,3′-diindolylmethane (DIM; Fig. 1), formed in the stomach upon digestion, have shown significant effects against prostate cancer.[1,2] We discovered that DIM inhibits cancer cell proliferation and invasion, as well as angiogenesis of prostate tumors *in vitro* and *in vivo*. I3C and DIM were also shown to be potent inducers of apoptosis via inhibition of Akt and NF-κB.[3–7] In addition, DIM downregulates uPA, MMP-9 and PDGF-D in mice, and potentiates the efficacy of erlotinib in pancreas cancer.[8–10] Special formulations of DIM led to a stabilization of the prostate tumor marker protein PSA and to a partial response in prostate cancer patients.[11] In breast cancer cells DIM also inhibited nuclear translocation of NF-κB, it induced $p27^{kip}$, which contributed to its apoptosis-inducing properties, and it enhanced taxotere activity via NF-κB inactivation and FoxM1 downregulation.[12–15]

Safe and co-workers investigated a series of anticancer-active *para*-substituted 3,3′-diindolyl(aryl)methane derivatives.[16–19] The anticancer potential of the ferrocene moiety has been reviewed recently.[20,21] For instance, our group could enhance the tumor selectivity of the fungal cytotoxin illudin M by esterification with ferrocene-1,1′-dicarboxylate.[22–24] Organometallic conjugates with natural products have revealed high activity in resistant cancer cells.[25,26] Similar studies of ferrocene-linked DIM derivatives have not been reported, so far. Hence, we prepared a series of ferrocene-substituted DIM analogues and investigated their activity against various tumor cell lines, including melanoma, pancreas, prostate, breast, cervix and colon carcinoma.

Experimental

Materials and Methods

All chemicals and solvents were of analytical grade quality from commercial sources and were used without further purification.

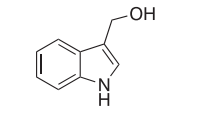
Fourier transform infrared (FT-IR) spectra were recorded using a PerkinElmer Spectrum One FT-IR spectrophotometer with attenuated total reflectance (ATR) sampling unit. Melting points (uncorrected) were determined with an Electro Thermal IA 9100 apparatus using a capillary tube. NMR spectra were recorded in $CDCl_3$ or MeOD using a Bruker AVANCE spectrometer at 300 MHz. Chemical shifts are given as parts per million (ppm) downfield from tetramethylsilane as internal standard. Mass spectra were obtained with a Thermo Finnigan MAT 8500 (EI). All tested compounds were >98% pure by elemental analysis (PerkinElmer 2400 CHN elemental analyzer). Column chromatography was performed on silica gel 60 (230–400 mesh, Merck) using ethyl acetate–*n*-hexane mixtures.

* *Correspondence to:Bernhard Biersack, Organic Chemistry Laboratory, University of Bayreuth, 95440 Bayreuth, Germany. E-mail: bernhard.biersack@yahoo.com*

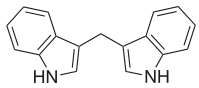
a *Organic Chemistry Laboratory, University of Bayreuth, Universitaetsstrasse 30, 95440, Bayreuth, Germany*

b *Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201, USA*

c *Abeda Inamdar Senior College, University of Pune, 2390 K. B. Hidayatullah Road, Azam Campus, Pune 411 001, India*



I3C



DIM

Figure 1. Chemical structures of I3C and DIM.

Synthesis and Characterization

*Bis(3-indolyl)(ferrocenyl)methane (**2a**)*[27]

Indole (412 mg, 3.52 mmol) was suspended in water (25 ml) and ferrocenecarboxaldehyde (380 mg, 1.78 mmol) was added. The mixture was treated with a catalytic amount of conc. Sulfuric acid (3 drops) and then stirred at 90 °C for 1 h. After cooling to room temperature, ethyl acetate was added to dissolve the precipitate. The ethyl acetate phase was separated, dried over Na_2SO_4, filtered and the filtrate was concentrated in vacuum. The residue was purified via column chromatography. Yield: 350 mg (0.81 mmol, 46%). Analytical data of **2a** corresponded with published data.[27]

