Letters

A Novel Atypical Retinoid Endowed with Proapoptotic and Antitumor Activity

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Abstract: The novel atypical retinoid *E*-3-(4′-hydroxy-3′-adamantylbiphenyl-4-yl)acrylic acid (ST1926, **4**) exhibited a potent antiproliferative activity on a large panel of human tumor cells. Despite almost complete loss of ability to activate RARs, the compound was an effective apoptosis inducer and surprisingly produced DNA damage, that likely contributes to the proapoptotic activity. Following oral administration, **4** was well tolerated and caused tumor growth inhibition in the ovarian carcinoma, A2780/DX, and in the human melanoma, MeWo, growing in nude mice, thus supporting the therapeutic interest of the novel agent.

The efficacy of chemotherapeutic treatment of tumors is limited by the development of drug resistance. Among the multiple mechanisms involved, it is likely that defects in apoptotic pathways are responsible for reduced cell ability to undergo drug-induced apoptosis. Indeed, tumor progression has been associated with multiple changes including resistance to apoptosis.[1]

This evidence emphasizes the interest for identification of novel strategies to facilitate the cells to undergo apoptosis. Among novel apoptosis-based approaches, "atypical" retinoids display significant potential as therapeutic agents in tumor treatment.[2] The cellular basis of their proapoptotic activity remains unclear. However, it is evident that some novel retinoid-related compounds possess unique mechanism(s) of action. In particular, a relevant feature of these agents is the ability to activate p53-independent apoptosis, because p53 mutation, a frequent alteration of tumor cells, is implicated in resistance to DNA-damaging agents. Classical retinoids, structural analogues of vitamin A, are involved in several biological processes, but have a marginal role in the treatment of neoplastic diseases. Retinoids exert their biological effects through ability to bind to and activate specific receptors which function as transcriptional factors, but it is likely that the broad biological effects associated with retinoids are mediated by distinct pathways.[2]

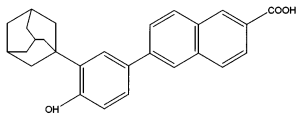
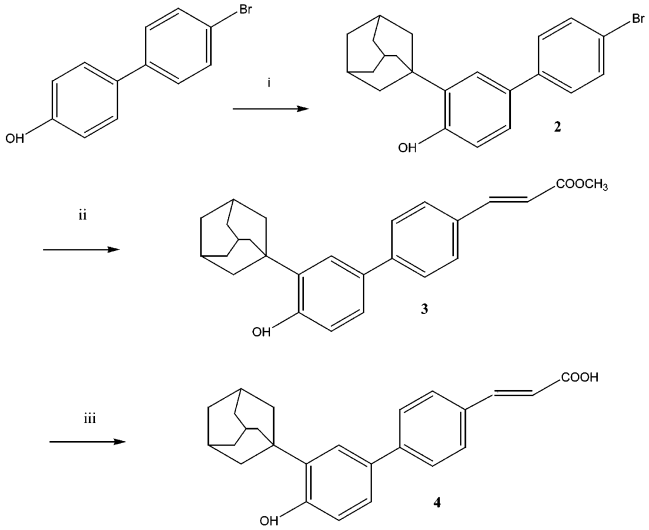


Figure 1. Chemical structure of **1**.

Scheme 1. Synthesis of **4**[a]



[a] Reagents and conditions: (i) 1-adamantanol, H_2SO_4/AcOH 1:9, 25 °C, 3 days (quantitative); (ii) methyl acrylate, tri-(*o*-tolyl)phosphine, $Pd(OAc)_2$, Et_3N, 100 °C, 4 h (98%); (iii) $LiOH \cdot H_2O$, THF/H_2O 1:1, 25 °C overnight (94%).

