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Synthesis and cytotoxic activity of resveratrol-based compounds

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Abstract In continuation of our studies on compounds with a resveratrol-based scaffold two compounds with *N*-containing functional groups have been synthesized and screened for their inhibitory effect on the growth of the human cancer cell lines HT29, 518A2, AsPC-1, BxPC-3, and PC-3. Compound **4**, but not **1**, demonstrated pronounced *in vitro* cytotoxicity against all these cancer cell lines, thus making this compound a promising candidate for further preclinical *in vivo* studies.

Keywords Antitumor agents; Amidoximes; Resveratrol; Drug research.

Introduction

Cancer is the second leading cause of death in the developed world, claiming over 6 million deaths each year. Chemoprevention in combination with anticancer treatment is therefore important to reduce mobidity and mortality [1]. Resveratrol (*trans*-3,4′,5-trihydroxystilbene) is a phytoalexin present in grapes, peanuts and pines. Besides antioxidant activity [2] and inhibitory potency against cyclooxygenases 1 and 2 [3], resveratrol inhibits the growth of various human tumor cells, including human colon [4], pancreatic [5], prostate [6] and melanoma [7] cancer cell lines. Furthermore, resveratrol is able to reduce incidences of carcinogen-induced development of cancers in experimental animals *via* the inhibition of tumor initiation, promotion and progression [8, 9]. It has also been demonstrated that resveratrol inhibits ribonucleotide reductase, an enzyme catalyzing the rate limiting step of *de novo DNA* synthesis [10]. Recent data showed that a hexamethoxylated derivative of resveratrol exerted no antiproliferaitve properites in HL-60 leukemia cells [11]. To increase the cytotoxic potency novel methoxylated compounds with *N*-containing functional groups in a resveratrol-like scaffold were synthesized and assayed for their inhibitory effect on the growth of the human cancer cell lines.

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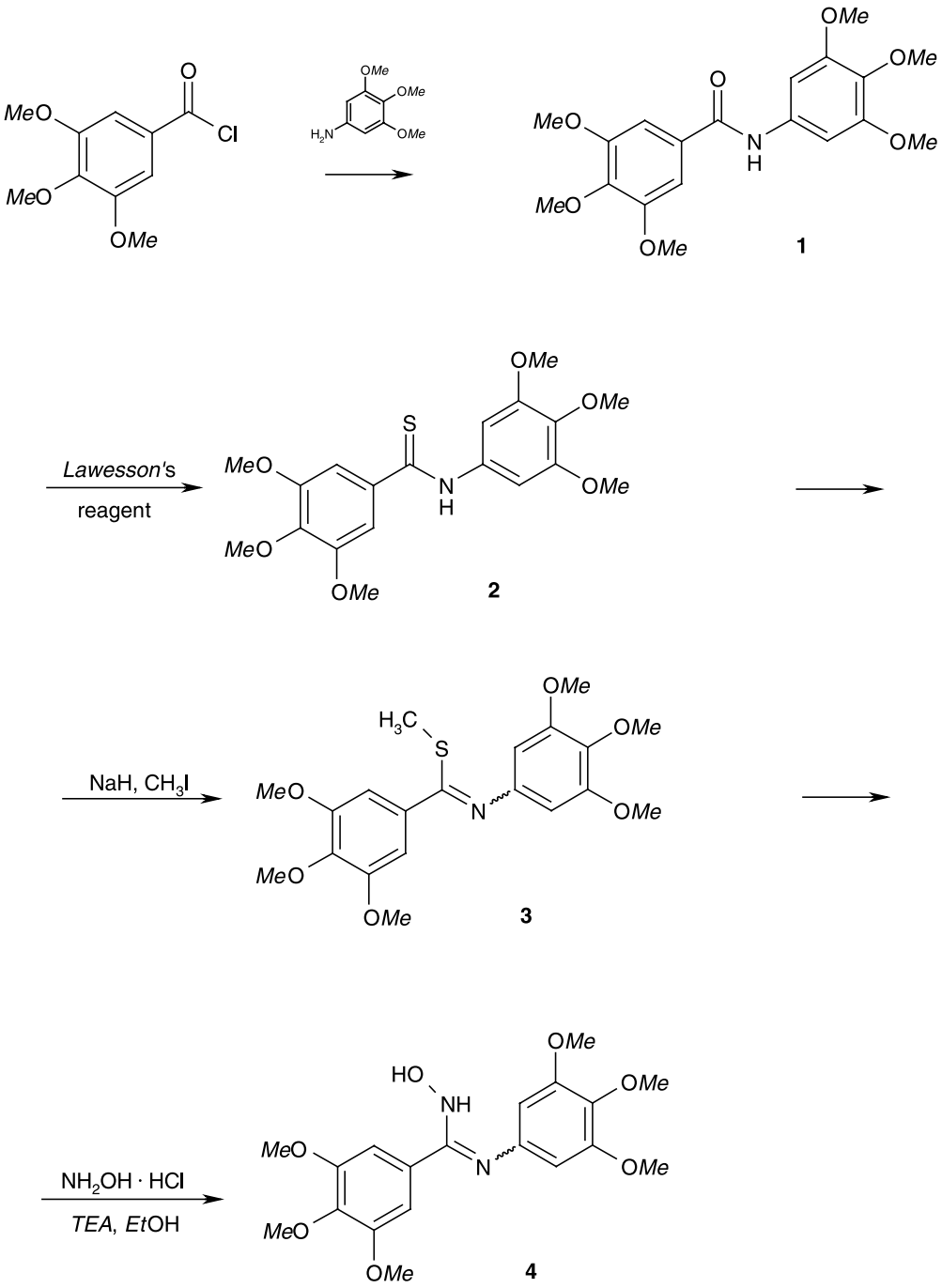
Results and discussion

