J Med Chem. 2006 May 18;49(10):3012-8. ACS PUBLICATIONS HIGH QUALITY. HIGH IMPACT. Links

Identification of a terphenyl derivative that blocks the cell cycle in the G0-G1 phase and induces differentiation in leukemia cells.

- **Roberti M**,
- **Pizzirani D**,
- **Recanatini M**,
- **Simoni D**,
- **Grimaudo S**,
- **Di Cristina A**,
- **Abbadessa V**,
- **Gebbia N**,
- **Tolomeo M**.

Dipartimento di Scienze Farmaceutiche, Universita di Bologna, Via Belmeloro 6, 40126 Bologna, Italy. marinella.roberti@unibo.it

To further explore the SAR of resveratrol-related trans-stilbene derivatives, here we describe the synthesis of (a) a series of 3,5-dimethoxy analogues in which a variety of substituents were introduced at positions 2', 3', 4', and 5' of the stilbene scaffold and (b) a second group of derivatives (2-phenylnaphthalenes and terphenyls) that incorporate a phenyl ring as a bioisosteric replacement of the stilbene alkenyl bridge. We thoroughly characterized all of the new compounds with respect to their apoptosis-inducing activity and their effects on the cell cycle. One of the new derivatives, 13g, behaved differently from the others, as it was able to block the cell cycle in the G(0)-G(1) phase and also to induce differentiation in acute myelogenous leukemia HL60 cells. Compared to resveratrol, the synthetic terphenyl 13g showed a more potent apoptotic and differentiating activity. Moreover, it was active on both multidrug resistance and Bcr-Abl-expressing cells that were resistant to resveratrol.

PMID: 16686543 [PubMed - indexed for MEDLINE]

Bioorg Med Chem Lett. 2006 Jun 15;16(12):3245-8. Epub 2006 Mar 31. ELSEVIER FULL-TEXT ARTICLE Links

Stilbene-based anticancer agents: resveratrol analogues active toward HL60 leukemic cells with a non-specific phase mechanism.

- **Simoni D**,
- **Roberti M**,
- **Invidiata FP**,
- **Aiello E**,
- **Aiello S**,
- **Marchetti P**,
- **Baruchello R**,
- **Eleopra M**,
- **Di Cristina A**,
- **Grimaudo S**,
- **Gebbia N**,
- **Crosta L**,

- **Dieli F**,
- **Tolomeo M**.

Dipartimento di Scienze Farmaceutiche, Via Fossato di Mortara 17-19, Universita di Ferrara, 44100 Ferrara, Italy. smd@unife.it

Several stilbenes, related to known resveratrol, have been synthesized and tested for their anticancer effect on HL60 leukemia cell line, taking particular care of the cell cycle analysis. The most potent compound was the known (Z)-3,4',5-trimethoxystilbene (6b) which was active as apoptotic agent at 0.24 microM. Differently from other stilbenes (including resveratrol) that induced a prevalent recruitment of cells in S phase of cell cycle, we found a peculiar behavior of 6b that caused a decrease of cells in all phases of cell cycle (G0-G1, S, and G2-M) and a proportional increase of apoptotic cells. The potent pro-apoptotic activity shown by compound 6b and its effects on cell cycle make this compound of great interest for further investigations.

PMID: 16580204 [PubMed - indexed for MEDLINE]

Int J Biochem Cell Biol. 2005 Aug;37(8):1709-26. Epub 2005 Apr 26.

Pterostilbene and 3'-hydroxypterostilbene are effective apoptosis-inducing agents in MDR and BCR-ABL-expressing leukemia cells.

Tolomeo M, **Grimaudo S**, **Di Cristina A**, **Roberti M**, **Pizzirani D**, **Meli M**, **Dusonchet L**, **Gebbia N**, **Abbadessa V**, **Crosta L**, **Barucchello R**, **Grisolia G**, **Invidiata F**, **Simoni D**.