"Atypical retinoids" do not fit into the classical concept of ligand−receptor interaction: they activate certain RARs (retinoic acid receptors) as well as exert a growth-regulatory or apoptogenic activity that is not receptor mediated. 4-Hydroxyphenylretinamide (4HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalenecarboxylic acid (AHPN/CD437) belonging to this class are considered retinoids because they bind to and transactivate RARs.[3] In particular, CD437 (**1**, Figure 1) is known as RARγ-selective retinoid. However, the involvement of RARs in biological activity of **1** remains uncertain. Compound **1** is active in retinoid-resistant cells and retinoid antagonists cannot block this activity.[4] Preclinical and clinical data have already supported the potential of atypical retinoids as cancer therapeutic and chemopreventive agents.[5,6]

Focusing our attention on the RAR-independent induction of apoptosis, we synthesized new atypical retinoids. Here we report the discovery of a new lead compound endowed with potent antiproliferative and pro-apoptotic activities.

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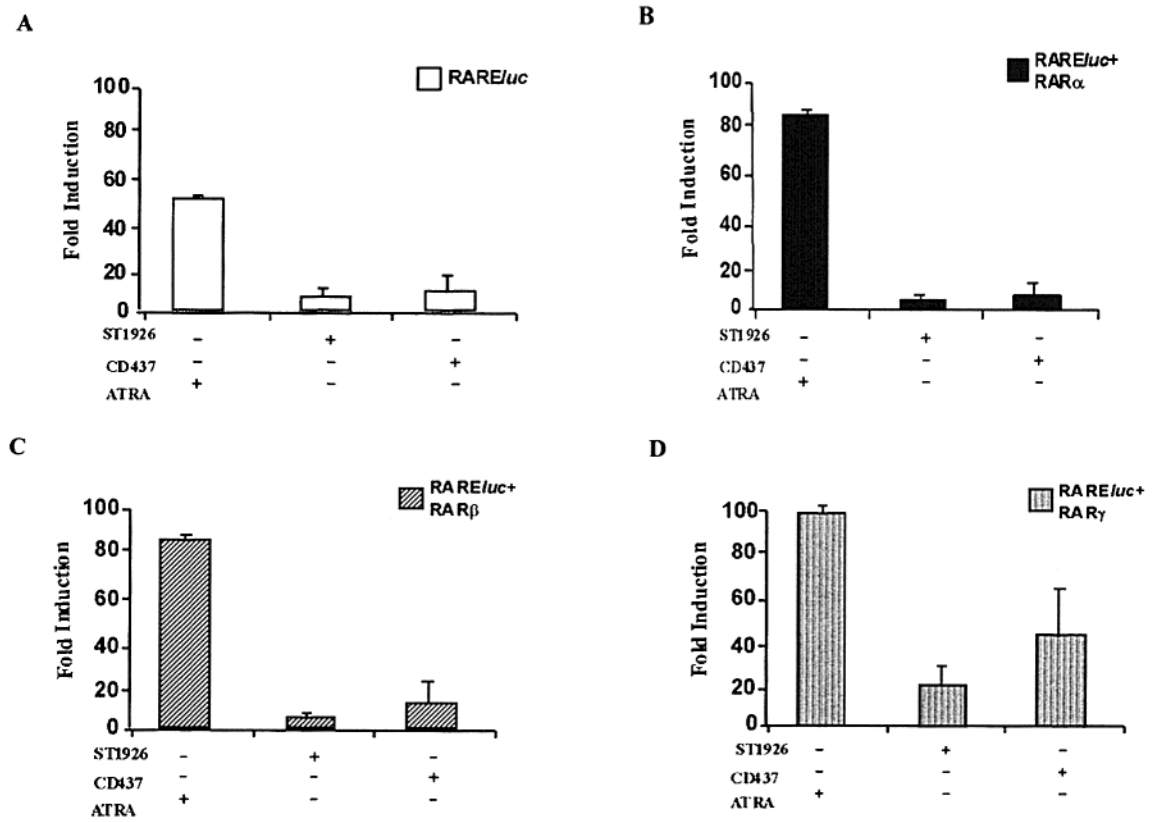

