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Halogenated naphthochalcones and structurally related naphthopyrazolines with antitumor activity

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ABSTRACT

Three 3-(3-halo-4,5-dimethoxyphenyl)-1-(2-naphthyl)prop-2-en-1-ones **1** and three structurally related 2-pyrazolines **2** were prepared and assessed in vitro for anticancer activity. The chalcones **1** were antiproliferative with low double-digit micromolar IC_{50} values against six tumor cell lines whereas the pyrazolines **2** showed low single-digit micromolar IC_{50} values against this panel. The pyrazolines inhibited ATP-binding cassette efflux transporters of types P-gp and BCRP while the chalcones inhibited selectively BCRP. All test compounds induced an accumulation of HT-29 colon carcinoma cells in the G2/M phase of the cell cycle and they interfered with the microtubule and F-actin dynamics, but only the chalcones induced apoptosis in 518A2 melanoma cells after 24 h.

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A critical hurdle to a successful chemotherapy is multidrug resistance (MDR) of cancer cells which may be inherent or acquired during previous lines of therapy. The energy-dependent efflux of xenobiotics, mediated by adenosine triphosphate-binding cassette (ABC) transporters, plays a major role in the process of cellular resistance to chemotherapeutics.[1] An overexpression of these transporters in tumor cells decreases the efficacy of drugs by reducing their intracellular concentration. The multidrug resistance-associated protein 1 (MRP1), P-glycoprotein (P-gp), and the breast cancer resistance protein (BCRP) are the most important ABC-transporters involved in MDR.[2] Effective and selective ABC-transporter inhibitors can help to restore the impact of anticancer drugs in MDR tumors. A good deal of 1,3-diphenyl-prop-2-en-1-ones (a.k.a. chalcones), precursors in the biosynthesis of flavonoids,[3] are known to inhibit ABC-transporters.[1,2,4] We recently reported a combretastatin A4 derived chalcone and its platinum complex that inhibited BCRP and P-gp.[4] Structure–activity relationship (SAR) studies by others showed that chalcones bearing basic functional groups are likely to be P-gp inhibitors,[5] whereas non-basic chalcones displayed no P-gp inhibition but selectively inhibited BCRP.[2] A strong BCRP inhibition was noted for naphthylchalcones with a chloro substituted 3-phenyl ring. Moreover, chalcones may also exert other cancer-relevant effects, including apoptosis induction, antiproliferative effects, and an interference with the cell cycle progression.[3] Herein, we report on a series of halogenated naphthylchalcones **1** and structurally related 2-pyrazolines **2**, on their potential as selective ABC-transporter inhibitors, and on further anticancer properties.

The new chalcones **1a** and **1c** were obtained as colorless solids from a Claisen–Schmidt condensation of 2-acetylnaphthalene and 3,4-dimethoxy-5-chloro-benzaldehyde or 3,4-dimethoxy-5-iodo-benzaldehyde, respectively (Scheme 1).[6] The synthesis of the chalcones **1b** and **1d** was reported previously.[7–9] The new acetylpyrazolines **2a–c** were prepared by reaction of **1a–c** with hydrazine hydrate in acetic acid and obtained as colorless solids (Scheme 1).[6]

The effect of the chalcones **1a–d** and of the 2-pyrazolines **2a–c** on the growth of cancer cells of five different entities was assessed using the MTT assay.[10] All compounds showed dose-dependent cell growth inhibition against the entire panel of cell lines (Table 1). The chalcones featured low double-digit micromolar IC_{50} concentrations with the 3-halophenyl chalcones **1a–c** being on average slightly more active than the trimethoxyphenyl derivative **1d**. It is known that 3,4-dimethoxy substituted chalcones inhibit ABC efflux transporters of the BCRP type.[2] Hence, it is not surprising that the multidrug-resistant cancer cell line MCF-7/Topo, which overexpresses these particular efflux pumps,[11] was approximately 10-times more sensitive to the chalcones than the other cell lines,

Abbreviations: ABC, adenosine triphosphate binding cassette; BCRP, breast cancer resistance protein; FTC, fumitremorgin C; MDR, multidrug resistance; P-gp, P-glycoptotein; SD, standard deviation; TdT, Terminal deoxyribonucleotide Transferase; Topo, topotecan; TUNEL, Terminal deoxyribonucleotide Transferase dUTP Nick End Labeling; Vbl, vinblastin.