Dipartimento di Ematologia, Policlinico, Universita di Palermo, via del Vespro 129, 90127 Palermo, Italy. mt4536@policlinico.pa.it

Pterostilbene and 3,5-hydroxypterostilbene are the natural 3,5-dimethoxy analogs of trans-resveratrol and piceatannol, two compounds which can induce apoptosis in tumor cells. In previous studies we demonstrated the importance of a 3,5-dimethoxy motif in conferring pro-apoptotic activity to stilbene based compounds so we now wanted to evaluate the ability of pterostilbene and 3,5-hydroxypterostilbene in inducing apoptosis in sensitive and resistant leukemia cells. When tested in sensitive cell lines, HL60 and HUT78, 3'-hydroxypterostilbene was 50-97 times more potent than trans-resveratrol in inducing apoptosis, while pterostilbene appeared barely active. However, both compounds, but not trans-resveratrol and piceatannol, were able to induce apoptosis in the two Fas-ligand resistant lymphoma cell lines, HUT78B1 and HUT78B3, and the multi drug-resistant leukemia cell lines HL60-R and K562-ADR (a Bcr-Abl-expressing cell line resistant to imatinib mesylate). Of note, pterostilbene-induced apoptosis was not inhibited by the pancaspase-inhibitor Z-VAD-fmk, suggesting that this compound acts through a caspase-independent pathway. On the contrary, 3'-hydroxypterostilbene seemed to trigger apoptosis through the intrinsic apoptotic pathway: indeed, it caused a marked disruption of the mitochondrial membrane potential delta psi and its apoptotic effects were inhibited by Z-VAD-fmk and the caspase-9-inhibitor Z-LEHD-fmk. Moreover, pterostilbene and 3'-hydroxypterostilbene, when used at concentrations that elicit significant apoptotic effects in tumor cell lines, did not show any cytotoxicity in normal hemopoietic stem cells. In conclusion, our data show that pterostilbene and particularly 3'-hydroxypterostilbene are interesting antitumor natural compounds that may be useful in the treatment of resistant hematological malignancies, including imatinib, non-responsive neoplasms.

PMID: 15878840 [PubMed - indexed for MEDLINE]

J Med Chem. 2005 Jun 30;48(13):4293-9.

Studies on the apoptotic activity of natural and synthetic retinoids: discovery of a new class of synthetic terphenyls that potently support cell growth and inhibit apoptosis in neuronal and HL-60 cells.

Simoni D, **Giannini G**, **Roberti M**, **Rondanin R**, **Baruchello R**, **Rossi M**, **Grisolia G**, **Invidiata FP**, **Aiello S**, **Marino S**, **Cavallini S**, **Siniscalchi A**, **Gebbia N**, **Crosta L**, **Grimaudo S**, **Abbadessa V**, **Di Cristina A**, **Tolomeo M**.

Dipartimento di Scienze Farmaceutiche, Universita di Ferrara, Via Fossato di Mortara 17-19, 44100 Ferrara, Italy. smd@dns.unife.it

New terphenyl derivatives have been synthesized and tested for their effect on cell survival in serum-free cultures. These compounds protected HL60 cells from death and supported their growth with an activity higher than that of the natural 14-hydroxy-retro-retinol. Terphenyls 26 and 28 also possess antiapoptotic activity on neuronal cells, proving them as possible candidates for the treatment of neurodegenerative and ischemic diseases.

PMID: 15974583 [PubMed - indexed for MEDLINE]

J Med Chem. 2005 Feb 10;48(3):723-36.

Heterocyclic and phenyl double-bond-locked combretastatin analogues possessing potent apoptosis-inducing activity in HL60 and in MDR cell lines.

Simoni D, Grisolia G, Giannini G, Roberti M, Rondanin R, Piccagli L, Baruchello R, Rossi M, Romagnoli R, Invidiata FP, Grimaudo S, Jung MK, Hamel E, Gebbia N, Crosta L, Abbadessa V, Di Cristina A, Dusonchet L, Meli M, Tolomeo M.