*Bis(2-methylindol-3-yl)(ferrocenyl)methane (**2b**)*

Analogously to compound **2a**, derivative **2b** (350 mg, 0.76 mmol, 43%) was obtained as a brown solid of m.p. > 210 °C (dec.) from 2-methylindole (462 mg, 3.52 mmol) and ferrocenecarboxaldehyde (380 mg, 1.78 mmol). FT-IR (ν_{max} (ATR), cm^{-1}): 3408, 3390, 1613, 1585, 1484, 1461, 1423, 1297, 1218, 1102, 1038, 1012, 999, 832, 811, 740, 729. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.25 (6 H, s, 2 × Me), 4.1–4.3 (9H, m, 9 × Fc-H), 5.59 (1 H, s, methine-H), 6.8–7.0 (4 H, m, indole-H), 7.1–7.2 (2 H, m, indole-H), 7.2–7.3 (2 H, m, indole-H). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ, ppm): 12.7 (Me), 34.4 (methine-CH), 67.0 (Fc-CH), 68.6 (Fc-CH), 68.7 (Fc-CH), 93.2 (Fc-C), 111.0 (indole-C7), 112.9 (indole-C3), 119.8 (indole-C4), 120.9 (indole-C6), 121.6 (indole-C5), 127.1 (indole-C3a), 131.4 (indole-C2), 136.3 (indole-C7a). MS: *m/z* (EI) 458 (100) [M^+], 390 (48), 377 (42). Anal. Calcd for $C_{29}H_{26}FeN_2$ (%): C, 75.99; H, 5.72; N, 6.11. Found (%): C, 75.63; H, 5.58; N, 6.01.

*Bis(5-hydroxyindol-3-yl)(ferrocenyl)methane (**2c**)*

Analogously to compound **2a**, derivative **2c** (100 mg, 0.22 mmol, 36%) was obtained as a brown solid of m.p. > 180 °C from 5-hydroxyindole (162 mg, 1.22 mmol) and ferrocenecarboxaldehyde (131 mg, 0.61 mmol). FT-IR (ν_{max} (ATR), cm^{-1}): 3404, 1626, 1580, 1487, 1455, 1421, 1356, 1302, 1218, 1186, 1167, 1102, 1049, 1025, 999, 937, 800, 769, 729, 723. ^1H NMR (300 MHz, $CDCl_3$/MeOD, δ, ppm): 3.9–4.0 (5 H, m, 5 × Fc-H), 4.0–4.1 (2 H, m, 2 × Fc-H), 4.2–4.3 (2 H, m, 2 × Fc-H), 5.39 (1 H, s, methine-H), 6.59 (2 H, dd, *J* = 8.6 Hz, 2.3 Hz, 2 × indole-6H), 6.82 (2 H, d, *J* = 2.3 Hz, 2 × indole-4H), 6.89 (2 H, s, 2 × indole-2H), 7.08 (2 H, d, *J* = 8.6 Hz, 2 × indole-7H). ^{13}C NMR (75.5 MHz, $CDCl_3$/MeOD, δ, ppm): 34.7 (methine-CH), 67.2 (Fc-CH), 68.9 (Fc-CH), 69.0 (Fc-CH), 94.0 (Fc-C), 104.2 (indole-C4), 110.3 (indole-C6), 111.3 (indole-C3), 111.7 (indole-C7), 123.6 (indole-C2), 128.0 (indole-C3a), 131.8 (indole-C7a), 149.4 (indole-C5). MS: *m/z* (EI) 462 (36) [M^+], 331 (52), 329 (50), 264 (8), 207 (9), 133 (100), 104 (32), 78 (13). Anal. Calcd for $C_{27}H_{22}FeN_2O_2$ (%): C, 70.14; H, 4.80; N, 6.06. Found (%): C, 69.94; H, 4.61; N, 5.87.

*Bis(5-methoxyindol-3-yl)(ferrocenyl)methane (**2d**)*

Analogously to compound **2a**, derivative **2d** (320 mg, 0.65 mmol, 38%) was obtained as an amber solid of m.p. 110–112 °C from 5-methoxyindole (500 mg, 3.40 mmol) and ferrocenecarboxaldehyde (364 mg, 1.70 mmol). FT-IR (ν_{max} (ATR), cm^{-1}): 3412, 2928, 2825, 1622, 1580, 1482, 1451, 1438, 1349, 1294, 1266, 1248, 1207, 1166, 1104, 1092, 1050, 1023, 1000, 923, 818, 798, 769, 725, 716. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 3.67 (6 H, s, 2 × OMe), 3.9–4.0 (5 H, m, 5 × Fc-CH), 4.1–4.2 (2 H, m, 2 × Fc-CH), 4.2–4.3 (2 H, m, 2 × Fc-CH), 5.54 (1 H, s, methine-H), 6.76 (2 H, dd, *J* = 8.7 Hz, 2.5 Hz, 2 × indole-6H), 6.9–7.0 (4 H, m, 2 × indole-4H, 2 × indole-H2), 7.16 (2 H, d, *J* = 8.7 Hz, 2 × indole-7H), 7.78 (2 H, s, 2 × indole-NH). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ, ppm): 34.5 (methine-CH), 55.7 (OMe), 67.1 (Fc-CH), 68.7 (Fc-CH), 68.8 (Fc-CH), 68.9 (Fc-CH), 93.1 (Fc-C), 101.9 (indole-C4), 111.6 (indole-C6), 112.5 (indole-C3), 120.5 (indole-C7), 123.0 (indole-C2), 127.5 (indole-C3a), 131.5 (indole-C7a), 153.5 (indole-C5). MS: *m/z* (EI) 490 (100) [M^+], 422 (23). Anal. Calcd for $C_{29}H_{26}FeN_2O_2$ (%): C, 71.03; H, 5.34; N, 5.71. Found (%): C, 70.78; H, 5.16; N, 5.52.

