Handbook 8

Targretin

1. Chemical and Physical Characteristics

1.1 Nomenclature

See General Remarks, section 1.4,

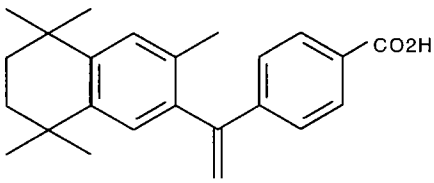
1.2 Name

Chemical Abstracts Services Registry number
153559-49-0

IUPAC Systematic name
4-[1-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)ethenyl]benzoic acid

Synonyms
Bexarotene, LG1069, LGD1069, LGD1001069, SR11247

1.3 Structural formula



Composition: $C_{24}H_{28}O_2$
Relative molecular mass: 348.2

1.4 Physical and chemical properties

Description
Fine white crystals (ethyl acetate or hexane)

Melting-point
234 °C

Spectroscopy
UV spectrum: λ_{max} 264 nm (ε 16 400 in methane)

^{1}H-NMR spectrum ($CDCl_3$): δ 1.28 (s, 6H, CH_3), 1.31 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 1.95 (s, 3H, CH_3), 5.35 and 5.83 (s, 2H, C=CH_2), 7.08 (s, 1H, ArH), 7.13 (s, 1H, ArH), 7.28 (d, *J* = 8.1 Hz, 2H, ArH), 8.03 (d, *J* = 8.1 Hz, 2H, ArH)
High-resolution mass spectrum: (FAB-MS): (M + H) calculated for $C_{14}H_{29}O_2$ 349.2168, found 349.2178

Infrared spectrum:
(KBr) 2959, 1677, 1278 cm^{-1}

Solubility
Soluble in organic solvents (see section 1.4 on all-*trans*-retinoic acid)

Stability
More stable than all-*trans*-retinoic acid

2. Occurrence, Production, Use, Human Exposure and Analysis

2.1 Occurrence

Human exposure to this synthetic retinoid is limited to patients receiving it as a drug. It does not occur in the diet.

2.2 Production

Targretin was prepared in four steps from toluene by the general route outlined in Figure 1 (Boehm *et al.*, 1994).

The procedure of Dawson *et al.* (1995) has only minor differences, including conversion of **2** to **3** with $ClCH_2CH_2Cl$ as the solvent, addition at 0 °C, reaction at room temperature and conversion of **3** to **4** with $KN(SiMe_3)_2$ as the base and reaction at room temperature.

2.3 Use

Several clinical trials have been conducted to assess the efficacy of targretin in the treatment of cancers, including advanced breast cancer (capsules in a phase-II trial); cutaneous T-cell lymphoma (gel in a phase-I and capsules in a phase-II/III trial); advanced lung cancer (capsules in a phase-II/III trial); Kaposi sarcoma (capsules in a phase-II/III trial); prostate cancer (phase-II trial);