Dipartimento di Scienze Farmaceutiche, Universita di Ferrara, Via Fossato di Mortara 17-19, 44100 Ferrara, Italy. smd@dns.unife.it

Two new series of combretastatin (CA-4) analogues have been prepared. The alkenyl motif of CA-4 was replaced either by a five-membered heterocyclic (isoxazoline or isoxazole) or by a six-membered ring (pyridine or benzene). The new compounds have been evaluated for their effects on tubulin assembly and for cytotoxic and apoptotic activities. Five compounds (18b, 20a, 21a, 34b, and 35b) demonstrated an attractive profile of cytotoxicity (IC50 < 1 microM) and apoptosis-inducing activity but poor antitubulin activity. The isoxazoline derivatives 18b, 20a, and 21a, demonstrated potent apoptotic activity different from that of natural CA-4. Their ability to block most cells in the G2 phase suggests that these compounds could act on targets different from the mitotic spindle. This would indicate activation of both the intrinsic and the extrinsic apoptotic pathways. The data suggest unambiguously that structural alteration of the stilbene motif of CA-4 can be extremely effective in producing potent apoptosis-inducing agents.

PMID: 15689156 [PubMed - indexed for MEDLINE]

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
The International Journal of Biochemistry & Cell Biology 37 (2005) 1709–1726
IJBCB
www.elsevier.com/locate/biocel

Pterostilbene and 3′-hydroxypterostilbene are effective apoptosis-inducing agents in MDR and BCR-ABL-expressing leukemia cells

Manlio Tolomeo [a,*], Stefania Grimaudo [a], Antonietta Di Cristina [a], Marinella Roberti [b], Daniela Pizzirani [b], Maria Meli [c], Luisa Dusonchet [c], Nicola Gebbia [d], Vincenzo Abbadessa [a], Lucia Crosta [d], Riccardo Barucchello [e], Giuseppina Grisolia [e], Francesco Invidiata [f], Daniele Simoni [e]

[a] *Dipartimento di Ematologia, Policlinico, Università di Palermo, via del Vespro 129, 90127 Palermo, Italy*
[b] *Dipartimento di Scienze Farmaceutiche, Università di Bologna, Italy*
[c] *Dipartimento di Scienze Farmacologiche, Università di Palermo, Italy*
[d] *Consorzio di Ricerca sul Rischio Biologico in Agricoltura (Co.Ri.Bi.A.), Regione Siciliana, Italy*
[e] *Dipartimento di Scienze Farmaceutiche, Università di Ferrara, Italy*
[f] *Dipartimento Farmacochimico, Tossicologico e Biologico, Università di Palermo, Italy*

Received 6 December 2004; received in revised form 2 March 2005; accepted 15 March 2005

Abstract

Pterostilbene and 3,5-hydroxypterostilbene are the natural 3,5-dimethoxy analogs of *trans*-resveratrol and piceatannol, two compounds which can induce apoptosis in tumor cells. In previous studies we demonstrated the importance of a 3,5-dimethoxy motif in conferring pro-apoptotic activity to stilbene based compounds so we now wanted to evaluate the ability of pterostilbene and 3,5-hydroxypterostilbene in inducing apoptosis in sensitive and resistant leukemia cells. When tested in sensitive cell lines, HL60 and HUT78, 3′-hydroxypterostilbene was 50–97 times more potent than *trans*-resveratrol in inducing apoptosis, while pterostilbene appeared barely active. However, both compounds, but not *trans*-resveratrol and piceatannol, were able to induce apoptosis in the two Fas-ligand resistant lymphoma cell lines, HUT78B1 and HUT78B3, and the multi drug-resistant leukemia cell lines HL60-R and K562-ADR (a Bcr-Abl-expressing cell line resistant to imatinib mesylate). Of note, pterostilbene-induced apoptosis was not inhibited by the pancaspase-inhibitor Z-VAD-fmk, suggesting that this compound acts through a caspase-independent pathway. On the contrary, 3′-hydroxypterostilbene seemed to trigger apoptosis through the intrinsic apoptotic

Abbreviations: SAR, structure-activity relationships; MDR, multidrug resistance; FCS, foetal calf serum; Z-LEHD-fmk, Z-Leu-Glu(Ome)-His-Asp(Ome)-fmk; Z-IETD-fmk, *N*-acetyl-Ile-Glu-Thr-Asp-fmk; Z-VAD-fmk, acetyl-Tyr-Val-Ala-Asp-chloromethyl ketone; DMSO, dimethylsulphoxide; PHA-LCM, phytohemagglutinin-leucocyte culture medium; CFU-GM, colony forming units-granulocyte macrophage; PBS, phosphate buffered saline; $DiOC_6$, 3,3′-dihexyloxacarbocyanine iodide; Fas-L, Fas-ligand; DISC, death-inducing signalling complex

* Corresponding author. Tel.: +39 091 655 4348; fax: +39 091 655 3249.
E-mail address: mt4536@policlinico.pa.it (M. Tolomeo).