*Bis(5-benzoxyindol-3-yl)(ferrocenyl)methane (**2e**)*

Analogously to compound **2a**, derivative **2e** (260 mg, 0.41 mmol, 37%) was obtained as a brown solid of m.p. 95–100 °C from 5-benzoxyindole (500 mg, 2.24 mmol) and ferrocenecarboxaldehyde (240 mg, 1.12 mmol). FT-IR (ν_{max} (ATR), cm^{-1}): 3409, 3088, 3027, 2951, 2860, 1622, 1578, 1479, 1451, 1378, 1292, 1269, 1213, 1187, 1104, 1089, 1049, 1024, 998, 941, 816, 799, 769, 732, 695. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 3.9–4.0 (5 H, m, 5 × Fc-CH), 4.0–4.1 (2 H, m, 2 × Fc-CH), 4.1–4.2 (2 H, m, 2 × Fc-CH), 4.90 (4 H, s, 2 × Bn-CH_2), 5.48 (1 H, s, methine-H), 6.83 (2 H, dd, *J* = 8.7 Hz, 2.3 Hz, 2 × indole-6H), 6.89 (2 H, m, 2 × Bn-CH), 6.96 (2 H, d, *J* = 2.3 Hz, 2 × indole-4H), 7.16 (2 H, d, *J* = 8.7 Hz, 2 × indole-H7), 7.2–7.4 (10 H, m, 8 × Bn-CH, 2 × indole-2H), 7.73 (2 H, s, 2 × NH). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ, ppm): 34.6 (methine-CH), 67.1 (Fc-CH), 68.6 (Fc-CH), 68.7 (Fc-CH), 70.7 (Bn-CH_2), 93.0 (Fc-C), 103.5 (indole-C4), 111.6 (indole-C6), 112.5 (indole-C3), 120.4 (indole-C7), 123.0 (indole-C2), 127.4 (Bn-C2, C6), 127.5 (indole-C3a), 127.6 (Bn-C4), 128.4 (Bn-C3, C5), 131.7 (indole-C7a), 137.7 (Bn-C1), 152.6 (indole-C5). MS: *m/z* (EI) 642 (100) [M^+], 419 (72), 328 (79), 262 (32), 223 (46), 132 (55), 91 (65). Anal. Calcd for $C_{41}H_{34}FeN_2O_2$ (%): C, 76.64; H, 5.33; N, 4.36. Found (%): C, 76.22; H, 5.08; N, 4.19.

*Bis(5-nitroindol-3-yl)(ferrocenyl)methane (**2f**)*

Analogously to compound **2a**, derivative **2f** (100 mg, 0.19 mmol, 17%) was obtained as an off-white solid of m.p. 115–120 °C from 5-nitroindole (363 mg, 2.24 mmol) and ferrocenecarboxaldehyde (240 mg, 1.12 mmol). FT-IR (ν_{max} (ATR), cm^{-1}): 3355, 1621, 1514, 1470, 1323, 1275, 1098, 1042, 891, 815, 788, 777, 751, 737, 674. ^1H NMR (300 MHz, $CDCl_3$/MeOD, δ, ppm): 4.0–4.1 (5 H, m, 5 × Fc-CH), 4.2–4.4 (4 H, m, 4 × Fc-CH), 5.61 (1 H, s, methine-H), 7.28 (2 H, s, 2 × indole-2H), 7.34 (2 H, d, *J* = 9.0 Hz, 2 × indole-7H), 7.91 (2 H, dd, *J* = 9.0 Hz, 2.2 Hz, 2 × indole-6H), 8.24 (2 H, d, *J* = 2.2 Hz, 2 × indole-4H). ^{13}C NMR (75.5 MHz, $CDCl_3$/MeOD, δ, ppm): 35.4 (methine-CH), 68.8 (Fc-CH), 69.9 (Fc-CH), 70.2 (Fc-CH), 93.5 (Fc-C), 112.0 (indole-C3), 117.4 (indole-C7), 117.5 (indole-C6), 122.1 (indole-C4), 126.6 (indole-C2), 130.0 (indole-C3a), 141.2 (indole-C5), 141.5 (indole-C7a). MS: *m/z* (EI) 520 (100) [M^+], 490 (18), 305 (18), 162 (51), 116 (62), 104 (43), 89 (60). Anal. Calcd for $C_{27}H_{20}FeN_4O_4$ (%): C, 62.33; H, 3.87; N, 10.77. Found (%): C, 62.10; H, 3.65; N, 10.35.