Figure 2. Transactivation and luciferase assay in HeLa cells. RARE*luc* reporter construct (2 μg) was cotransfected with 0.5 μg of pcDNA3 RARα, pcDNA3 RARβ, and pcDNA3 RARγ expression vectors together with 0.5 μg of CMV βgal expression vector for evaluation of transfection efficiency. 24 h after transfection, cells were incubated respectively with 0.1 μM *all-trans*-retinoic acid (ATRA) or ST1926 (**4**) or CD437 (**1**) or equivalent amount of control solvent. Luciferase assays were performed after additional 24 h using the Luciferase Assay System (Promega). Luciferase activity was measured using an A9904V TopCount luminometer (Packard). Counts per second were corrected for protein concentration and transfection efficiency. Data are mean values of three independent experiments (±SD) performed in duplicate.

Table 1. Antiproliferative Effect of **4** and ***1***[a]

			IC_{50} (μM)	
cell line	tumor type	p53 status	**4**	**1**
IGROV-1	ovarian carcinoma	wild-type	0.23 ± 0.09	0.35 ± 0.04
A2780/DX			0.1 ± 0.05	n.d.
DU145	prostate carcinoma	mutant	0.1 ± 0.02	0.28 ± 0.05
LNCaP		wild-type	0.12 ± 0.09	0.32 ± 0.05
PC3		null	0.21 ± 0.06	0.43 ± 0.01
H460	NSCLC	wild-type	0.19 ± 0.06	0.43 ± 0.05
HCT116	colon carcinoma	wild-type	0.32 ± 0.05	0.68 ± 0.02
LoVo		wild-type	0.15 ± 0.03	0.49 ± 0.04
A431	cervix carcinoma	mutant	0.25 ± 0.07	0.51 ± 0.1
GBM	glioblastoma	mutant	0.18 ± 0.02	0.55 ± 0.03
Me665/2/21	melanoma	mutant	0.25 ± 0.02	0.42 ± 0.12
SAOS	osteosarcoma	null	0.25 ± 0.03	0.67 ± 0.07
U2OS		wild-type	0.26 ± 0.09	0.44 ± 0.03

[a] Cells were exposed to **4** or **1** for 72 h. Antiproliferative activity was measured by cell counting and expressed as concentration required for 50% inhibition of cell growth (IC_{50}). IC_{50} values were determined by dose−response curves.

Table 2. Time Course of Apoptosis in Response to **4** or **1**[a]

	IGROV-1		DU145	
	4	**1**	**4**	**1**
24 h	42 ± 9	22 ± 6	24 ± 3	16 ± 1
48 h	46 ±11	15 ± 5	44 ± 4	33 ± 8
72 h	44 ± 5	38 ± 2	50 ± 2	30 ± 5

[a] Apoptosis was determined by morphological analysis of propidium iodide-stained cells following exposure to IC_{80} (0.5 and 1 μM for **4** and **1**, respectively). The results are expressed as percentage of apoptotic cells versus total cell number. Values are the mean ± standard deviation of three independent experiments.

The strategy for the design was devised using as a reference compound **1**. We assumed that functional groups critical for activity were the hydroxy group, the lipophilic adamantyl moiety, and the carboxylic function. On the basis of molecular mechanics calculations we obtained a minimal energy conformation for **1**, in which the distance between the C1 of the adamantyl group and the carboxylic carbon was calculated to be ca. 11.4 Å. Thus we planned to prepare a series of analogues keeping the key structural and pharmacologically important groups of **1** connected by different linkers. These spacers were designed in order to obtain conformationally minimized structures in which the distance between the two groups was maintained in a range of values as close as possible to 11.4 Å. Among the numerous compounds synthesized, we identified a very promising derivative in which the naphthalene ring was replaced by a styrene moiety.