1357-2725/$ – see front matter
doi:10.1016/j.biocel.2005.03.004

Heterocyclic and Phenyl Double-Bond-Locked Combretastatin Analogues Possessing Potent Apoptosis-Inducing Activity in HL60 and in MDR Cell Lines

Daniele Simoni,*[,†] Giuseppina Grisolia,[†] Giuseppe Giannini,[‡] Marinella Roberti,[§] Riccardo Rondanin,[†] Laura Piccagli,[†] Riccardo Baruchello,[†] Marcello Rossi,[†] Romeo Romagnoli,[†] Francesco Paolo Invidiata,[||] Stefania Grimaudo,[⊥] M. Katherine Jung,[#] Ernest Hamel,[▽] Nicola Gebbia,[○] Lucia Crosta,[○] Vincenzo Abbadessa,[⊥] Antonietta Di Cristina,[⊥] Luisa Dusonchet,[¶] Maria Meli,[¶] and Manlio Tolomeo[⊥]

Dipartimento di Scienze Farmaceutiche, Università di Ferrara, Via Fossato di Mortara 17-19, 44100 Ferrara, Italy, R & D, Sigma-Tau, Pomezia, Italy, Dipartimento di Scienze Farmaceutiche, Università di Bologna, Bologna, Italy, Dipartimento Farmacochimico Tossicologico e Biologico, Università di Palermo, Palermo, Italy, Cattedra di Ematologia e Servizio AIDS, Policlinico di Palermo, Palermo, Italy, SAIC-Frederick, Inc., Frederick, Maryland 21702, Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702, Consorzio di Ricerca sul Rischio Biologico in Agricoltura (Co.Ri.Bi.A.), and Dipartimento di Scienze Farmacologiche, Università di Palermo, Palermo, Italy

Received May 24, 2004

Two new series of combretastatin (CA-4) analogues have been prepared. The alkenyl motif of CA-4 was replaced either by a five-membered heterocyclic (isoxazoline or isoxazole) or by a six-membered ring (pyridine or benzene). The new compounds have been evaluated for their effects on tubulin assembly and for cytotoxic and apoptotic activities. Five compounds (**18b**, **20a**, **21a**, **34b**, and **35b**) demonstrated an attractive profile of cytotoxicity (IC_{50} < 1 μM) and apoptosis-inducing activity but poor antitubulin activity. The isoxazoline derivatives **18b**, **20a**, and **21a**, demonstrated potent apoptotic activity different from that of natural CA-4. Their ability to block most cells in the G2 phase suggests that these compounds could act on targets different from the mitotic spindle. This would indicate activation of both the intrinsic and the extrinsic apoptotic pathways. The data suggest unambiguously that structural alteration of the stilbene motif of CA-4 can be extremely effective in producing potent apoptosis-inducing agents.

Introduction

Apoptosis, also known as programmed cell death, is a physiologic cell suicide mechanism that controls cell number in the tissues of metazoans.[1] Apoptosis is involved in embryo development, during morphogenesis, and in the adult animal, during tissue turnover. Moreover, it plays an important role in the development of the immune response. Because apoptosis is a major modality by which tumor cells can be eliminated, the identification of new drugs able to induce the death program in different tumor cell types is an important goal in cancer therapy and may provide new useful tools for the treatment of patients with drug-resistant malignancies.[2,3]

