TLC – ANTIPROLIFERATIVE EFFECTS OF A SERIES OF SYNTHETIC 4-AMINOCOUMARINS IN A PANEL OF HUMAN TUMOR CELL LINES

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Abstract. A series of 4-aminocoumarins were assayed for antiproliferative/cytotoxic effects in a panel of tumor cell lines after 72 h exposure using the MTT-dye reduction assay as a read-out system and the IC_{50} as an end-point to evaluate efficacy. The tested compounds exerted cytotoxic properties at a high micromolar range of concentrations. In general, the number of cell lines is limited for broad and definitive conclusions to be drawn out, the study has shown that the presented compounds exert antiproliferative potential and could be subset to further modification aimed at identifying prospective antineoplastic lead compounds.

Key words: 4-aminocoumarins, cytotoxicity, MTT-assay, human tumor cell lines

Introduction

Coumarins (derivatives of 2H-1-benzopyran-2-one) comprise a large group of important plant-derived polyphenolic secondary metabolites [1]. Based on the successful commercialization of 4-hydroxycoumarin-based anticoagulants, there has been tremendous exploration of this class of compounds as potential pharmacological agents, which has unraveled a wide range of biological activities, including some of potential clinical translation [1, 2]. Natural coumarins (incl., furanocoumarines, pyranocoumarins, prenylated coumarins and more complex structures) have attracted intense interest in recent years because of their diverse pharmacological properties, and moreover the core coumarin structure has been exhaustedly exploited as a scaffold for structural modification in order to identify novel prospective leads [1-4]. The constellation of biological activities established with coumarins include, but is not limited to antineoplastic, anti-inflammatory, anticoagulant, antibacterial, antihypertensive, antituberculous, anticonvulsant, antiadipogenic, antihyperglycemic, antioxidant, neuroprotective, fungicidal [2, 4, 5], and antiviral [1], including antiretroviral properties, mediated by inhibition of HIV-integrase or reverse transcriptase [6].

Among the pleiotropic pharmacological activities much attention has been paid to the tumor-inhibiting and cytotoxic properties of natural coumarins [1, 4], their derivatives and structural analogs [3, 7, 8], or metal coordination compounds [9-16]. Thus it is well appreciated, that the cytotoxic coumarins represent a feasible source of new antineoplastic leads, which by virtue of the complex modes of action (incl. anti-mitotic, antiangiogenic, proapoptotic etc.) might also help addressing the issues of side effects, toxicity and the emerging multi-drug resistance [9, 17].