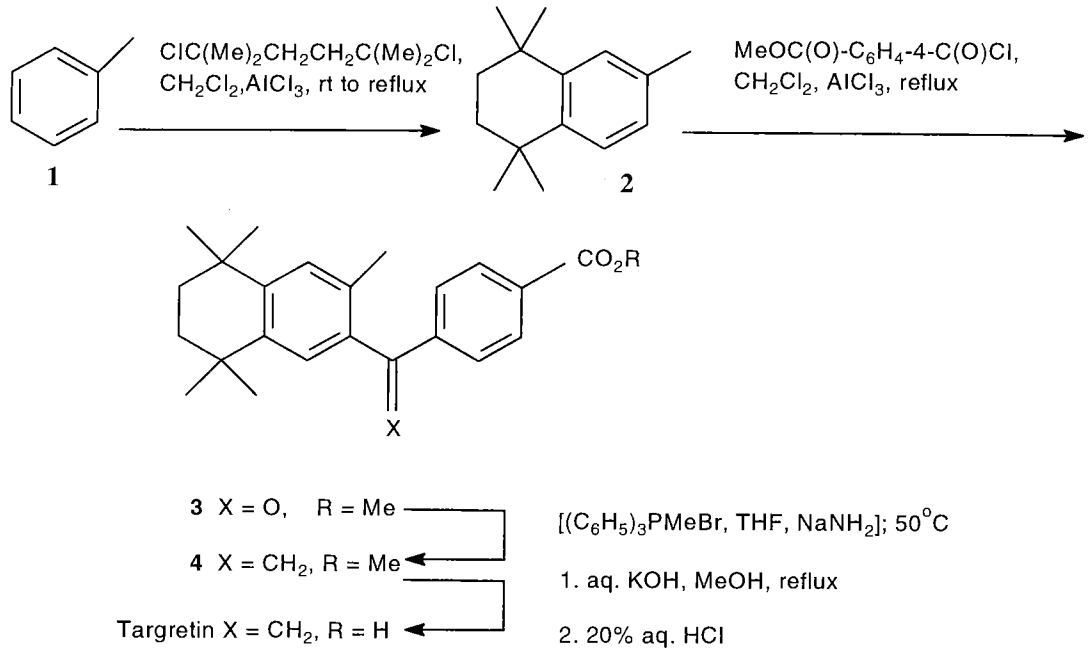


Figure 1. Synthesis of Targretin

renal cancer (phase-II trial); ovarian cancer (phase-I trial) and head-and-neck cancer (phase-I trial) (Rizvi *et al.*, 1996; Rigas *et al.*, 1997). Patients with cutaneous T-cell lymphoma responded well, whereas patients with advanced cancers showed mild retinoid toxicity (Miller *et al.*, 1997).

2.4 Human exposure

The maximum tolerated dose was considered to be 300 mg/m^2 (Miller *et al.*, 1997).

2.5 Analysis

Targretin can be separated by thin-layer chromatography (10% methane and 90% chloroform; R_f 0.5) (Boehm *et al.*, 1994). Gas chromatography–mass spectrometry has been used to determine targretin and its metabolites in studies of human pharmacokinetics (Miller *et al.*, 1997). High-performance liquid chromatography is also appropriate for the analysis of targretin (Howell *et al.*, 1998).

3. Metabolism, Kinetics and Genetic Variation

3.1 Humans

Studies of pharmocokinetics provide no evidence of auto-induction of metabolism. At a dose of 400 mg/m^2, the concentration of targretin in blood was about 300 ng/ml. The elimination half-life was 1–2 h (Miller *et al.*, 1997).

3.2 Experimental models

Although targretin increases the activity of hepatic cytochrome P450 enzymes in rats, little is known about its metabolism. It is converted to the 6- or 7-monohydroxy and glucuronide derivatives by rat liver microsomes (Howell *et al.*, 1998).

4. Cancer-preventive Effects

4.1 Humans

No data were available to the Working Group.

4.2 Experimental models

4.2.1 Cancer and preneoplastic lesions

Groups of 17–18 female rats, 50 days of age, received intravenous injections of *N*-methyl-*N*-nitrosourea (MNU) at a dose of 50 mg/kg bw, and, one week later, the vehicle or targretin was administered five times per week at a dose of 30 or 100 mg/kg bw by oral gavage. After 12 weeks, rats in the control group had a 100% incidence of mammary carcinomas and an average of three carcinomas per rat, whereas rats given the low and high doses of targretin had incidences of 22% and 12% and tumour multiplicities of 0.33 and 0.18, respectively (Table 1; $p < 0.001$, ANOVA) (Gottardis *et al.*, 1996). [The Working Group noted the short duration of the experiment.]

4.2.2 Intermediate biomarkers

No data were available to the Working Group.