After inhibition of cell proliferation by inducing a block in one of the cell cycle phases, most anticancer drugs induce programmed cell death through a mechanism involving cell cycle checkpoint proteins such as p53. This process includes activation of key elements of apoptosis, the cell's intrinsic death program. A failure of this mechanism or the activation of apoptosis-inhibitor proteins (Bcl-2, Bcl-XL) in neoplastic cells can induce resistance to anticancer chemotherapy.[4,5] Genetic and epigenetic alterations that are important in carcinogenesis often disable the cell death pathway, thereby affecting the way that cancer cells respond to therapeutic insult. Therefore, factors affecting apoptosis activation might be important determinants of drug sensitivity.[2,3,6,7] New drugs able to modulate the expression of molecules involved in the apoptotic pathway and able to induce apoptosis in multidrug-resistant or apoptosis-resistant tumor cell lines are of great importance in cancer chemotherapy.

Combretastatins are natural antimitotic agents isolated from the bark of the South African tree *Combretum caffrum*. Among these compounds, combretastatin A-4 (CA-4, **1**, Figure 1) possesses the most potent and interesting antitumor activity.[8−14] From a structure−activity relationship point of view, CA-4 belongs to the class of natural compounds related to biphenyls and contains, as a key structural feature, the *cis*-stilbene motif. CA-4 is an exceptionally strong inhibitor of tubulin polymerization and is potently cytotoxic against murine lymphocytic leukemia and against human ovarian and colon cancer cell lines.[15−20] Its mechanism of action is thought to be related to the tubulin-binding properties that result in rapid tumor endothelial cell damage, neovascular shutdown, and subsequent haemorrhagic necrosis.[21−23] It has been recently demon-

* Author to whom correspondence should be addressed. Phone: +39-0532-291291. Fax: +39-0532-291296. E-mail: smd@dns.unife.it.
† Dipartimento di Scienze Farmaceutiche, Università di Ferrara.
‡ R & D, Sigma-Tau.
§ Dipartimento di Scienze Farmaceutiche, Università di Bologna.
|| Dipartimento Farmacochimico Tossicologico e Biologico, Università di Palermo.
⊥ Cattedra di Ematologia e Servizio AIDS, Policlinico di Palermo.
SAIC-Frederick, Inc.
▽ National Cancer Institute at Frederick, National Institutes of Health.
○ Consorzio di Ricerca sul Rischio Biologico in Agricoltura (Co.Ri.Bi.A.).
¶ Dipartimento di Scienze Farmacologiche, Università di Palermo.

10.1021/jm049622b CCC: $30.25
Published on Web 01/12/2005

Studies on the Apoptotic Activity of Natural and Synthetic Retinoids: Discovery of a New Class of Synthetic Terphenyls That Potently Support Cell Growth and Inhibit Apoptosis in Neuronal and HL-60 Cells

Daniele Simoni,*,# Giuseppe Giannini,† Marinella Roberti,|| Riccardo Rondanin,# Riccardo Baruchello,# Marcello Rossi,# Giuseppina Grisolia,# Francesco Paolo Invidiata,‡ Stefania Aiello,‡ Silvia Marino,§ Sabrina Cavallini,§ Anna Siniscalchi,§ Nicola Gebbia,⊥ Lucia Crosta,⊥ Stefania Grimaudo,● Vincenzo Abbadessa,● Antonietta Di Cristina,● and Manlio Tolomeo●

Dipartimento di Scienze Farmaceutiche, Università di Ferrara, Via Fossato di Mortara 17-19, 44100 Ferrara, Italy, R & D, Sigma-tau, Pomezia, Italy, Dipartimento di Scienze Farmaceutiche, Università di Bologna, Bologna, Italy, Dipartimento Farmacochimico Tossicologico e Biologico, Università di Palermo, Palermo, Italy, Dipartimento di Medicina Clinica e Sperimentale, Sezione di Farmacologia, Università di Ferrara, Ferrara, Italy, Consorzio di Ricerca sul Rischio Biologico in Agricoltura (Co.Ri.Bi.A.), Palermo, Italy, and Divisione di Ematologia e Servizio A.I.D.S., Policlinico, Università di Palermo, Palermo, Italy

Received November 15, 2004

New terphenyl derivatives have been synthesized and tested for their effect on cell survival in serum-free cultures. These compounds protected HL60 cells from death and supported their growth with an activity higher than that of the natural 14-hydroxy-*retro*-retinol. Terphenyls **26** and **28** also possess antiapoptotic activity on neuronal cells, proving them as possible candidates for the treatment of neurodegenerative and ischemic diseases.