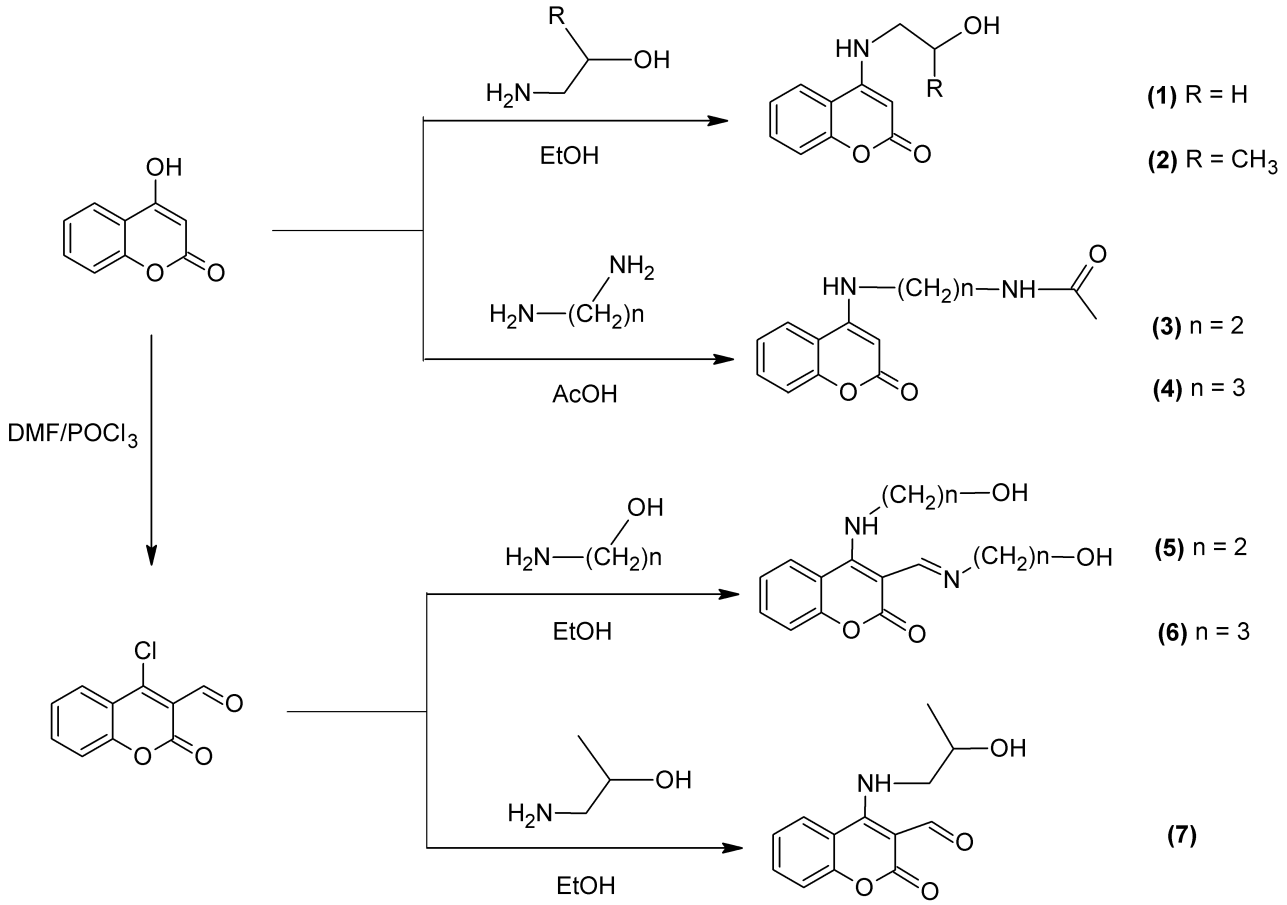
This report is aimed at assessment of the antiproliferative potential of as series of 4-aminocoumarins, whose synthesis and characterization have been recently presented elsewhere [18] in a panel of human tumor cell lines, representative for some important types of human neoplastic disease.

Materials and methods

Drugs, chemicals and reagents.

Formic acid, 2-propanol, L-glutamine were purchased from AppliChem GmbH, Darmstadt, Germany. Fetal calf serum (FCS) and RPMI 1640 medium were purchased from Sigma – Aldrich Co., the USA. The tetrazolium salt 3-(4,5-dimethylthi¬azol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was supplied from Merck Co., Germany. The referent antineoplastic drug melphalan was used as a commercially available pharmaceutical grade substance.

The tested compounds were synthesized as previously described [18]. The procedure is briefly summarized in Scheme 1.



Scheme 1. *Synthesis of the tested compounds: reaction of 4-hydroxycoumarin with aminoalcohols and diaminoalkanes and reaction of 4-chlorocoumarin-3-carbaldehyde with aminoalcohols [18].*

Cell lines and culture conditions.