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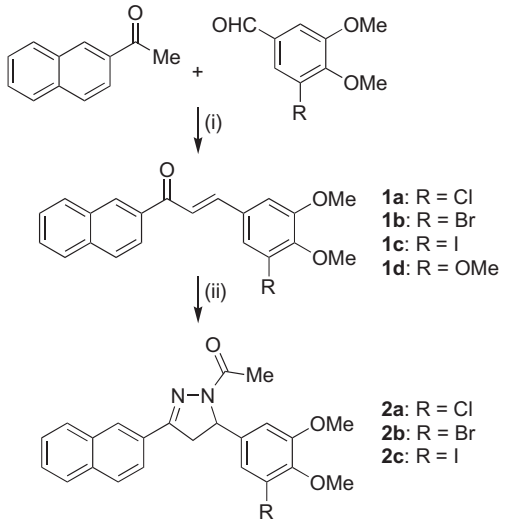

Scheme 1. Synthesis of chalcones **1a–d** and of acetylpyrazolines **2a–c**. Reagents and conditions: (i) NaOH, MeOH/H_2O, rt, 16 h, 80–85%; (ii) $N_2H_4 \times H_2O$, AcOH, reflux, 3 h, 32–71%.

with IC_{50} values in the low single-digit micromolar range. The 3-halophenyl substituted napthylpyrazolines **2** were more active compared with their chalcone congeners. They showed low single-digit micromolar IC_{50} values against all tested cancer cell lines including the multidrug-resistant HT-29 colon, MCF-7/Topo breast, and KB-V1/Vbl cervix carcinoma cells.

The chalcones **1a–d** and the pyrazolines **2a–c** were then screened for inhibition of the ABC-transporters P-gp/ABCB1 and BCRP/ABCG2 by means of the calcein-AM accumulation assay in KB-V1/Vbl cells and the mitoxantrone accumulation assay in MCF-7/Topo cells, respectively (Table 2).[12,13] MCF-7/Topo breast cancer cells, which overexpress BCRP, were treated with the test compounds (**1a–d**, **2a–c**; 0–100 μM) or the known BCRP inhibitor fumitremorgin C (0.1–100 μM) for 30 min in the presence of the fluorescent BCRP substrate mitoxantrone. The mitoxantrone fluorescence intensity was taken as a measure of the degree of BCRP inhibition and used to obtain dose–response curves which allow the determination of IC_{50} values. The halophenyl chalcones **1a–c** and their pyrazoline analogues **2a-c** proved to be equally effective inhibitors of BCRP on average with IC_{50} values in the range of 5–9 μM, while the trimethoxy-substituted chalcone **1d** was two to three times more active. These findings are in line with the results of the MTT assay and the lower IC_{50} values of the chalcones against MCF-7/Topo breast cancer cells compared with the other tested cell lines. P-gp overexpressing KB-V1/Vbl cervix carcinoma cells were also treated with the test compounds (**1a–d**, **2a–c**; 0–500 μM) or the P-gp inhibitor verapamil (0.1–500 μM) for 15 min in the presence of the non-fluorescent P-gp substrate calcein acetoxymethyl ester (calcein-AM). Inhibition of P-gp impedes the efflux of calcein-AM and allows esterases to hydrolyze calcein-AM to give the intensely fluorescent calcein which is not a P-gp substrate anymore and thus accumulates in the cytosol.[14] The calcein fluorescence intensity as a measure of P-gp inhibition was used to calculate the IC_{50} values of the test compounds. In line with the weaker antiproliferative activities of the halophenyl chalcones **1a–c** against KB-V1/Vbl cancer cells in the MTT assays, compared with those of their 2-pyrazoline analogues, no P-gp inhibition was observed for the former, whereas the inhibitory activities of the latter even exceeded that of the established P-gp inhibitor verapamil, clinically used to re-sensitize resistant tumors.[15] Interestingly, the selectivity for BCRP was lost when the substituent in the *meta* position of the phenyl ring was changed from halide (**1a–c**) to methoxy (**1d**). In terms of IC_{50} values, **1d** and the 2-pyrazolines **2a-c** were on average four to five times better inhibitors of P-gp when compared to verapamil.