*Bis(5-aminoindol-3-yl)(ferrocenyl)methane (**2 g**)*

Compound **2f** (50 mg, 0.095 mmol) was dissolved in methanol and 10% Pd/C (100 mg) was added. The reaction flask was filled with hydrogen gas and the reaction mixture was stirred under 1 atm hydrogen at room temperature for 3 h. The suspension was filtered over celite, the filtrate was concentrated in vacuum and the residue was repeatedly washed with CH_2Cl_2–*n*-hexane mixtures. Yield: 30 mg (0.065 mmol, 69%); off-white gum. FT-IR (ν_{max} (ATR), cm^{-1}): 3399, 2920, 2853, 1625, 1582, 1488, 1458, 1421, 1342, 1221, 1205, 1104, 1024, 1000, 931, 801, 770, 719. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 3.9–4.0 (5 H, m, 5 × Fc-CH), 4.0–4.1 (2 H, m, 2 × Fc-CH), 4.1–4.2 (2 H, m, 2 × Fc-CH), 5.44 (1 H, s, methine-H), 6.56 (2 H, dd, J = 8.3 Hz, 2.1 Hz, 2 × indole-6H), 6.78 (2 H, s, 2 × indole-2H), 6.89 (2 H, d, J = 2.1 Hz, 2 × indole-4H), 7.09 (2 H, d, J = 8.3 Hz, 2 × indole-7H), 7.72 (2 H, s, 2 × indole-NH). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ, ppm): 34.6 (methine-CH), 67.3 (Fc-CH), 68.8 (Fc-CH), 69.9 (Fc-CH), 93.0 (Fc-C), 103.7 (indole-C4), 110.9 (indole-C6), 112.0 (indole-C3), 119.4 (indole-C7), 123.1 (indole-C2), 127.7 (indole-C3a), 131.5 (indole-C7a), 144.0 (indole-C5). MS: *m/z* (EI) 492 (100) [M^+]. Anal. Calcd for $C_{27}H_{24}FeN_4O_4$ (%): C, 70.44; H, 5.25; N, 12.17. Found (%): C, 70.10; H, 5.03; N, 11.99.

Biological Studies

Cell lines and culture conditions

BxPC-3 pancreas cancer cells (gemcitabine-sensitive) and MDA-MB-231 breast cancer cells (triple-negative) were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum, 100 U ml^{-1} of penicillin and 100 μg ml^{-1} of streptomycin in a 5% CO_2 atmosphere at 37 °C. The prostate cancer cell line PC-3 (androgen receptor negative, obtained from the ATCC) was maintained in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS), 100 U ml^{-1} of penicillin and 100 μg ml^{-1} of streptomycin. The human melanoma cell line 518 A2 (Department of Radiotherapy and Radiobiology, University Hospital Vienna), the human colon adenocarcinoma cell line HT-29 (German Centre of Biological Materials, DSMZ, Braunschweig, Germany) and the KB-V1/Vbl cervix cancer cell line (German Centre of Biological Materials, DSMZ, Braunschweig, Germany) were grown in DMEM or RPMI (HT-29) medium, supplemented with 10% FBS, 1% antibiotic–antimycotic solution (both from Gibco) and 250 μg ml^{-1} gentamycin (SERVA). Human pancreatic duct epithelial (HPDE) cells were a generous gift of Dr. Paul J. Chiao (MD Anderson Cancer Center, Houston, TX). These cells were maintained in keratinocyte serum-free medium with 5 ng ml^{-1} epidermal growth factor and 50 μg ml^{-1} bovine pituitary extract, and cultured in DMEM with 10% FBS for the experiments. All cells were cultured in a 5% CO_2-humidified atmosphere at 37 °C.

Determination of tumor cell growth (MTT assay)

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; ABCR) was used to identify viable cells which reduce it to a violet formazan. Cells (3 × 10^3 cells per well) were seeded and cultured for 24 h in 96-well microplates. Incubation (5% CO_2, 95% humidity, 37 °C) of cells following treatment with the freshly dissolved test compounds (dilution series from 1 to 50 μM in dimethylsulfoxide) was continued for 72 h. MTT stock solution (25 μl), containing 5 mg ml^{-1} in phosphate-buffered saline, was added to a final concentration of 0.05% and incubated for a further 2 h at 37 °C. The supernatant was aspirated, and the formazan was dissolved in isopropanol or dimethylsulfoxide (100 μl). The absorbance at 595 nm was measured with an Ultra Multifunctional Microplate Reader (TECAN, Durham, NC). The data are presented as the mean values ± standard deviation. Two-sided two-sample *t* test was used for statistical analyses (using PRISM).