4.2.3 In-vitro models

4.2.3.1 Cellular studies

(a) Inhibition of cell proliferation

In HL-60 myelocytic leukaemia cells, targretin had low antiproliferative activity at doses between 10^{-8} and 10^{-6} mol/L. At the highest concentration tested, it reduced proliferation by about 20%, an effect that requires only about 10^{-9} mol/L of all-*trans*-retinoic acid (Kizaki *et al.*, 1996).

(b) Inhibition of cell differentiation

Targretin has been evaluated in two human myelocytic leukaemia cell lines used as models of differentiation: the HL-60 and the U937 lines. It was active at high concentrations in both. In HL-60 cells, expression of a differentiation marker CD11b was increased after six days of culture. Activity was seen at doses between 10^{-9} and 10^{-7} mol/L, the highest concentration tested. Simultaneous treatment with all-*trans*-retinoic acid caused marked synergy of response (Kizaki *et al.*, 1996). When targretin was tested in U937 cells, it was marginally active, inducing differentiation (measured as an oxidative burst) at concentrations of 10^{-8} to 10^{-6} mol/L. This action was strongly enhanced by the retinoic acid receptor (RAR)-specific retinoid Ro 13-7410 at 10^{-7} mol/L (Defacque *et al.*, 1997).

4.2.3.2 Antimutagenicity in short-term tests

No data were available to the Working Group.

4.3 Mechanisms of cancer prevention

4.3.1 Receptor selectivity

Targretin was initially reported to bind selectively to retinoid X receptors (RXRs). It was most active in the presence of RXRs α, β and γ and was inactive in the presence of the RARs α, β and γ (Boehm *et al.*, 1994, 1995). Targretin was subsequently reported to show RAR cross-reactivity (Dawson *et al.*, 1995; Umemiya *et al.*, 1997). Targretin has a 12–100 times higher relative binding affinity for RXRs than for RARs (K_d = 16–31 nmol/L) (Table 2). Table 2 also shows the results of co-transfection assays, which measure the capacity of compounds to activate gene expression through each of the six known retinoid receptors. In these assays, targretin (median effective concentration, 20–28 nmol/L) was > 600 times more active with RXRs than with RARs (Gottardis *et al.*, 1996; Umemiya *et al.*, 1997).

4.3.2 Studies in animals

The study of MNU-induced breast carcinogenesis in rats (see section 4.2.1) provides some information about the mechanism whereby targretin can reduce tumour incidence. Since treatment began after administration of the carcinogen, effects on initiation can be excluded. Although the plasma concentrations of oestradiol, progesterone and prolactin were unchanged, targretin reduced the uterine weight and partially inhibited the increases in uterine weight resulting from treatment with oestrogen or tamoxifen (Gottardis *et al.*, 1996). [The Working Group noted that targretin appears to have anti-oestrogenic activity in the uterus and may have similar effects in the breast.]

4.3.3 Cell cultures

Targretin was only moderately active in cell culture models of differentiation, but its activity was greatly increased by addition of RAR or vitamin D receptor agonists (Kizaki *et al.*, 1996; Defacque *et al.*, 1997), which suggests that differentiation in these cells is driven by RAR–RXR or vitamin D receptor–RXR heterodimers.

5. Other Beneficial Effects

No data were available to the Working Group.

Table 1. Effects of targretin on mammary gland carcinogenesis in animals

Species sex, age at carcinogen treatment	No. of animals per group	Carcinogen dose/route	Targretin dose and route	Duration in relation to carcinogen	Incidence		Multiplicity		Efficacy	Reference
					Control	Treated	Control	Treated		
Female Sprague-Dawley rat	18	MNU (50 mg/kg bw, i.v.)	30 mg/kg b.w. (5 per wk p.o. for 12 wks)	– 1 wk – 12 wks	100	22*	3.0	0.33*	Effective	Gottardis *et al.* (1996)
			100 mg/kg bw (5 per wk p.o. for 12 wks)	– 1 wk – 12 wks		12*		0.18*	Effective	

MNU, *N*-methyl-*N*-nitrosourea; i.v., intravenous; p.o., oral
* Significantly different from controls (see text)

