DROLOXIFENE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Droloxifene

Chem. Abstr. Serv. Reg. No.: 82413-20-5

Chem. Abstr. Name: (*E*)-3-(1-(4-(2-(Dimethylamino)ethoxy)phenyl)-2-phenyl-1-butenyl)phenol

IUPAC Systematic Name: (*E*)-α-[*para*-[2-(Dimethylamino)ethoxy]phenyl]-α′-ethyl-3-stilbenol

Synonyms: (*E*)-1-(4′-(2-Dimethylaminoethoxy)phenyl)-1-(3-hydroxyphenyl)-2-phenylbut-1-ene; *trans*-1-(4-β-dimethylaminoethoxyphenyl)-1-(3-hydroxyphenyl)-2-phenylbut-1-ene; 3-hydroxytamoxifen

Droloxifene citrate

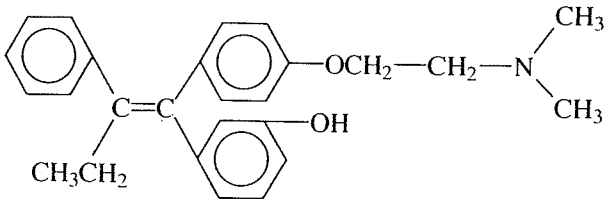
Chem. Abstr. Serv. Reg. No.: 97752-20-0

Chem. Abstr. Name: (*E*)-3-(1-(4-(2-(Dimethylamino)ethoxy)phenyl)-2-phenyl-1-butenyl)phenol, 2-hydroxy-1,2,3-propanetricarboxylate (1:1)

IUPAC Systematic Name: (*E*)-α-[*para*-[2-(Dimethylamino)ethoxy]phenyl]-α′-ethyl-3-stilbenol citrate

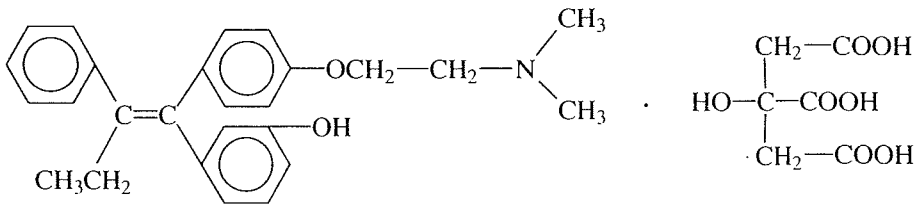
Synonyms: (*E*)-1-(4′-(2-Dimethylaminoethoxy)phenyl)-1-(3-hydroxyphenyl)-2-phenylbut-1-ene citrate; *trans*-1-(4-β-dimethylaminoethoxyphenyl)-1-(3-hydroxyphenyl)-2-phenylbut-1-ene citrate; 3-hydroxytamoxifen citrate

1.1.2 *Structural and molecular formulae and relative molecular mass*



Droloxifene

$C_{26}H_{29}NO_2$ Relative molecular mass: 387.52



Droloxifene citrate

$C_{26}H_{29}NO_2 . C_6H_8O_7$ Relative molecular mass: 579.65

1.1.3 *Chemical and physical properties of the pure substances*

From Budavari (1995); Pfizer (1996)

Droloxifene

(*a*) *Description*: Colourless crystals

(*b*) *Melting-point*: 160–163 °C

Droloxifene citrate

(*a*) *Description*: Off-white, crystalline powder

(*b*) *Melting-point*: 142 °C

(*c*) *Solubility*: Slightly soluble in water (0.078 mg/mL (unbuffered, pH 3.4)); soluble in methanol; sparingly soluble in ethanol; insoluble in chloroform

(*d*) *Stability*: Stable in aqueous solutions at pHs 3–7 at temperatures up to 50 °C

1.1.4 *Technical products and impurities*

In its pharmaceutical preparations, droloxifene is normally formulated as the citrate salt.

Trade names and designations of droloxifene citrate and its pharmaceutical preparation include: *E*-Droloxifene; FK 435; K 060; K 060E; K 21.060E.