Table 2
Concentrations IC_{50} [μM][a] for the inhibition of BCRP and P-gp transporters by the test compounds **1a–d** and **2a–c**, the specific BCRP inhibitor fumitremorgin C, and the specific P-gp inhibitor verapamil

	BCRP	P-gp
Fumitremorgin C	0.96 ± 0.24[b]	–
Verapamil	–	65.6 ± 10.1
1a	8.7 ± 1.3	>500
1b	4.9 ± 0.3	>500
1c	7.8 ± 1.6	>500
1d	2.4 ± 0.5	13.0 ± 1.6
2a	7.4 ± 0.3	12.1 ± 0.1
2b	8.9 ± 1.9	12.6 ± 1.6
2c	7.1 ± 1.8	16.3 ± 1.4

[a] Determined in MCF-7/Topo breast cancer cells after 30 min or in KB-V1-V1/Vbl cervix carcinoma cells after 15 min exposure to the compounds. The results are the mean ± SD of three independent experiments and derived from dose–response curves.
[b] Results of two independent experiments.

The interference of the test compounds **1a–d** and **2a–c** with the cell cycle progression of HT-29 colon carcinoma cells was determined by flow cytometry using propidium iodide staining. Treatment of the cells with 15 μM of the chalcones or pyrazolines led to a significant accumulation of cells in G2/M phase of the cell cycle whereas the population of cells in G1-phase was drastically reduced (Fig. 1). This effect was more pronounced for the pyrazolines.

The extent of apoptosis in 518A2 melanoma cells treated with **1a**, **1d**, or **2a** for 24 h, was analyzed by flow cytometry using the TUNEL (Terminal deoxyribonucleotide Transferase dUTP Nick End Labeling) technique (Fig. 2) to visualize DNA fragmentation typical of apoptosis. Interestingly, apoptosis was only induced by the chalcones **1a** and **1d**, whereas cells treated with the pyrazoline **2a** did not differ from untreated control cells. These findings were confirmed by DNA ladder assays using agarose gel electrophoresis to

Table 1
Inhibitory concentrations IC_{50} (μM, 72 h) of compounds **1a–d** and **2a–c** when applied to human cancer cell lines[a]

Cell line/compound	HT-29	HCT-116	518A2	MCF-7/Topo	Panc-1	KB-V1/Vbl
1a	23.9 ± 2.5	12.9 ± 0.7	16.4 ± 1.6	1.5 ± 0.4	10.3 ± 0.2	13.1 ± 1.7
1b	22.8 ± 4.8	11.2 ± 1.3	20.2 ± 2.5	2.3 ± 0.3	9.3 ± 0.2	10.5 ± 0.7
1c	13.3 ± 1.3	14.4 ± 1.3	18.1 ± 1.3	1.3 ± 0.4	10.4 ± 0.1	16.5 ± 1.0
1d[7]	28.1 ± 7.4	13.7 ± 2.7	22.9 ± 5.6	5.6 ± 1.0	13.3 ± 0.4	13.9 ± 2.4
2a	3.5 ± 0.1	3.8 ± 0.2	4.6 ± 0.6	2.1 ± 0.1	3.1 ± 0.1	4.7 ± 0.2
2b	2.5 ± 0.1	2.7 ± 0.2	3.8 ± 0.9	2.6 ± 0.4	1.5 ± 0.1	2.2 ± 0.1
2c	2.3 ± 0.6	1.6 ± 0.1	3.8 ± 0.5	2.5 ± 0.4	1.1 ± 0.1	1.9 ± 0.1

[a] Human cancer cell lines: HT-29 and HCT-116 colon carcinomas, 518A2 melanoma, MCF-7/Topo breast cancer adenocarcinoma, Panc-1 pancreatic ductular adenocarcinoma and KB-V1/Vbl cervix carcinoma. Values are the means ± SD determined in four independent experiments and derived from dose–response curves (percentage of viable cells relative to untreated controls) after 72 h incubation using the MTT assay.

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