Novel pteridine-based inhibitors of cAMP phospho-diesterases: promising antineoplastic agents

K.-H. Merz, N. Graf v. Keyserlingk*, S. Vatter, M. Habermeyer, G. Eisenbrand

Department of Chemistry, Division of Food Chemistry and Environmental Toxicology, University of Kaiserslautern, Erwin-Schrödinger-Str. 52, 67663 Kaiserslautern, Germany

* Faustus Forschungs Compagnie Translational Cancer Research GmbH, Grimmaische Strasse 2-4, 04109 Leipzig, Germany

Introduction

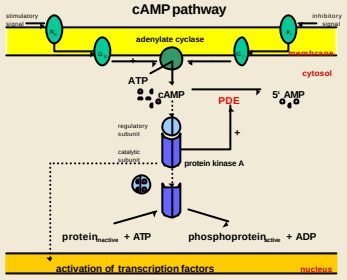
The second messenger cAMP is known to be involved in cell growth and differentiation. The intracellular level of cAMP is regulated by adenylate cyclases and phosphodiesterases (PDEs). Many tumor cells exhibit significantly decreased cAMP levels resulting from increased cAMP-PDE activity.

Alkylamino substituted pteridines have been identified as potent inhibitors of the cAMP specific isoenzyme family PDE4 [J. Med. Chem. **41** (1998), 4733-43], inducing efficient growth inhibition in a panel of cell lines.

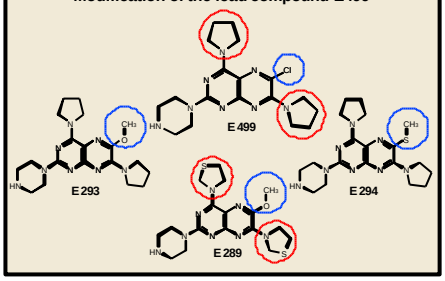
The synthesis of these pteridines is achieved by successive nucleophilic substitution of the chlorine atoms of 2,4,6,7-tetrachloropteridine.

In this structure-activity study we investigated compounds bearing identical heterocyclic substituents in position 4 and 7 and varying substituents in position 6.

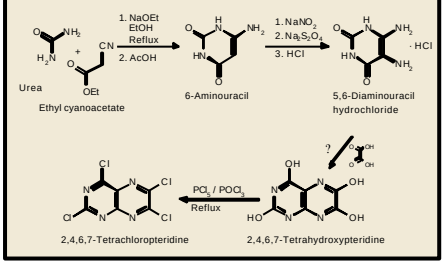
cAMP pathway



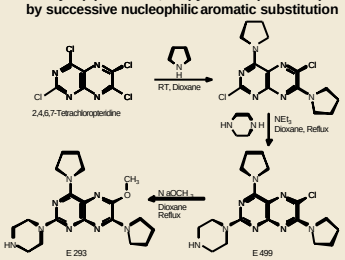
Modification of the lead compound E499



Synthesis of 2,4,6,7- Tetrachloropteridine



Synthesis of 6-Methoxy-2-piperazino-4,7- dipyrrolidinopteridine (E 293) by successive nucleophilic aromatic substitution



Structure-activity data
obtained from SRB and XTT assays

SRB assay:

All tested compounds were found to be growth inhibitors in the low µM range for human LXFL529L cells, 6-chloro-2-piperazino-4,7-dipyrrolidinopteridine (E499) serving as the lead compound in this study (IC_{50} value 4.7 µM).

→ Replacement of the chloro substituent in 6-position by methoxy (E293) or methylthio (E294) enhanced the growth inhibitory activity (IC_{50} values ~ 3 µM).

→ Exchange of the pyrrolidino versus thiazolidino substituents in 4- and 7-position reduced the growth inhibitory properties (IC_{50} value 6.7 µM).

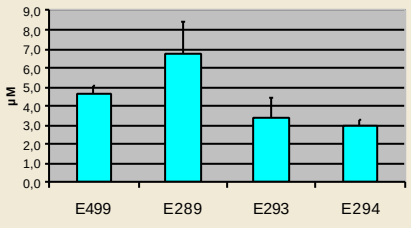
XTT assay:

The IC_{50} values are comparable to those of the SRB assay.

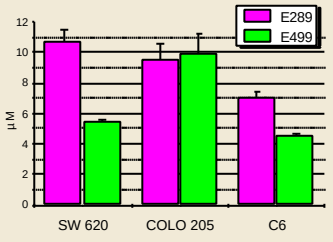
→ Rat glioblastoma cell line C6 :
7.0 µM for E289
4.6 µM for E499

→ Human colon carcinoma cells:
SW 620: 5.5 µM; Colo 205: 9.9 µM for E499
SW 620: 8.0 µM; Colo 205: 9.5 µM for E289

Growth inhibition of LXFL529L cells
IC_{50} values of SRB assay



Cytotoxicity
IC_{50} values of XTT assay



Material and Methods

Cell culture:

The lung xenograft tumour cell line LXFL529L was cultivated in RPMI1640 (supplemented with 10% FCS and 1% penicillin/streptomycin) at 37 °C and 5% CO_2.

The human colon cancer cell lines COLO 205 and SW260 were cultivated in RPMI and IMDM; the rat glioblastoma cell line C6 was cultivated in DMEM. Each medium was supplemented with 10% FCS.

Sulforhodamine B assay:

LXFL529L cells were seeded in 24-well tissue culture plates at a density of 6000 cells per well. After 24 h the medium was removed and the cells were incubated with the test compounds for 72 h.

The assay was performed as described by Skehan et al., JNCI 82 (1990), 1107-1112, with slight modifications.

Material and Methods

XTT assay:

XTT, a tetrazolium salt, is cleaved by succinate-tetrazolium reductase, a mitochondrial redox-system that is exclusively active in living cells. The cleavage results in a soluble formazan salt that can be quantified by colorimetric measurement at 450 nm. The intensity is directly linked to the number of metabolically active cells.

Cells were seeded into 96-well tissue culture plates at a density of 15000 cells for COLO 205, 10000 cells for SW260 and 5000 cells for C6 per well. The cells were incubated at 37 °C and 5% CO_2 for 24 hours. Then 100 µl of each dilution of the substances were added to the cells. For a negative control, 100 µl of cell culture medium were applied and for a positive control all cells were deadened with phenol. The cells were incubated with the substances for 72 hours. 40 µl of XTT solution were added to each well and the cells were incubated for further 8 hours. Formed formazan was quantified at 450 nm. The IC_{50} values were determined by linear regression of the data points.

Conclusions

In this study the impact of structural elements of alkylaminopteridines bearing identical heterocyclic substituents in positions 4 and 7 on inhibitory properties has been investigated. These pteridines are potent inhibitors of tumor cell growth in a panel of cell lines with IC_{50} values in the low micromolar range. Compounds with thiazolidine substituents in 4- and 7-position exhibit diminished growth inhibitory potency compared to 4,7- dipyrrolidinopteridines.

In 6-position, a methoxy- or methylthio-substituent slightly enhances growth inhibition. This kind of substituents with the ability to be easily metabolized might be of advantage, as will be investigated in further *in vivo*-studies.

Acknowledgement

The project this report is based on was supported by the EFRE of the EU and by funds from the Freistaat Sachsen (P-Nr.: 6306). The responsibility for the contents of this publication rests with the authors.