The lead compound of this class of new retinoids, ST 1926 (**4**), was prepared in a high-yielding three-step sequence on a multigram scale (Scheme 1). Friedel−Crafts reaction of 1-adamantanol with commercially available 4-hydroxy-4′-bromobiphenyl gave quantitatively compound **2** that was subjected to Heck reaction with methyl acrylate to afford the ester **3** (98%). *E*-3-(4′-Hydroxy-3′-adamantylbiphenyl-4-yl)acrylic acid (**4**) was obtained (94%) by hydrolysis of **3** with LiOH in THF/H_2O.

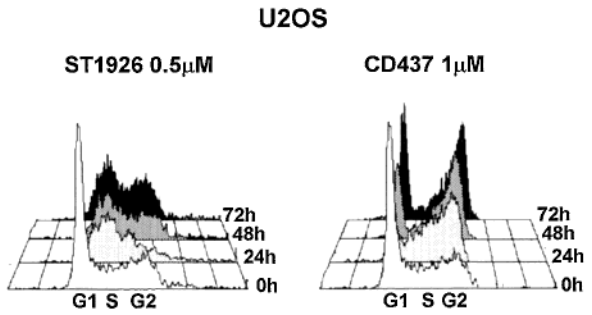

Figure 3. Cell cycle perturbation. Cell cycle distribution of U2-OS cells at different times of treatment with the IC_{80} of the two drugs. FACS analysis was performed on propidium iodide stained cells.

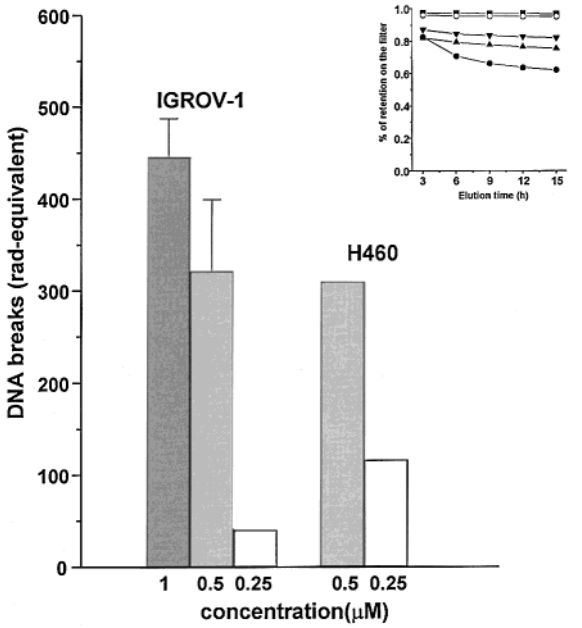

Figure 4. ST1926 **4**-induced DNA damage in IGROV-1 and H460 cells as determined by alkaline elution. Cells were treated for 6 h with the drug, lysed, and processed as reported by Supino et al.[9] For IGROV-1 cells, the data are mean values of three independent experiments. In the inset: percentage of DNA retention on the filter in function of the elution time in IGROV-1 cells. ■, untreated cells; ●, 1000 rad; ▲, **4**, 1 μM; ▼, **4**, 0.5 μM; ○, **4**, 0.25 μM.

The capacity of **4** and **1** to transcriptionally activate a retinoic acid response element reporter construct (RARE*luc*) in the presence or absence of specific retinoic acid receptor isoforms were studied, in comparison to ATRA, in transient transfection experiments performed in HeLa cells. While ATRA strongly activated all receptor isoforms (about 100 fold), **4** failed to significantly stimulate transcription in presence of RARα and RARβ receptors compared to RARE*luc* alone (Figure 2). A marginal increase in luciferase activity, however, was observed in the presence of RARγ overexpression. Thus, although structurally related to retinoids, **4** was characterized by an almost complete loss of typical retinoid features.