Results and Discussion

Synthesis and Characterization

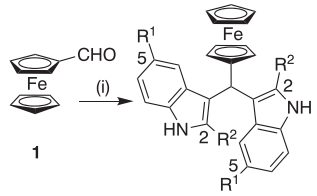
The synthesis of compound **2a** has been reported previously.[27] However, we used a procedure by Gruber and co-workers for the synthesis of **2a** and the new derivatives **2b**–**f** which were obtained by heating substituted indoles with half an equivalent of ferrocenecarboxaldehyde and a few drops of sulfuric acid in hot water (green chemistry conditions) (Scheme 1).[28] The amino derivative **2 g** was obtained from nitro compound **2f** and catalytic reduction at 10% Pd on charcoal. The compounds **2a**–**g** were characterized using NMR, FT-IR and MS. The characteristic methine protons of derivatives **2a**–**g** (5.39–5.65 ppm) and methine carbon signals of **2a**/**c**–**f** (34.4–35.4 ppm) are observed in the NMR spectra. The FT-IR spectra of compounds **2a**–**g** show distinct NH bands at 3400 cm^{-1} aside from a strong aromatic band at 1500 cm^{-1}. The alkoxy derivatives **2d** and **2e** reveal small CH_2 or CH_3 bands at 2800–3000 cm^{-1}. The mass spectra of compounds **2a**–**g** obtained under EI conditions display distinct molecule ion signals.

Biological Evaluation

The growth inhibitory activity of DIM and of the compounds **2a**–**g** was tested in MDA-MB-231 breast cancer and BxPC-3 pancreas cancer cells (Fig. 2). Compounds **2a** (IC_{50} = 6.9 μM) and **2b** (IC_{50} = 9.8 μM) show four- to six-fold higher growth inhibitory activity than DIM (IC_{50} = 37.8 μM) in triple-negative MDA-MB-231 breast cancer cells.

Compounds **2c**–**g** are distinctly less active (IC_{50} > 15 μM). In the BxPC-3 pancreas cancer cells **2a**, **2b** and **2f** reach activities with IC_{50} values below 5 μM and thus exceeding that of DIM by far (IC_{50} = 32.1 μM). Compounds **2c**–**e** and **2 g** exhibit lower activity (IC_{50} > 10 μM) in these cells, as well.

Both pancreas and prostate cancer cells belong to those cancer cell types with elevated reactive oxygen species levels leading to aggressive cancer phenotypes.[29,30] Due to the selective activity



2a: R^1, R^2 = H (46%)
2b: R^1 = H, R^2 = Me (43%)
2c: R^1 = OH, R^2 = H (36%)
2d: R^1 = OMe, R^2 = H (38%)
2e: R^1 = OBn, R^2 = H (37%)
2f: R^1 = NO_2, R^2 = H (17%)
(ii) → **2g**: R^1 = NH_2, R^2 = H (69%)

Scheme 1. Synthesis of DIM derivatives **2a**–**g**. Reagents and conditions: (i) indole derivative (2 equiv.), H_2SO_4 (cat.), H_2O, 90 °C, 1–3 h; (ii) H_2 (1 atm), 10% Pd/C, MeOH, r.t., 3 h.

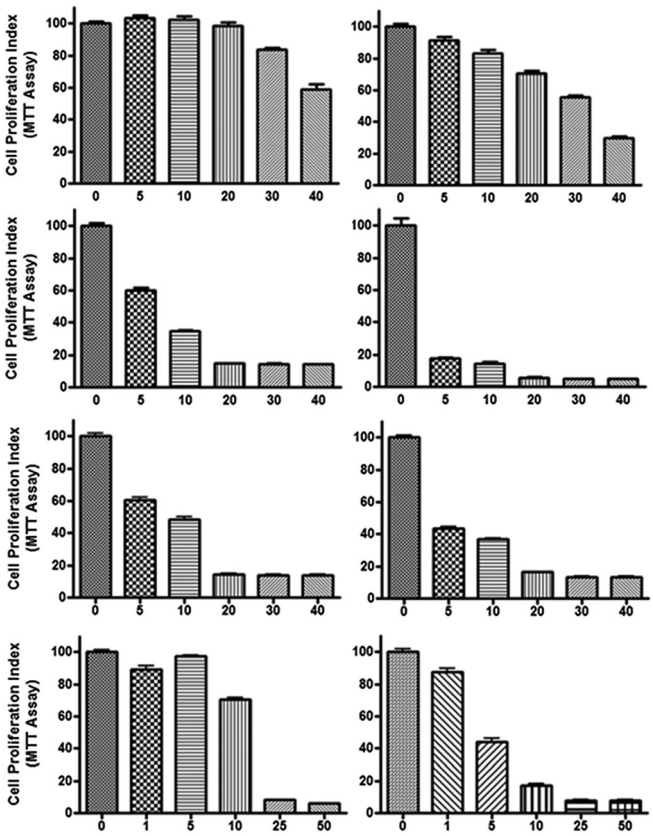


Figure 2. Growth inhibition by DIM (top), **2a** (second row), **2b** (third row) and **2f** (bottom) against MDA-MB-231 breast cells (left) and BxPC-3 pancreas carcinoma cells (right). *x*-axis: concentration in μM; *y*-axis: cell proliferation in %.