Table 2. Relative binding affinity of targretin to retinoic acid receptors (RARs) and retinoid X receptors (RXRs)

Receptor	Relative binding activity (nmol/L)		Median effective concentration (nmol/L)[a]
	Gottardis *et al.* (1996)	Umemiya *et al.* (1997)	Gottardis *et al.* (1996)
RARα	5950	180	> 10 000
RARβ	7624	50	> 10 000
RARγ	4221	130	> 10 000
RXRα	31	16	30–46
RXRβ	16	6	30–46
RXRγ	20	8	30–46

[a]At 10 µmol/L in transient transfections, targretin gives a response equal to 20% of the maximal response to all-*trans*-retinoic acid.

6. Carcinogenicity

No data were available to the Working Group.

7. Other Toxic Effects

7.1 Adverse effects

7.1.1 Humans

A study was performed in 52 patients with advanced cancer to determine the safety, clinical tolerance and pharmacokinetics of targretin at doses of 5–500 mg/m² for 1–41 weeks. Reversible, asymptomatic increases in the activity of liver enzymes were the most common dose-limiting adverse effect. The cumulative incidence of increased alkaline phosphatase activity and hypercalcaemia also increased with dose. Overall, 17% of the study group was so affected. Dry mucous membranes and dry skin occurred in 41% of the patients, and two developed a diffuse maculopapular skin rash. Other reactions included leukopenia and hyperlipidaemia. Reduction of the dose by 25–50% resolved the more severe neutropenia and the increases in aspartate aminotransferase activity. Toxic effects characteristic of the retinoids, such as cheilitis, headache, myalgia and arthralgia were mild or absent. Most of the headaches required no or occasional analgesia. The maximum tolerated dose was considered to be 300 mg/m² on the basis of the haematological and hepatic biochemical abnormalities at higher doses. The mucocutaneous toxicity of targretin was less frequent and milder than that seen in most patients treated with all-*trans*- or 13-*cis*-retinoic acid (Miller *et al.*, 1997).

In a preliminary report of a phase-I/II study of targretin in 26 patients with recurrent squamous-cell carcinoma of the head and neck, the dose was escalated from 10 mg/m² to 300 mg/m² twice daily. Two cases of pancreatitis were seen at the highest dose, including one episode of fatal haemorrhagic pancreatitis in a patient who continued to drink alcohol during the study. Hypertriglyceridaemia of grade 2 or higher was also seen in six patients (Papadimitrakopoulou *et al.*, 1998).

As reported in an abstract, treatment with targretin produced alterations primarily in cholesterol metabolism, with effects on plasma cholesterol and low-density lipoprotein cholesterol (Nervi *et al.*, 1997).

7.1.2 Experimental models

In a model of mammary carcinoma in which Sprague-Dawley rats received targetrin at 30 or 100 mg/kg bw per day on five days per week for 13 weeks, one week after a single dose of MNU, none of the classical signs of retinoid-associated toxicity was seen, except for mild alopecia. In particular, targretin did not induce osteopathy at doses similar to those of all-*trans*-retinoic acid needed to achieve the same degree of chemoprevention in this model within the same time (Gottardis *et al.*, 1996).

7.2 Reproductive and developmental effects

7.2.1 Humans

No data were available to the Working.

7.2.2 Experimental models

Targretin was not teratogenic in mice given single doses of 5–20 mg/kg bw on day 8 or 11 of gestation. When it was administered with a below-threshold dose of Am580, a ligand with relative selectivity for the RARα, or CD437, with relative selectivity for the RARγ, synergistic effects on a number of structural defects were observed. Spina bifida and ear and mandible defects, but not exencephaly, were induced when this regimen was given on day 8 and cleft palate and limb defects when it was given on day 11 of gestation. The synergistic response increased with increasing doses of targretin (Elmazar *et al.*, 1996). [The Working Group noted that these results were confirmed with additional RXR ligands and indicate that RXR ligands of low teratogenic potency can induce a strong teratogenic response when combined with low doses of RAR ligands which do not induce structural defects.]

