CYTOTOXICITY ASSAY ON SEVERAL THEOPHYLLINE-7-ACETIC ACID AMIDES WITH AMINO ACIDS

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Summary. Theophylline-7-acetic acid (7-TAA) derivatives with several amino acids were screened for antiproliferative effects on acute (HL-60) and chronic (K-562) myeloid leukemia cells as well as on non-malignant cells derived from embryonic human kidney (HEK 293T). The anti-proliferative effects (IC_{50}) were revealed at relatively high concentrations, between 330.4 and 1051.9 μM, but the tested compounds proved to be devoid of cytotoxicity on HEK 293T in the evaluated concentration range (50-1200 μM). The amino acid esters demonstrated higher cytotoxicity towards the myeloid leukemia cells compared to the carboxylic acid derivatives.

Key words: theophylline-7-acetic acid, cytotoxicity, amino acid

Introduction

Methylxanthine derivatives are known to increase cAMP, by virtue of multiple mechanisms including antagonism at adenosine receptors and inhibition of phosphodiesterase [1]. Non-selective phosphodieterase inhibitors such as theophylline can effectively kill cancer cells in vitro by elevating intracellular cAMP and by modulating the DNA-repair enzymatic machinery. Both caffeine and theophylline have been found to induce apoptosis, and to enhance doxorubicin-induced cytotoxicity [2]. Likewise theophylline (at 15–25 ng/ml) was found to synergize with gemcitabine or cisplatin to induce programmed cell death in 4 different carcinoma cell lines derived from human ovarian, prostate and lung cancer and in a malignantly transformed granulosa cell line. The mechanism of the apoptogenic effect of theophylline has been found to involve reduction of intracellular levels of the antiapoptotic mediator Bcl2. Therefore, theophylline-based structures comprise an attractive scaffold for structural modification in search of innovative antineoplastic agents with multimodal pharmacodynamics [3, 4].

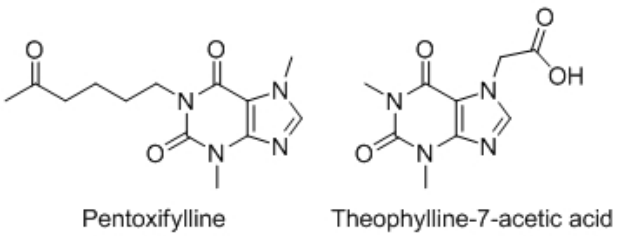


Figure 1.*Structures of pentoxifylline and theophylline-7-acetic acid.*

Besides theophylline and its congeners other methylxanthines have been also evaluated for anticancer and chemosensitivity-modulating agents. Thus pentoxifylline (Fig. 1) has been shown to increase the effectiveness of both radiotherapy and chemotherapy [5, 6]. A combination regimen of pentoxifylline with liposomal doxorubicin, exhibited synergistic activity and inhibited cellular proliferation to a greater extent with regard to each drug used alone [7].

Among the abundance of methylxanthines promising purine-based lead compound is theophylline-7-acetic acid (7-TAA) (Fig. 1), also called acefylline,which is characterized by an beneficial safety profile as compared to theophylline, but suffers from poor absorption [8]. Creating an amino acid-linked molecule is an approach for designing analogs with higher bioavailability. Hence peptides and proteins are involved in numerous biological processes and play important roles in the development and progression of various diseases, important biological processes such as kinase activation and inhibition can be mimicked with peptide-based drugs [9].

The aforementioned biological relevance prompted us to probe the amino acid conjugates with 7-TAA as potential antiproliferative agents.

Materials and Methods

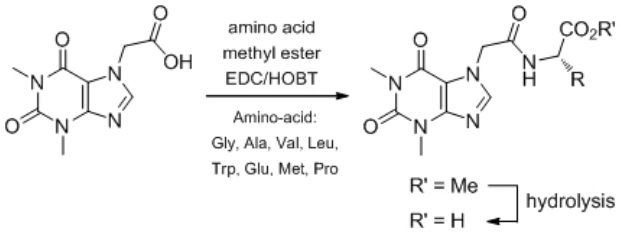
Cell lines and culture conditions

The cell lines used in this study, namely HL-60 (acute myelocyte leukemia) and K-562 (chronic-myelocyte leukemia) were obtained from the German Collection of Microorganisms and Cell Cultures

(DSMZ GmbH, Braunschweig, Germany). They were cultured in controlled environment – RPMI-1640 liquid medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine, in cell culture flasks, housed at 37 ºC in an incubator 'BB 16-Function Line' Heraeus (Kendro, Hanau, Germany) with humidified atmosphere and 5% CO_2. Cell cultures were maintained in logarithmic growth phase by supplementation with fresh medium two or three times weekly. Heraeus (Kendro, Hanau, Germany) with humidified atmosphere and 5% CO_2. Cell cultures were maintained in logarithmic growth phase by supplementation with fresh medium two or three times weekly.