in the BxPC-3 pancreas cancer cells, the derivative **2f** was also evaluated against aggressive androgen-refractory PC-3 prostate cancer cells (Fig. 3). Compound **2f** reveals significant activity in these cancer cells (IC_{50} below 5 μM) and is thus more active than DIM (IC_{50} = 25.2 μM). The close amino-congener **2 g** exhibits reduced activity (IC_{50} > 10 μM), as do the other 5-substituted derivatives **2c**–**e** (Fig. 3).

The enhanced activity by compound **2f** in the pancreas and prostate cancer cell lines can be explained by an activation of the ferrocene derivative by reactive oxygen species. As shown previously for anticancer-active ferrocene-modified drug candidates,[31,32] the formation and stabilization of emerging molecular radicals by the various indole scaffolds used in this study

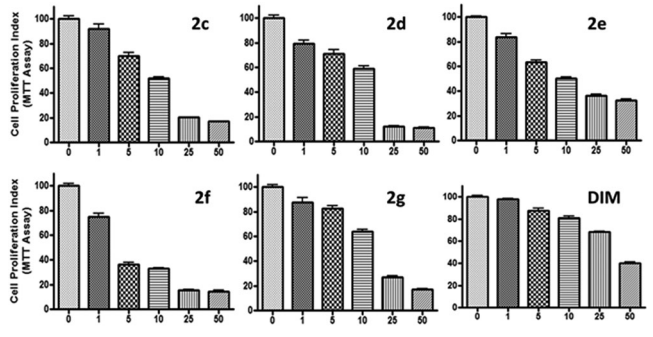


Figure 3. Inhibition of the growth of PC-3 prostate carcinoma cells by **2c**–**g** and DIM. x-axis: concentration in μM; *y*-axis: cell proliferation in %.

Table 1. Inhibitory concentration values (IC_{50} in μM, 72 h) of DIM and compounds **2a**–**f** when applied to 518 A2 melanoma, KB-V1/Vbl cervix carcinoma and HT-29 colon carcinoma cells

Compound	518 A2	KB-V1/Vbl	HT-29
DIM	>100	>100	>100
2a	1.0 ± 0.3	3.0 ± 0.3	6.3 ± 0.3
2b	23.2 ± 2.9	10.8 ± 2.6	13.7 ± 1.6
2c	15.2 ± 2.8	>100	21.5 ± 0.5
2d	>100	10.9 ± 0.7	21.5 ± 0.5
2e	57.9 ± 4.8	>100	>100
2f	9.5 ± 0.5	12.6 ± 0.4	15.6 ± 1.6

may play a role in the anticancer activity of the respective ferrocene DIM derivatives **2** in these cell lines.

DIM has shown promising effects against various drug-resistant human cancer cell lines including hormone-insensitive and epidermal growth factor receptor-mutant cancer cells.[33–35] However, we found that DIM is virtually inactive (IC_{50} > 100 μM) against 518 A2 melanoma, vinblastine-resistant KB-V1/Vbl cervix carcinoma and HT-29 colon carcinoma cells. Hence, compounds **2a**–**f** were also tested on these three DIM-resistant human cancer cell lines. Compounds **2b** and **2d** are particularly active against the vinblastine-resistant KB-V1/Vbl cells, while **2f** exhibits high activity against the melanoma cells (Table 1). Compound **2f** shows also distinct activity against the KB-V1/Vbl and HT-29 cells. However,