7.3 Genetic and related effects

No data were available to the Working Group.

8. Summary of Data

8.1 Chemistry, occurrence and human exposure

Targretin {4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)ethenyl]benzoic acid} is a synthetic aromatic retinoid. It is a lipophilic molecule that is more stable than all-*trans*-retinoic acid. Human exposure to targretin is limited to patients participating in clinical trials.

8.2 Metabolism and kinetics

Few data are available. Certain metabolites have been implicated *in vitro*.

8.3 Cancer-preventive effects

8.3.1 Humans

No data were available to the Working Group.

8.3.2 Experimental models

In a single study of three months' duration, targretin was effective in preventing mammary cancer induced by *N*-methyl-*N*-nitrosourea in rats.

In two models of differentiation in human cells *in vitro*, targretin was less active than retinoic acid receptor-selective agonists; however, in both models supra-additive activity was seen when the cells were treated simultaneously with targretin and the retinoic acid receptor-selective agonists.

8.3.3 Mechanisms of cancer prevention

There are insufficient data to establish the mechanism of action of targretin.

8.4 Other beneficial effects

No data were available to the Working Group.

8.5 Carcinogenicity

No data were available to the Working Group.

8.6 Other toxic effects

8.6.1 Humans

Reversible, asymptomatic increases in the activity of hepatic enzymes were the dose-limiting adverse side-effects observed most commonly in phase I studies of targretin. Other reactions included leukopenia, hyperlipidaemia and hypercalcaemia. Mild mucocutaneous lesions and headache were seen infrequently after exposure to targretin.

8.6.2 Experimental models

In one short-term study in rats, targretin caused mild alopecia. In a single study, it was not teratogenic in mice when administered alone, but synergistic effects were seen when it was given in conjunction with agonists of retinoic acid receptors.

9. Recommendations for Research

9.1 General recommendations for targretin and other retinoids

See section 9 of the Handbook on all-*trans*-retinoic acid.

9.2 Recommendations specific to targretin

1. Evaluate the role of retinoid X receptors in the inhibition of experimental carcinogens using targretin (and other synthetic ligands) selective for retinoid X receptors.
2. Elucidate the mechanisms by which retinoic acid receptors and selective agonists enhance the effect of targretin.

10. Evaluation

10.1 Cancer-preventive activity

10.1.1 Humans

There is *inadequate evidence* that targretin has cancer-preventive activity in humans.

10.1.2 Experimental animals

There is *inadequate evidence* that targretin has cancer-preventive activity in experimental animals.

10.2 Overall evaluation

There are no data on the cancer-preventive activity of targretin in humans.

11. References

Boehm, M.F., Zhang, L., Badea, B.A., White, S.K., Mais, D.E., Berger, E., Suto, C.M., Goldman, M.E. & Heyman, R.A. (1994) Synthesis and structure–activity relationships of novel retinoid X receptor-selective retinoids. *J. Med. Chem.*, **37**, 2930–2941

Boehm, M.F., Zhang, L., Zhi, L., McClurg, M.R., Berger, E., Wagoner, M., Mais, D.E., Suto, C.M., Davies, J.A., Heyman, R.A. & Nadzan, A.M. (1995) Design and synthesis of potent retinoid X receptor selective ligands that induce apoptosis in leukemia cells. *J. Med. Chem.*, **38**, 3146–3155

Dawson, M.I., Jong, L., Hobbs, P.D., Cameron, J.F., Chao, W.R., Pfahl, M., Lee, M.O. & Shroot, B. (1995) Conformational effects on retinoid receptor selectivity. 2. Effects of retinoid bridging group on retinoid X receptor activity and selectivity. *J. Med. Chem.*, **38**, 3368–3383