1.1.5 *Analysis*

Droloxifene and its metabolites can be analysed in biological fluids by high-performance liquid chromatography (Lien *et al.*, 1995).

1.2 Production and use

1.2.1 *Production*

Droloxifene can be prepared by the reaction of 3-(tetrahydropyran-2-yloxy)phenyl bromide with magnesium or *n*-butyllithium followed by addition of the resulting reagent to 1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-phenylbutan-1-one. Acid-catalysed dehydration of the resulting tertiary alcohol gives a mixture of droloxifene and its Z-isomer,

which can be separated by chromatography and recrystallization (Ruenitz *et al.*, 1982; Foster *et al.*, 1985).

1.2.2 *Use*

Droloxifene (a phenolic analogue of tamoxifen) is a new non-steroidal antioestrogenic drug which has a high affinity for oestrogen receptors (see Glossary, p. 448) (Roos *et al.*, 1983), a low oestrogenic to antioestrogenic activity ratio (Löser *et al.*, 1985) and rapid pharmacokinetics (Grill & Pollow, 1991).

Phase I and II trials (see Glossary, p. 449) of droloxifene in postmenopausal women with metastatic breast cancer in various countries (Breitbach *et al.*, 1987; Stamm *et al.*, 1987) have shown that the drug has very little short-term toxicity (Bellmunt & Solé, 1991; Deschênes 1991; Miller *et al.*, 1991; Bruning, 1992; Haarstad *et al.*, 1992; Rauschning & Pritchard, 1994). Response (see Glossary, p. 449) rates and durations are similar, within the limits of cross-study comparison, to those seen with tamoxifen. Doses of up to 300 mg daily seem to be well tolerated (Buzdar *et al.*, 1993). A large multicentre phase III trial (see Glossary, p. 449) comparing droloxifene (40 mg) with tamoxifen (20 mg) is in progress in Europe, North and South America, South Africa and India (Pfizer, 1996). Droloxifene has not yet been tested for use in adjuvant therapy.

1.3 Occurrence

Droloxifene is not known to occur as a natural product.

1.4 Regulations and guidelines

Droloxifene is not registered for use as a pharmaceutical in any country (Pfizer, 1996).

2. Studies of Cancer in Humans

No report of carcinogenicity or preventive activity of droloxifene in humans has been published.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Rat: In a study that also included tamoxifen, groups of 49–50 male and 50 female Sprague-Dawley rats, 4–5 weeks old, after 13 weeks of adaptation, were given 0 (control), 4, 12, 36 or 90 mg/kg bw droloxifene citrate (> 99.1% pure) by gastric instillation daily for 24 months. No increase in the incidence of liver tumours was reported (Dahme & Rattel, 1994; Hasmann *et al.*, 1994). [The Working Group noted the inadequate information on dose selection and inadequate reporting.]

3.2 Administration with known carcinogens

3.2.1 *Mouse*

Groups of five or six male and female BALB/c/Bln mice, 8–12 weeks of age, were given intraperitoneal injections of diluted serum from leukaemic mice infected with the Rauscher murine leukaemia virus. Starting one day later, mice were given intraperitoneal injections of 0.5, 1.0 or 2 mg per animal droloxifene citrate [purity not specified] in dimethyl sulfoxide three times a week for 1–2 weeks. The mice were killed when moribund and spleens were weighed as an index of leukaemic disease. Compared to the controls, the spleen weights of the droloxifene-treated animals were about 70%, 60% and 45% in the low-, mid- and high-dose groups, respectively (Sydow & Wunderlich, 1994).

3.2.2 *Rat*

Three groups of 10 female Sprague-Dawley rats, seven weeks of age, were given 20 mg per animal 7,12-dimethylbenz[*a*]anthracene in sesame oil orally; starting one day later, two of the groups were given 1.0 or 10.0 mg/kg bw droloxifene citrate [purity not specified] in methylcellulose orally for seven days, while the third group received no further treatment. At 20 weeks, mammary tumours were found in 8/9 controls, 7/9 low-dose droloxifene citrate-treated rats and 3/10 ($p < 0.05$) high-dose droloxifene citrate-treated rats (Kawamura *et al.*, 1991).