The study was carried out with the following cell lines: SKW-3 (ACC 53) – human T-cell leukemia, originally described to be established from the peripheral blood of a 61-year-old man with T cell chronic lymphocytic leukemia (CLL) in 1977; however, DNA fingerprinting and cytogenetic analysis showed cross-contamination with cell line KE-37; KE-37 was established from a 27-year-old man with acute lymphoblastic leukemia (ALL) in 1979; HL-60 (ACC 3) - human acute myeloid leukemia (AML), established from the peripheral blood of a 35-year-old woman with acute myeloid leukemia (AML FAB M2) in 1976; cells are apparently tetraploid derivates of the hypodiploid original; K-562 (ACC 10) – human chronic myeloid leukemia (CML) established from the pleural effusion of a 53-year-old woman with CML in blast crisis in 1970; cells carry the BCR-ABL1 e14-a2 (b3-a2) fusion gene; the used subclone weakly expresses MHC class I (image); MDA-MB-231 (ACC 732) – human adenocarcinoma, established in 1973 from the pleural effusion of a 51 years old Caucasian woman with metastatic mammary carcinoma; cells were described of being negative for cytoplasmic estrogen receptors.

The cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig, Germany). They were cultured under standard conditions - RPMI-1640 liquid medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine, in cell culture flasks, housed at 37^{o}C in an incubator 'BB 16-Function Line' Heraeus (Kendro, Hanau, Germany) with humidified atmosphere and 5% CO2. Cell cultures were maintained in logarithmic growth phase by supplementation with fresh medium two or three times weekly.

Cytotoxicity assessment (MTT-dye reduction assay).

The cell viability after exposure to the tested compounds was assessed using the MTT-dye reduction assay, based on the biotransformation of the yellow dye 3-(4,5-dimethylthi¬azol-2-yl)-2,5-diphenyl tetrazolium bromide to a violet formazan product via the mitochondrial succinate dehydrogenase in viable cells. The procedure was carried out as described elsewhere [19], with some modifications [20]. Exponentially growing cells were seeded in 96-well flat-bottomed microplates (100 μl/well) at a density of $1x10^5$ cells per ml and after a 24 h incubation period at 37°C they were treated with graded concentrations of the extract (or its fractions) for 72h. For each concentration a set of 8 wells was used. After the exposure period 10μl MTT solution (10mg/ml in PBS) aliquots were added to each well. The microplates were further incubated for 4h at 37°C and the MTT-formazan crystals formed were dissolved through addition of 100 μl/well 5% formic acid in 2-propanol. The MTT-formazan absorption was determined using a microprocessor controlled microplate reader (Labexim LMR-1) at 580 nm.

Bioassay data processing and statistics.

The cell survival data were normalized as percentage of the untreated control (set as 100% viability), were fitted to sigmoidal dose response curves and the corresponding IC50 values (concentrations causing 50% suppression of cellular viability) were calculated using non-linear regression analysis (GraphPad Prizm Software for PC). The statistical processing of biological data included the Student's t-test whereby values of $p \leq 0.05$ were considered as statistically significant. The in vivo data development is described in the corresponding section of the Materials and methods section.

Results

The synthesis of the tested compounds was described elsewhere [18]. All compounds were characterized by NMR, IR, Mass, Elemental Analysis and melting points. The precise assignment of the ^{1}H and ^{13}C NMR spectra was accomplished by measurement of 2D homonuclear correlation (COSY), DEPT-135 and 2D inverse detected heteronuclear (C–H) correlations (HMQC and HMBC). The spectroscopic data was in accordance with the assigned structures. The predominant tautomeric form and the true geometry (E or Z) of the molecule (6) were solved by means of X-ray crystallographic analysis.

The antiproliferative/cytotoxic effects of the tested series of 4-aminocoumarines were evaluated using the standard MTT-dye reduction assay in a panel of human tumor cell lines representative for some important neoplastic diseases. The panel consisted of the acute lymphoid leukemia derived KE-37, the acute myeloid leukemia derived HL-60, the chronic myeloid leukemia derived K-562, and ER-negative breast cancer MDA-MB-231 cell lines.