Defacque, H., Sévilla, C., Piquemal, D., Rochette-Egly, C., Marti, J. & Commes, T. (1997) Potentiation of VD-induced monocytic leukemia cell differentiation by retinoids involves both RAR and RXR signaling pathways. *Leukemia*, **11**, 221–227

Elmazar, M.M., Reichert, U., Shroot, B. & Nau, H. (1996) Pattern of retinoid-induced teratogenic effects: Possible relationship with relative selectivity for nuclear retinoid receptors RARα, RAR β, and RARγ. *Teratology*, **53**, 158–167

Gottardis, M.M., Bischoff, E.D., Shirley, M.A., Wagoner, M.A., Lamph, W.W. & Heyman, R.A. (1996) Chemoprevention of mammary carcinoma by LGD1069 (targretin): An RXR-selective ligand. *Cancer Res.*, **56**, 5566–5570

Howell, S.R., Shirley, M.A. & Ulm, E.H. (1998) Effects of retinoid treatment of rats on hepatic microsomal metabolism and cytochromes P450. Correlation between retinoic acid receptor/retinoid X receptor selectivity and effects on metabolic enzymes. *Drug Metab. Dispos.*, **26**, 234–239

Kizaki, M., Dawson, M.I., Heyman, R., Elster, E., Morosetti, R., Pakkala, S., Chen, D.L., Ueno, H., Chao, W., Morikawa, M., Ikeda, Y., Heber, D., Pfahl, M. & Koeffler, H.P. (1996) Effects of novel retinoid X receptor-selective ligands on myeloid leukemia differentiation and proliferation in vitro. *Blood*, **87**, 1977–1984

Miller, V.A., Benedetti, F.M., Rigas, J.R., Verret, A.L., Pfister, D.G., Straus, D., Kris, M.G., Crisp, M., Heyman, R., Loewen, G.R., Truglia, J.A. & Warrell, R.P.J. (1997) Initial clinical trial of a selective retinoid X receptor ligand, LGD1069. *J. Clin. Oncol.*, **15**, 790–795

Nervi, A.M., Rigas, J.R., Miller, V.A., Levine, D.M., Dain, B.J. & Warrell, R.P.J. (1997) Plasma lipoproteins associated with three novel retinoids: all-*trans* Retinoic acid, 9-*cis* retinoic acid, and oral targretin™. *J. Invest. Med.*, **45**, 260A–260A

Papadimitrakopoulou, V., Khuri, F.R., Lippman, S.M., Shin, D.M., Ginsberg, L., Glisson, B.S., Martinez, L., Loewen, G., Truglia, J. & Hong, W.K. (1998) Phase I/II evaluation of targretin (LGD1069), a novel, RXR-specific retinoid, in patients with recurrent squamous cell carcinoma of the head and neck. *Proc. Am. Soc. Clin. Oncol.*, **17**, 1512–1512

Rigas, J.R., Maurer, L.H., Meyer, L.P., Hammond, S.M., Crisp, M.R., Parker, B.A. & Truglia, J.A. (1997) Targretin, a selective retinoid X receptor ligand (LGD1069), vinorelbine and cisplatin for the treatment of non-small cell lung cancer (NSCLC): A phase I/II trial. *Proc. Am. Soc. Clin. Oncol.*, 16, A1724–A1724

Rizvi, N.A., Marshall, J.L., Loewen, G.R., Ulm, E.H., Gill, G.M., Truglia, J.A., Ness, E., Clarke, R. & Hawkins, M.J. (1996) A phase I study of targretin™, an RXR-selective retinoid agonist. *Proc. Am. Assoc. Cancer Res.*, **37**, 165–165

Umemiya, H., Kagechika, H., Fukasawa, H., Kawachi, E., Ebisawa, M., Hashimoto, Y., Eisenmann, G., Erb, C., Pornon, A., Chambon, P., Gronemeyer, H. & Shudo, K. (1997) Action mechanism of retinoid-synergistic dibenzodiazepines. *Biochem. Biophys. Res. Commun.*, **233**, 121–125