Introduction

Apoptotic processes are widespread biochemical events, being involved in, for example, development, differentiation, cell proliferation/homoeostasis, regulation and function of the immune system, and the removal of defective and therefore harmful cells.[1] Thus, dysfunction or deregulation of the apoptotic program is implicated in a variety of pathological conditions. Defects in apoptosis can result in cancer,[2,3] autoimmune diseases, and spreading of viral infections, while neurodegenerative disorders such as Alzheimer and Parkinson diseases, AIDS, and ischemic diseases are caused or enhanced by excessive apoptosis.[4,5] Increasing evidence suggests that inhibition of apoptosis in these severe pathologies may be beneficial as a therapeutic approach to slow disease progression and to improve a patient's prospects.[4] To this end, the discovery of new compounds able to modulate the apoptotic process, both as activators or inhibitors, represents an important challenge in medicinal chemistry.

Retinoids, a class of natural and synthetic compounds structurally related to vitamin A (**1**, Figure 1), are known to modulate cell proliferation, apoptosis, and differentiation, with different effects depending on the cellular context. Thus, *all-trans*-retinoic acid (ATRA, **2**) and its naturally occurring retinoid analogue 9-*cis*-retinoic acid (**3**), are known[6] to be involved in cell differentiation, morphogenesis, proliferation, and antineoplastic processes.

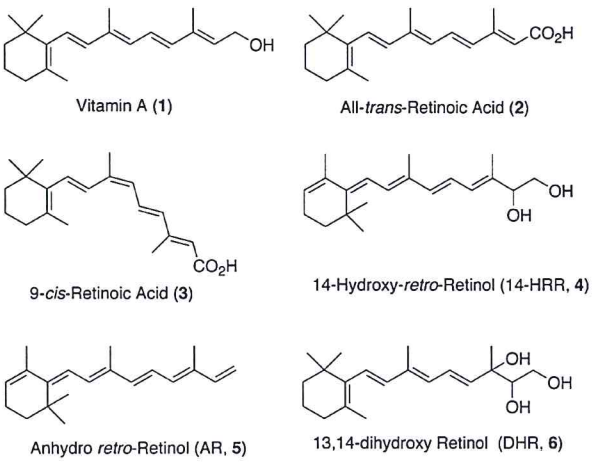

Figure 1. Natural retinoids and *retro*-retinoids.

Two other bioactive metabolites of vitamin A, namely, 14-hydroxy-*retro*-retinol (14-HRR) (**4**) and anhydroretinol (AR) (**5**), are involved in cell proliferation, with growth-supporting properties for normal lymphocyte proliferation being ascribed to **4**, while **5** was discovered to act as a natural antagonist triggering growth arrest and death by apoptosis.[7,8] A mutually reversible relationship exists between these two *retro*-retinoids because one can reverse the effects of the other when given in pharmacological doses.[9–11] Another reported metabolite of vitamin A sharing similar activity as **4** is the 13,-14-dihydroxy retinol (DHR, **6**).[12] Exploitation of this relationship holds much promise for therapeutic control of diseases such as cancer.

* To whom correspondence should be addressed. Phone: +39-0532-291291. Fax: +39-0532-291296. E-mail: smd@dns.unife.it.
# Dipartimento di Scienze Farmaceutiche, Università di Ferrara.
† Sigma-tau.
|| Università di Bologna.
‡ Dipartimento Farmacochimico Tossicologico e Biologico, Università di Palermo.
§ Dipartimento di Medicina Clinica e Sperimentale, Università di Ferrara.
⊥ Consorzio di Ricerca sul Rischio Biologico in Agricoltura (Co.Ri.Bi.A.).
● Divisione di Ematologia e Servizio A.I.D.S., Policlinico, Università di Palermo.

10.1021/jm049080y CCC: $30.25
Published on Web 06/03/2005