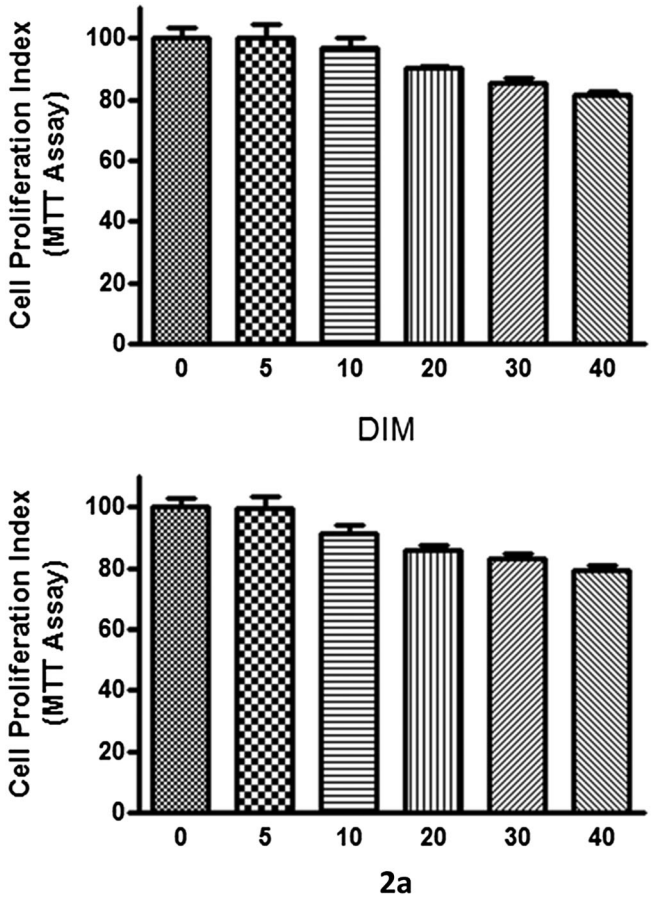


Figure 4. Inhibition of the growth of immortalized HPDE cells by DIM and **2a**. *x*-axis: concentration in μM; *y*-axis: cell proliferation in %.

2a is the most active derivative displaying IC_{50} values in the low one-digit micromolar range (1–6 μM). Compound **2a** is particularly active against the 518 A2 melanoma cells (IC_{50} = 1.0 μM) and the Pgp-transporter expressing multi-drug-resistant KB-V1/Vbl cervix carcinoma cells (IC_{50} = 3.0 μM). In contrast to that, the benzyloxy derivative **2e** displays the lowest activity of this series of organometallic DIM derivatives. Nevertheless, the high activity of **2a** and, to a lesser extent, of **2b** and **2f** against 518 A2 melanoma cells and Pgp-expressing KB-V1/Vbl cancer cells coincides well with our previous reports about ferrocene derivatives with distinct activity in these cancer cell lines.[23,26]

Finally, the tumor selectivity of **2a** was evaluated in comparison with DIM. The growth inhibitory activity of DIM and of ferrocene derivative **2a** against immortalized HPDE cells was tested (Fig. 4). Despite the significant activity of **2a** against various cancer cells, this ferrocene compound is virtually inactive against HPDE cells (cell growth inhibition of *ca* 20% at a dose of 40 μM). There is no difference in activity between **2a** and the parent compound DIM in these HPDE cells, despite the distinctly higher activity of **2a** against various cancer cells. Thus, **2a** represents a highly tumor-selective anticancer agent.

Conclusions

The results obtained add significantly to previous works on DIM derivatives and might lead to the design of more powerful DIM-related organometallic anticancer drugs. Both the ferrocene moiety and substituents at the indole rings play an important role concerning anticancer activity. In particular, ferrocene compounds **2a**, **2b** and **2f** might be suitable candidates for further tests in solid cancers, especially in pancreas and prostate cancers. The strong impact of **2a** on DIM-resistant melanoma cells as well as on Pgp-transporter expressing multi-drug-resistant cancer cells is remarkable and paves the way to a reasonable strategy that will overcome DIM resistance in cancer cells in the end.

Acknowledgments

We are grateful to the Deutsche Forschungsgemeinschaft for a grant (Scho 402/12-1).