Two groups of 21 and 20 female Sprague-Dawley rats, 50 days of age, were given 50 mg/kg bw *N*-methyl-*N*-nitrosourea as a single intravenous injection. When at least one mammary tumour reached a diameter of 10 mm, the animals were given 6 or 12 mg/kg bw droloxifene citrate [purity not specified] in Tween 80/distilled water by gastric instillation on five and three days a week, respectively, for four weeks. A control group of 50 female rats received *N*-methyl-*N*-nitrosourea alone. At 207 days, the multiplicity of mammary tumours was 1.1 in controls, 1.1 in low-dose droloxifene citrate-treated rats and 1.4 in high-dose droloxifene citrate-treated rats (Winterfeld *et al.*, 1992).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Droloxifene is rapidly and completely absorbed from the intestinal tract. Results from a phase II clinical trial (Kvinnsland, 1991) in postmenopausal women with metastatic breast cancer treated with droloxifene at 20 mg (34 patients), 40 mg (43 patients) or 100 mg (71 patients) once daily for up to one year showed that, with the 20-mg dose, peak plasma concentrations were reached after 2–4 h and the terminal half-life was 24 h. The mean plasma levels at steady-state concentration were about 15, 30 and 80 ng/mL with the 20-, 40- and 100-mg doses, respectively. These results are consistent with the plasma

levels of 81 ng/mL droloxifene, 1 ng/mL 4-methoxydroloxifene and 6 ng/mL *N*-desmethyldroloxifene measured in a breast cancer patient following a single oral dose of 100 mg droloxifene (Lien *et al.*, 1995).

In humans, droloxifene is rapidly metabolized to droloxifene glucuronide, *N*-desmethyldroloxifene and 4-methoxydroloxifene (Löser *et al.*, 1989; Hasmann *et al.*, 1994) (see Figure 1). Consequently, accumulation of droloxifene or its metabolites, if it occurs, is slight.

4.1.2 *Experimental systems*

In rats and mice, droloxifene is metabolized to droloxifene glucuronide, *N*-desmethyldroloxifene, 4-methoxydroloxifene, 3-methoxy-4-hydroxytamoxifen (4-hydroxydroloxifene) and droloxifene *N*-oxide. The proportion of 3-methoxy-4-hydroxytamoxifen formed is much higher in mice (> 40%) than in rats (< 20%) (Hasmann *et al.*, 1994).

White *et al.* (1993) showed that droloxifene (0.12 mmol/kg bw per day for four days) administered by gastric instillation to rats had little or no effect on the metabolism of ethoxy-, benzyloxy- or pentoxyresorufin.