Cytotoxicity assessment (MTT-dye reduction assay)

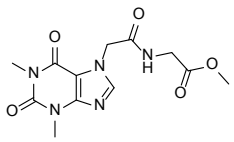
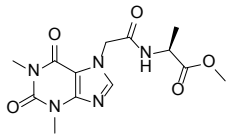
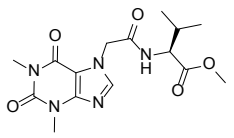
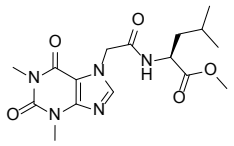
The cell viability, after continuous exposure to the tested compounds was assessed using the standard MTT-dye [10] with minor modifications [11]. The method is based on the biotransformation of the yellow tetrazolium salt MTT to a violet formazan by the mitochondrial succinate dehydrogenase in viable cells. For the MTT-assay exponentially growing cells

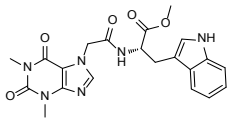
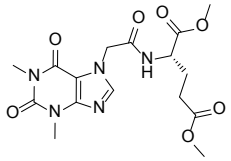
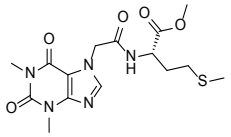
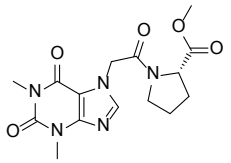
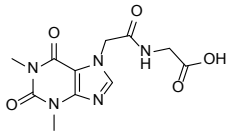
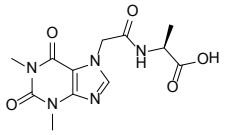
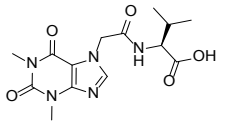
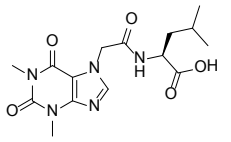
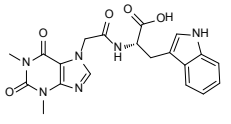
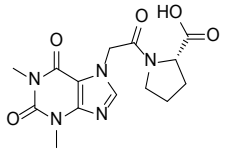


Scheme 1. *Synthesis of the tested compounds.*

were seeded in 96-well flat-bottomed microplates after 24 h incubation at 37 ºC they were exposed to various concentrations of the tested extracts for 72 h. For each concentration a set of at least 8 wells were used. After the treatment 10 ml MTT solution (10 mg/ml in PBS) aliquots were added to each well. The microplates were further incubated for 4 h at 37 ºC and the MTT-formazan crystals formed were dissolved through addition of 100 ml/well 5% formic acid (in 2-propanol). The absorption was read on a microprocessor controlled Labexim LMR-1 microplate reader at 580 nm.

Table 1.*Antiproliferative activity,* IC_{50} *(μM)*

Compounds	MW	Cell cultures		
		HL-60	K-562	HEK-293T
	309.28	842.1	893.0	N.Q.
	323.30	743.0	868.0	N.Q.
	351.15	330.4	549.5	N.Q.
	365.17	480.0	663.2	N.Q.

Compounds	MW	Cell cultures		
		HL-60	K-562	HEK-293T
	438.44	561.0	733.1	N.Q.
	395.14	581.5	707.4	N.Q.
	383.42	858.2	982.9	N.Q.
	349.34	575.2	675.2	N.Q.
	295.25	961.2	1051.9	N.Q.
	309.11	954.9	1024.0	N.Q.
	337.14	659.5	860.2	N.Q.
	351.15	472.6	719.5	N.Q.
	424.41	571.5	741.7	N.Q.
	335.32	685.5	776.4	N.Q.

Results and Discussion

The synthesis and characterization of the 7-TAA derivatives with amino acids have been described earlier [12]. The amide bond was created viacoupling reactionscarried out using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and hydroxybenzotriazole (EDC/HOBt) in dichloromethane. Consequent hydrolysis of the ester function to carboxylic group of the amino acid moiety was achieved with aqueous LiOH (Scheme 1).

The compounds were screened for antiproliferative effects against 2 human leukemic cell lines, namely HL-60 and K-562. Table 1 lists the data from the cytotoxic assays on the 2 malignant cells. For the purpose of a comparison, the antiproliferative effects against non-malignant cells derived from embryonic human kidney – HEK 273T, documented in our previous study [12], are also included.

The tested compounds exhibited concentration-dependent reduction in the proliferative activity and the viability of the malignant cells. The anti-proliferative effects were revealed at relatively high concentrations, between 330.4 and 1051.9 μM. In general, the amino acid esters demonstrated higher cytotoxicity compared to the carboxylic acid derivatives. Higher chemosensitivity has been observed towards the acute myeloid leukemia (HL-60), compared to the chronic myeloid leukemia (K-562). The tested compounds did not achieve their IC_{50} towards the non-malignant cell line HEK-293T in the concentration range tested (50 - 1200μM). This is indicative for the selective cytotoxic activity against the malignant transformed cells used in this study.

Conclusion

Amino acid derivatives of 7-TAA were evaluated for their antiproliferative activity against 2 human leukemic cell lines. The data indicate that the antiproliferative effects (IC_{50}) are achieved at relatively high concentrations. The tested compounds were not toxic to the embryonic human kidney cells.

Acknowledgement

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References

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