Compound **4** was studied in a large panel of human tumor cell lines including p53-defective cells (Table 1). Treatment for 72 h with **4** caused a dose-dependent growth inhibition in all tumor cell lines. **4** was at least 2-fold more potent than **1**. The concentrations of **4** required for 50% growth inhibition (IC_{50}) ranged from

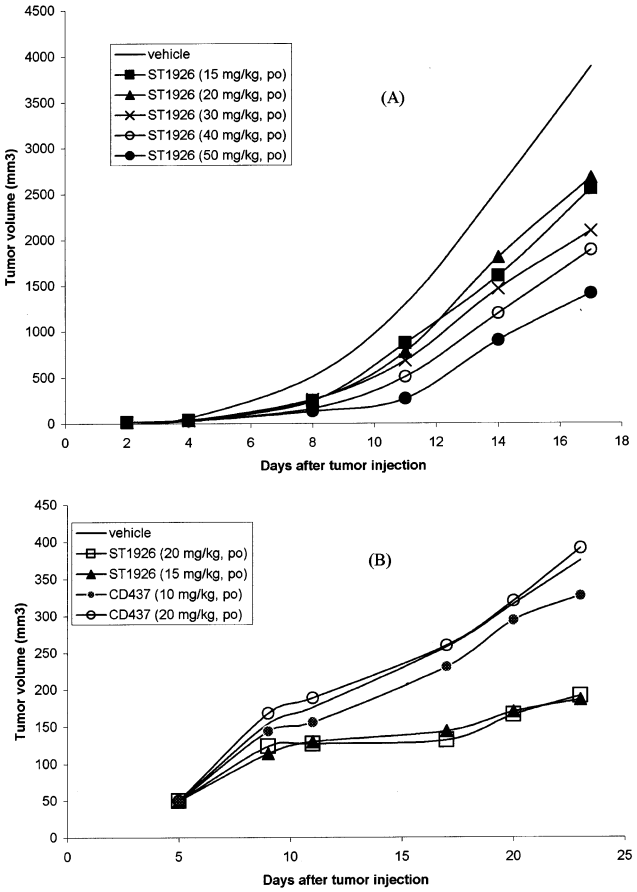

Figure 5. (A) Effect of ST1926 **4** delivered orally (qdx2/wx3w) starting from day 1, against the A2780/DX human ovarian carcinoma. (B) Effect of **4**, delivered orally (qdx5/wx2w) starting from day 6, against the MeWo human melanoma.

0.1 to 0.3 μM, which are pharmacologically relevant. The growth-inhibitory activity of **4** was apparently p53-independent, because the cellular effects were comparable in cells with functional or defective p53. Among the tested cell lines, prostate carcinoma cells exhibited an exquisite sensitivity to **4**. The cellular effects of **4** and **1** were studied in more detail in an ovarian carcinoma cell line, IGROV-1, carrying a functional wild-type p53[7] and a p53-mutant prostate carcinoma cell line, DU145.[8] In both cell systems **4** was an effective apoptosis inducer, since an induction of apoptosis (already evident at 24 h) was found at concentrations causing antiproliferative effects (IC_{80}) (Table 2). Again **4** was more potent and effective than **1** in both cell systems. **4** caused cell accumulation in G1/S or S phase of cell cycle depending on tumor cells. In osteosarcoma U2-OS cells, the **4**-induced arrest in S phase was more pronounced than that caused by **1** (Figure 3).

The impressive potency of **4** as an antiproliferative agent, and the accumulation of treated cells in S phase of cell cycle suggested a drug interference with DNA replication. Indeed, an appreciable extent of DNA breaks was detected in IGROV-1 ovarian carcinoma and H460 lung carcinoma cells following 6 h exposure to **4** (Figure 4). It is unlikely that these lesions reflect an early manifestation of apoptosis since apoptosis could be detected only at 24 h. Alkaline elution experiments in different conditions should provide additional information on the mechanism and nature of these DNA lesions. Despite the compound ability to induce poten-

tially lethal lesions and its potency, **4** was well tolerated in vivo following oral administration. Using a daily treatment schedule (qdx5/wx4w), oral administration of **4** caused a significant tumor growth inhibition in a human ovarian carcinoma, A2780/DX, and in a human melanoma, MeWo, growing in nude mice (Figure 5). Under similar conditions, **1** did not exhibit activity against the melanoma model.