Scheme 1 shows the synthesis pathway for compound **4** starting with the secondary amide **1**, which was obtained by reaction of 3,4,5-trimethoxybenzoylchloride with 3,4,5-trimethoxyaniline in good yield. This product reacted smoothly with *Lawesson*'s reagent in *THF* to give the corresponding thiocarboxamide **2**, which was activated by treatment with sodium hydride and iodo methane to yield the corresponding *S*-methyl derivative **3**. The product was used immediately without purification for the final



Scheme 1

step with hydroxylamine hydrochloride and triethylamine in absolute ethanol to obtain the desired *N*-hydroxy-*N*′-(3,4,5-trimethoxyphenyl)-3,4,5-trimethoxybenzamidine (**4**) in moderate yield. The exact configuration for **3** and **4** was not determined (Scheme 1).

Compounds **1** and **4** were tested for their antitumor activity on various cancer cell lines (Table 1). Substance **4** inhibited the formation of AsPC-1 pancreatic cell colonies with an IC_{50} (inhibition of 50% of the colonies) of 9 μM and the formation of pancreatic BxPC-3 cells with an IC_{50} of 8 μM, respectively. Similarly, the formation of 518A2 melanoma and PC-3 prostate cell colonies was inhibited with an IC_{50} of 10 μM and 6 μM, whereas an IC_{50} of 35 μM was found for inhibition of HT29 colon cells. On the

Table 1 Effect of **1** and **4** on the colony formation of five human cancer cell lines. Logarithmically growing cells were incubated with increasing concentrations of 1 for 7 days at 37°C under cell culture conditions. After trypan blue staining, colonies (>40 cells) were counted using an inverted microscope at 40× magnification

Cancer cell line	IC_{50} for *HHSt*[a]/ μM	IC_{50} for *HMSt*[b]/ μM	IC_{50} for compound **1**/ μM	IC_{50} for compound **4**/ μM
HL-60	4.2 [11]	>100 [11]	n.d.	n.d.
518A2	n.d.	n.d.	60	10
AsPC-1	n.d.	n.d.	>100	9
BxPC-3	n.d.	n.d.	>100	8
HT29	n.d.	n.d.	>100	35
PC-3	n.d.	n.d.	>100	6

[a] *HHSt* 3,3′,4,4′,5,5′-hexahydroxystilbene
[b] *HMSt* 3,3′,4,4′,5,5′-hexamethoxystilbene

other hand compound **1** inhibited the formation of 518A2 melanoma cell colonies with only 60 μM and an $IC_{50} > 100\ \mu M$ could be stated for the four other cell lines (Table 1).

Two other molecules, namely 3,3′,4,4′,5,5′-hexamethoxystilbene and 3,3′,4,4′,5,5′-hexahydroxystilbene have been synthesized by our group before [3]. The hexamethoxy derivative showed no cytotoxic effects in a HL-60 cell culture whereas the 3,3′,4,4′,5,5′-hexahydroxystilbene exhibited an IC_{50} value of 4.2 μM [11], but turned out to be unstable in solutions. Therefore, we tried to optimize the properties of our compounds resulting in molecule **4** with six methoxy groups and an amidoxime functional group on the stilbeneoid scaffold. The pronounced antitumor activity seemed to be based on the amidoxime group in the molecule, since the carboxamide **1** showed very weak inhibition in the cell colonies.

In summary, we synthesized a novel cytotoxic compound with broad and potent antiproliferative *in vitro* effects. Further efforts like *in vivo* experiments and synthesis of other new derivatives are in progress.

Experimental

General

Melting points were determined on a Kofler hof-stage apparatus. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian UnityPlus-200 (200 MHz). Chemical shifts are reported in δ values (ppm) relative to Me_4Si line as internal standard. Mass spectra were obtained by a *Shimadzu* GC/MS QP 1000 EX. The elemental analyses obtained were within ±0.4% of the theoretical values for the formulas given. Column chromatography was performed using silica gel 60, 70–230 mesh ASTM (Merck). Solutions in organic solvents were dried over anhydrous sodium sulfate.