4.2 Toxic effects

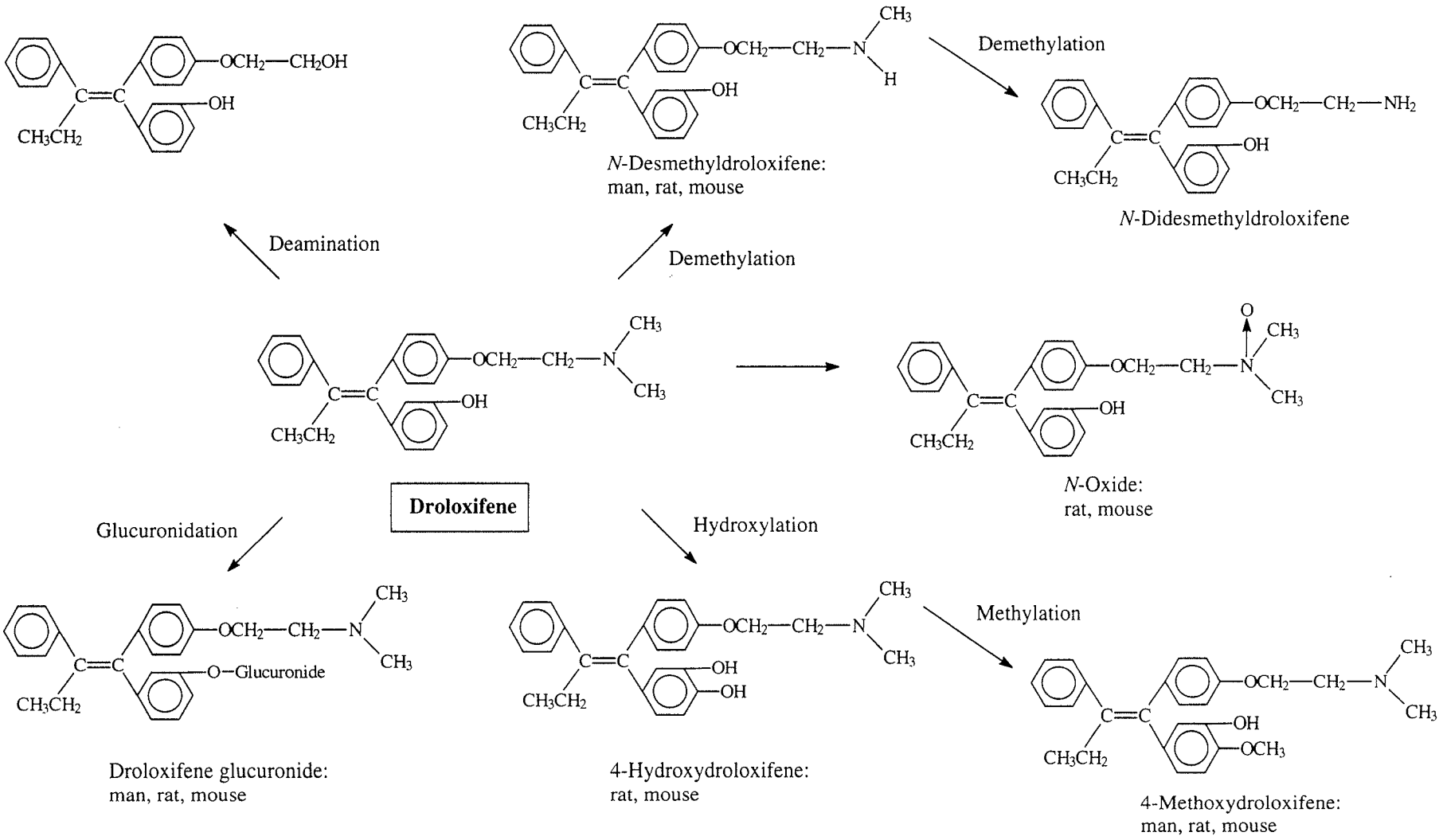
4.2.1 *Humans*

Results from the phase II clinical trial described in Section 4.1.1 showed dose-related decreases in luteinizing hormone and follicle-stimulating hormone levels. A rise in sex hormone-binding globulin level was found (particularly at doses of 40 and 100 mg). These changes were all observed during the first three months of therapy (Kvinnsland, 1991). When droloxifene was administered to 369 postmenopausal women with advanced breast cancer in a dose-finding phase II trial at levels of 20, 40 or 100 mg/day, common side-effects included hot flushes (32%, 32%, 29%), lassitude (28%, 23%, 26%) and nausea (22%, 24%, 29%) in the 20-mg, 40-mg and 100-mg dose groups, respectively. In 1200 patients who received 20, 40, 100 or more than 100 mg doses, signs of serious toxicity were infrequent, but pulmonary embolism in two patients and superficial venous thrombosis of the leg in one patient receiving 20 mg-doses were observed. In patients receiving 100-mg doses, these effects were observed in four and eight patients, respectively, and among those receiving more than 100 mg, they were each observed in one patient (Rauschning & Pritchard, 1994).

4.2.2 *Experimental systems*

Groups of 25 male or 25 female rats were given 1, 10 or 100 mg/kg bw droloxifene orally for four weeks. Droloxifene was tolerated at all doses, with no sign of systemic toxicity. A substance-related increase in the weight of the male and female sexual organs was found; this and histological changes in these organs can be explained by the anti-oestrogenic effect of droloxifene (Löser *et al.*, 1986).