The tested 4-aminocoumarin compounds exerted concentration-dependent cytotoxic activities, which allowed the construction of sigmoidal dose response curves, and calculation of the equieffective IC50 concentrations (the concentration leading to 50% reduc-

Table 1. *MTT-bioassay data for the series of 4-aminocoumarins against a panel of human tumor cell lines.*

Compounds[1]		IC_{50} (μM)[2]			
		HL-60[3]	KE-37[4]	K-562[5]	MDA-MB-231[6]
1	**4-[(2-Hydroxyethyl)amino]-2H-chromen-2-one**	251.5 ± 4.7	> 400.0	397.4 ± 7.9	> 400.0
2	**4-[(2-Hydroxypropyl)amino]-2H-chromen-2-one**	317.7 ± 4.7	> 400.0	385.9 ± 6.8	> 400.0
3	**N-{2-[(2-Oxo-2H-chromen-4-yl)amino]ethyl} acetamide**	245.7 ± 8.2	379.9 ± 7.5	377.3 ± 8.2	357.3 ± 7.7
4	**N-{3-[(2-Oxo-2H-chromen-4-yl)amino]propyl} acetamide**	> 400.0	> 400.0	300.4 ± 2.2	397.7± 7.9
5	**4-[(2-Hydroxyethyl)amino]-3-{(E)-[(2-hydroxyethyl)imino]methyl}-2H-chromen-2-one**	367 ± 8.2	> 400.0	299.9 ± 7.8	> 400.0
6	**4-[(3-Hydroxypropyl)amino]-3-{(E)-[(3-hydroxypropyl)imino]methyl}-2H-chromen-2-one**	237.2 ± 11.2	307.4 ± 9.9	227.5 ± 14.2	279.0 ± 10.7
7	**4-[(2-Hydroxypropyl)amino]-2-oxo-2H-chromene-3-Carbaldehyde**	343.2 ± 7.4	374.7 ± 11.1	299.9 ± 7.8	> 400.0
Contr.	Melphalan	11.2 ± 1.9	14.6 ± 3.1	28.3 ± 3.2	13.7 ± 1.9

[1]Synthesized as described elsewhere; [2]means ± sd from eight independent experiments. Cell lines: [3]acute myeloid leukemia derived HL-60, [4]acute lymphoid leukemia, [5]chronic myeloid leukemia, [6] ER-negative breast cancer.

tion of the cellular viability) which served as criteria for juxtaposition of the antiprolirferative/cytotoxic activity. Throughout the cytotoxicity screening the clinically applied anticancer alkylating agent melphalan was employed as a positive control.

The bioassay data for the series of 4-amino-substituted coumarins are summarized on table 1. Evident from the equieffective half-maximal inhibitory concentrations (IC_{50}), the tested compounds exerted cytotoxic properties at generally high micromolar range of concentrations.

The chemosensitivity of the individual cell lines little varies, bit generally the myeloid cell lines K-562 and HL-60 were more responsive than the lymphoid KE-37 and the breast cancer derived MDA-MB-231. In the sensitive cell lines however the cytotoxicity of the compounds was encountered at concentrations greatly exceeding 100 μM.

Conclusion

This study was commenced in an attempt to evaluate the anti-cancer potential of a series of 4-aminocoumarines. To meet this objective a detailed cytotoxicity screening was performed in a panel of tumor cell lines after 72 h exposure using the MTT-dye reduction assay as a read-out system and the IC_{50} as an end-point to evaluate efficacy.

Taken together our findings firmly indicate that the presented series of 4-aminocoumarin derivatives exhibit antiproliferative effects in a panel of human tumor cell lines of different origin and cellular type. The proven cytotoxic activity of the series supported the well-established antineoplastic potential of structurally diverse natural and synthetic coumarins

In general, the number of cell lines and the relatively close chemical structures of the tested analogues is limited for broad and definitive conclusions to be drawn out. Nevertheless, the study has shown that the presented compounds exert antiproliferative potential and could be subset to further modification aimed at identifying prospective antineoplastic lead compounds.

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