Conclusions

In comparison with **1**, the related compound **4** shows an even lesser ability to activate RARs. Notwithstanding its nonretinoid profile, **4** shows a potent antiproliferative activity on a large panel of human tumor cells. The large spectrum of growth-inhibitory activity and the evidence of antitumor activity in human tumor xenografts support the therapeutic potential of the novel agent for cancer treatment. The therapeutic interest of the novel compound is also emphasized by the compound ability to induce genotoxic lesions which likely contribute to the proapoptotic activity. The mechanism of the induction of DNA lesions is unclear, because a direct drug−DNA interaction is unexpected. Taking into account the unique pharmacological profile, characterized by good tolerability and broad spectrum of activity, **4** represents a lead agent of a novel series of potent antiproliferative agents with potential applicability in the treatment of a wide variety of tumor types.

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References

(1) Igney, F. H.; Krammer, P. H. Death and anti-death: tumour resistance to apoptosis. *Cancer* **2002**, *2*, 277−288.
(2) Altucci, L.; Gronemeyer H. The Promise of Retinoids to Fight against Cancer. *Nature Rev. Cancer* **2001**, *1*,181−193.
(3) Fontana, J. A.; Rishi, A. K. Classical and novel retinoids: their targets in cancer therapy. *Leukemia* **2002**, *16*, 463−472.
(4) Holmes, W. F.; Dawson, M. I.; Soprano, R. V.; Soprano, K. J. Induction of Apoptosis in Ovarian Carcinoma Cells by AHPN/CD437 is Mediated by Retinoic Acid Receptors. *J. Cell. Physiol.* **2000**, *185*, 61−67.
(5) Sun, S. Y.; Yue, P.; Hong, W. K.; Lotan, R. Augmentation of Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (Trail)-Induced Apoptosis by the Synthetic Retinoid 6-[3-(1-Adamantyl)-4-hydroxyphenyl]-2-naphthalenecarboxylic acid (CD437) through Upregulation of Trail Receptors in Human Lung Cancer Cells. *Cancer Res.* **2000**, *60*, 7149−7155.
(6) Sun, S. Y.; Yue, P.; Hong, W. K.; Lotan, R. Induction of FAS Expression and Augmentation of FAS/FAS Ligand-Mediated Apoptosis by the Synthetic Retinoid **1** in Human Lung Cancer Cells. *Cancer Res.* **2000**, *60*, 6537−6543.
(7) Perego, P.; Giarola, M.; Righetti, S. C.; Supino, R.; Camerini, C.; Delia, D.; Pierotti, M. A.; Miyashita, T.; Reed, J. C.; Zunino, F. Association between cisplatin resistance and mutation of p53 gene and reduced bax expression in ovarian carcinoma cell systems. *Cancer Res.* **1996**, *56*, 556−562.
(8) Lanzi, C.; Cassinelli, G.; Cuccuru, G.; Supino, R.; Zuco, V.; Ferlini, C.; Scambia, G.; Zunino, F. Cell cycle checkpoint efficiency and cellular response to paclitaxel in prostate cancer cells. *Prostate* **2001**, *48*, 254−264.
(9) Supino, R.; Polizzi, D.; Pavesi, R.; Pratesi, G.; Guano, F.; Capranico, G.; Palumbo, M.; Sissi, C.; Richter, S.; Beggiolin, G.; Menta, E.; Pezzoni, G.; Spinelli, S.; Torriani, D.; Carenini, N.; Dal Bo, L.; Facchinetti, F.; Tortoreto, M.; Zunino, F. A novel 9-aza-anthrapyrazole effective against human prostatic carcinoma xenografts. *Oncology* **2001,** *61*, 234−42.

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