Figure 1. Postulated metabolic pathways of droloxifene



From Grill and Pollow (1991)

Groups of 25 male and 25 female Sprague-Dawley rats, four to five weeks old, after 13 weeks of adaptation, were given 0, 2, 20 or 200 mg/kg bw droloxifene citrate (purity, > 99.1%) per day by gastric instillation in 0.25% agar suspension (volume 10 mg/mL) for six months [body weights, survival not reported.] Six weeks after the end of the treatment period, animals were killed and complete necropsy and histology were carried out but only liver histology was reported. No preneoplastic or neoplastic liver change was found at either dose level (Dahme & Rattel, 1994).

Droloxifene and its major metabolite, *N*-desmethyldroloxifene, exhibit high binding affinity to the oestrogen receptor in the oestrogen receptor-positive human breast cancer cell line MCF-7. The affinity of droloxifene for the oestrogen receptor was more than 60-fold higher than that of tamoxifen and the IC_{50} value of droloxifene for displacement of 17β-oestradiol from the receptor was approximately 1×10^{-8} M (maximal blood concentration, $1–4 \times 10^{-7}$ M) (Hasmann *et al.*, 1994).

Droloxifene inhibits lipid peroxidation in microsomal and liposomal membranes, but to a lower extent than 17β-oestradiol. The inhibition of lipid peroxidation by droloxifene may result from membrane stabilization that could be associated in cancer cells with decreased membrane fluidity, that might antagonize cell division (Wiseman *et al.*, 1992).

Other effects of droloxifene in rats include prevention of increased bone turnover and bone loss and reduced total serum cholesterol (Chen *et al.*, 1995a,b; Ke *et al.*, 1995a,b).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects (see also Table 1 for references and Appendices 1 and 2)

Droloxifene did not cause significant morphological transformation of Syrian hamster embryo cells.

DNA adducts were not detected by ^{32}P-postlabelling in the livers of female Fischer 344 rats given 0.12 mmol/kg bw droloxifene per day by gastric instillation for four days (White *et al.*, 1992).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Droloxifene is a phenolic analogue of tamoxifen which is undergoing clinical trials for the treatment of metastatic breast cancer, but is not yet registered in any country.

5.2 Human carcinogenicity data

No data were available to the Working Group.

Table 1. Genetic and related effects of droloxifene

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	–	NT	3.9	Metzler & Schiffmann (1991)
BVD, Binding (covalent) to DNA, Fischer 344/N rat liver *in vivo* (^{32}P-postlabelling)	–		47 po × 4	White *et al.* (1992)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day

5.3 Animal carcinogenicity data

Droloxifene was tested for carcinogenicity by oral administration in one study in rats. No increase in the incidence of tumours was reported.

Droloxifene was studied in two experiments in rats for its modulation of chemically induced mammary tumours. In one study with 7,12-dimethylbenz[*a*]anthracene, inhibition of mammary tumours was observed, while in another study with *N*-methyl-*N*-nitrosourea, there was no effect or a slight increase in the incidence of mammary tumours.

5.4 Other relevant data

Droloxifene is well absorbed in humans after oral doses. It undergoes both oxidative metabolism and direct glucuronidation, and the elimination half-life is about 24 h. Metabolites were identified in rats and mice that were not found in humans.

Toxic effects were infrequent in a phase II trial in postmenopausal women with metastatic breast cancer. Short- (four weeks) and longer- (six months) term toxicity studies in rats at doses of up to 200 mg/kg bw showed biochemical changes but little toxicity.

Droloxifene did not induce cell transformation *in vitro* or form DNA adducts in rat liver *in vivo*.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of droloxifene.

There is *inadequate evidence* in experimental animals for the carcinogenicity of droloxifene.

Overall evaluation

Droloxifene is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

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[1]For definition of the italicized terms, see Preamble, pp. 22–25.

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