References

[1] R. K. Tiwari, L. Guo, H. L. Bradlow, N. T. Telang, M. P. Osborne, *J. Natl. Cancer Inst.* **1994**, *86*, 126.
[2] C. Hong, H. A. Kim, G. L. Firestone, L. F. Bjeldanes, *Carcinogenesis* **2002**, *23*, 1297.
[3] K. M. Rahman, O. Aranha, A. Glazyrin, S. R. Chinni, F. H. Sarkar, *Oncogene* **2000**, *19*, 5764.
[4] K. M. Rahman, O. Aranha, F. H. Sarkar, *Nutr. cancer* **2003**, *45*, 101.
[5] M. M. Bhuiyan, Y. Li, S. Banerjee, F. Ahmed, Z. Wang, S. Ali, F. H. Sarkar, *Cancer Res.* **2006**, *66*, 10064.
[6] S. R. Chinni, F. H. Sarkar, *Clin. Cancer Res.* **2002**, *8*, 1228.
[7] L. M. Howells, B. Gallacher-Horley, C. E. Houghton, M. M. Manson, E. A. Hudson, *Mol. Cancer Ther.* **2002**, *1*, 1161.
[8] D. Kong, S. Banerjee, W. Huang, Y. Li, Z. Wang, H. R. Kim, F. H. Sarkar, *Cancer Res.* **2008**, *68*, 1927.
[9] D. Kong, Y. Li, Z. Wang, S. Banerjee, F. H. Sarkar, *Cancer Res.* **2007**, *67*, 3310.
[10] S. Ali, S. Banerjee, A. Ahmad, B. F. El-Rayes, P. A. Philip, F. H. Sarkar, *Mol. Cancer Ther.* **2008**, *7*, 1708.
[11] F. H. Sarkar, Y. Li, *Cancer Treat. Rev.* **2009**, *35*, 597.
[12] K. M. W. Rahman, F. H. Sarkar, *Cancer Res.* **2005**, *65*, 364.
[13] Z. Wang, B. W. Yu, K. M. W. Rahman, F. Ahmad, F. H. Sarkar, *Mol. Cancer Ther.* **2008**, *7*, 341.
[14] K. M. W. Rahman, S. Ali, A. Aboukameel, S. H. Sarkar, Z. Wang, P. A. Philip, W. A. Sakr, A. Raz, *Mol. Cancer Ther.* **2007**, *6*, 2757.
[15] A. Ahmad, S. Ali, Z. Wang, S. S. Ali, S. Sethi, W. A. Sakr, A. Raz, K. M. W. Rahman, *Int. J. Cancer* **2011**, *129*, 1781.
[16] P. Lei, M. Abdelrahim, S. D. Cho, X. Liu, S. Safe, *Mol. Cancer Ther.* **2008**, *7*, 3363.
[17] C. Qin, D. Morrow, J. Stewart, K. Spencer, W. Porter, R. Smith, III, T. Phillips, M. Abdelrahim, I. Samudio, S. Safe, *Mol. Cancer Ther.* **2004**, *3*, 247.
[18] S. Chintharlappalli, R. Burghardt, S. Papineni, S. Ramaiah, K. Yoon, S. Safe, *J. Biol. Chem.* **2005**, *280*, 24903.
[19] R. Contractor, I. J. Samudio, Z. Estrov, D. Harris, J. A. McCubrey, S. H. Safe, M. Andreeff, M. Konopleva, *Cancer Res.* **2005**, *65*, 2890.
[20] C. Ornelas, *New J. Chem.* **2011**, *35*, 1973.
[21] E. Meléndez, *Inorg. Chim. Acta* **2012**, *393*, 36.
[22] R. Schobert, S. Knauer, S. Seibt, B. Biersack, *Curr. Med. Chem.* **2011**, *18*, 790.
[23] S. Knauer, B. Biersack, M. Zoldakova, K. Effenberger, W. Milius, R. Schobert, *Anti-Cancer Drugs* **2009**, *20*, 676.
[24] R. Schobert, S. Seibt, K. Mahal, A. Ahmad, B. Biersack, K. Effenberger-Neidnicht, S. Padhye, F. H. Sarkar, T. Mueller, *J. Med. Chem.* **2011**, *54*, 6177.
[25] A. Gmeiner, K. Effenberger-Neidnicht, M. Zoldáková, R. Schobert, *Appl. Organometal. Chem.* **2011**, *25*, 117.
[26] C. Spoerlein-Guettler, K. Mahal, R. Schobert, B. Biersack, *J. Inorg. Biochem.* **2014**, *138*, 64.
[27] J. Meng, W. Daming, Y. Wang, H. Wang, *Chin. Chem. Lett.* **1992**, *3*, 247.
[28] D. Maciejewska, M. Rasztawicka, I. Wolska, E. Anuszewska, B. Gruber, *Eur. J. Med. Chem.* **2009**, *44*, 4136.
[29] E. C. Vaquero, M. Edderkaoui, S. J. Pandol, I. Gukovsky, A. S. Gukovskaya, *J. Biol. Chem.* **2004**, *279*, 34643.
[30] B. Kumar, S. Koul, L. Khandrika, R. B. Meacham, H. K. Koul, *Cancer Res.* **2008**, *68*, 1777.
[31] E. Hillard, A. Vessières, L. Thouin, G. Jaouen, C. Amatore, *Angew. Chem. Int. Ed.* **2006**, *45*, 285.
[32] M. Schikora, A. Reznikov, L. Chaykovskaya, O. Sachinska, L. Polyakova, A. Mokhir, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3447.
[33] M. Rahimi, K.-L. Huang, C. K. Tang, *Cancer Lett.* **2010**, *295*, 59.
[34] C. Hong, G. L. Firestone, L. F. Bjeldanes, *Biochem. Pharmacol.* **2002**, *63*, 1085.
[35] D. Chen, S. Banerjee, Q. C. Cui, D. Kong, F. H. Sarkar, Q. P. Dou, *PLoS One* **2012**, *7*, e47186.