Chemistry

*N-(3,4,5-Trimethoxyphenyl)-3,4,5-trimethoxybenzamide (**1**)*

To a solution of 2.30 g 3,4,5-trimethoxybenzoyl chloride (10 mmol) in absolute dioxane 1.83 g 3,4,5-trimethoxyaniline (10 mmol), dissolved in absolute dioxane, was added at once. The solution was shaken thoroughly and left for 10 minutes. Then the mixture was poured into 500 cm^3 ice-water, the precipitate was filtered off and recrystallized from ethanol (50%) to yield 2.80 g (74.2%) of **1**. Mp 218°C (Ref. [12] 211–212°C).

N-(3,4,5-Trimethoxyphenyl)-3,4,5-trimethoxybenzothioamide (**2**, $C_{19}H_{23}N_1O_6S_1$)

In a dry three-necked flask 1.13 g benzamide **1** (3 mmol) was dissolved in absolute *THF*, 1.01 g *Lawesson* reagent (2.5 mmol) was added and the mixture was refluxed with a $CaCl_2$ tube for 3 hours. The solvent was removed *in vacuo* and the raw product was purified by column chromatography (eluent: toluene/ethyl acetate 8 + 2) to yield 0.99 g (84.3%) of **2**. Mp 193°C; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 9.05$ (s, broad, 1H, NH), 7.13 (m, 2H, phenyl-H), 7.00 (s, 2H, phenyl-H), 3.90 (s, 6H, OCH_3), 3.87 (s, 12H, OCH_3) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): δ = CSN missing, 153.2, 152.9, 136.4, 134.8, 101.1, 60.9, 56.2 ppm; EI-MS: m/z (%) = 393 (M^+, 24), 360 (18), 211 (100), 144 (25).

S-Methyl-N-(3,4,5-trimethoxyphenyl)-3,4,5-trimethoxybenzothiocarboxamid (**3**, $C_{20}H_{25}N_1O_6S_1$)

In a dry three-necked flask 0.180 g (60%-suspension) sodium hydride (4.5 mmol) was suspended in absolute *THF* under argon. After removing the mineral oil from the mixture 1.18 g *N*-(3,4,5-trimethoxyphenyl)-3,4,5-trimethoxybenzothioamide (**2**) (3 mmol), solved in absolute *THF*, was added *via* syringe. Then 0.85 g (= 0.37 cm^3) iodomethane (6 mmol) was added and the mixture was stirred for one hour at room temperature. The solvent was removed to yield 0.79 g (64.8%) of **3** as pale oil. The product was used raw for the next step.

N-Hydroxy-N′-(3,4,5-trimethoxyphenyl)-3,4,5-trimethoxybenzamidine (**4**, $C_{19}H_{24}N_2O_7$)

In a dry three-necked flask 1.22 g *S*-methyl-*N*-(3,4,5-trimethoxyphenyl)-3,4,5-trimethoxybenzothiocarboxamide (**3**) (3 mmol) was dissolved in absolute ethanol, 0.42 g hydroxylamine hydrochloride (6 mmol) and 3.33 cm^3 triethylamine (12 mmol) were added and the mixture was refluxed for 4 h. Then the solvent was removed and the residue was extracted three times with water/ethyl acetate. The organic phase was dried and the solvent removed again. The raw product could be purified by recrystallization from diluted ethanol to yield 0.67 g (54.2%) **4**. Mp 161°C; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 9.30$ (s, broad, 1H, OH), 7.23 (s, broad, 1H, NH), 6.66 (s, 2H, phenyl-H), 5.95 (s, 2H, phenyl-H), 3.82 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.73 (s, 6H, OCH_3), 3.61 (s, 6H, OCH_3) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 153.1$, 151.9, 135.6, 133.8, 126.4, 105.7, 60.9, 99.1, 56.2, 55.8 ppm; EI-MS: m/z (%) = 392 (1), 375 (53), 359 (51), 344 (28), 330 (30), 168 (100).

Cell culture

The 518A2, HT29, AsPC-1, BxPC-3, and PC-3 human tumor cell lines were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 using a Cytoperm 8080 incubator (HERAEUS, Vienna, Austria). Cells were grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum (FCS) (GIBCO, Grand Island Biological Co., Grand Island, NY, USA), and with 1% penicillin-streptomycin. Cell counts were determined using a microcellcounter CC-108 (SYSMEX, Kobe, Japan). Cells growing in the logarithmic phase of growth were used for all studies described.

Clonogenic assay

Logarithmically growing cells (1000 per well) were plated in 24 well Costar plates and incubated with increasing concentrations of drugs for 7 days at 37°C under cell culture conditions. After trypan blue staining, colonies (>40 cells) were counted using an inverted microscope at 40× magnification. Results were calculated as number of viable cells. All experiments were performed in triplicates and were repeated two times.

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