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ORIGINAL ARTICLE

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Stereoselective pharmacokinetics of ifosfamide and its 2- and 3-*N*-dechloroethylated metabolites in female cancer patients

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Abstract The pharmacokinetics of the *R* and *S* enantiomers of ifosfamide (IFF) and of its 2- and 3-*N*-dechloroethylated metabolites (2-DCE-IFF and 3-DCE-IFF) were investigated in 14 cancer patients treated with a 3-h infusion of (*R*,*S*)-IFF (3 g/m^2) with mesna uroprotection. An enantioselective gas chromatographic-mass spectrometric (GC-MS) assay was used to determine the concentrations in plasma and urine. The AUCs of (*R*)-IFF were significantly larger than those of (*S*)-IFF (2480 ± 200 vs 1960 ± 150 μ*M*.h). The terminal half-lives (7.57 ± 0.99 h) and mean residence times (11.17 ± 1.10 h) of (*R*)-IFF were significantly longer than those of (*S*)-IFF, 6.03 ± 0.82 h and 9.37 ± 0.88 h, respectively. The mean volume of distribution at steady state of (*R*)-IFF (25.68 ± 0.80 l/m^2) was slightly smaller than that of (*S*)-IFF (27.35 ± 0.89 l/m^2). While the renal clearances of (*R*)-IFF and (*S*)-IFF were similar, the nonrenal clearance was significantly lower for (*R*)-IFF (30.20 ± 2.70 vs 41.40 ± 3.55 ml/m^2 per min) as was total clearance (41.52 ± 2.90 vs 52.37 ± 3.75 ml/m^2 per min). The AUC values for all of the DCE metabolites from (*S*)-IFF were significantly greater than those from (*R*)-IFF with 47% of the measured AUC accounted for by DCE from (*S*)-IFF compared to only 20% for (*R*)-IFF. Therefore, the enantioselective difference in IFF elimination can be partially explained by differences in *N*-dechloroethylation.

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Introduction

Many enantiomeric drugs are given as racemic (50:50) mixtures of the two stereoisomers. However, each enantiomer may have its own pharmacological properties and the two isomers may differ in their metabolism and disposition. Since these parameters have a bearing on efficacy and toxicity, it is important to establish the pharmacokinetic parameters of each enantiomer, especially when the drug in question has a narrow therapeutic index.

One example of this situation is ifosfamide (IFF), a structural isomer of cyclophosphamide, which is used in a wide range of malignancies, including adult soft-tissue sarcoma, osteosarcoma, small-cell lung cancer, and pediatric tumors [4, 6]. IFF, which contains a chiral center at the phosphorus atom, is clinically administered as a racemic mixture of its two isomers, (*R*)-IFF and (*S*)-IFF.

IFF is a prodrug which is metabolized in the liver by two major pathways (Fig. 1) [1]. The first pathway involves ring oxidation through cytochrome P450 enzymes (CYP) and leads to the production of the cytotoxic isophosphoramide mustard [5, 7, 20]. In the second pathway, up to 50% of the administered dose can undergo CYP-mediated side-chain oxidation resulting in two dechloroethylated metabolites, 2-DCE-IFF and 3-DCE-IFF [20]. This pathway has been associated with a central nervous system (CNS) toxicity [15, 18].

There have been very few reports regarding the stereoselective disposition of IFF and its dechloroethylated metabolites. Based on urinary data only, previous studies have suggested stereospecific differences in the metabolism of IFF [3, 9, 17–19]. Urine samples from two patients who had received a racemic mixture of IFF [(*R*, *S*)-IFF] contained higher

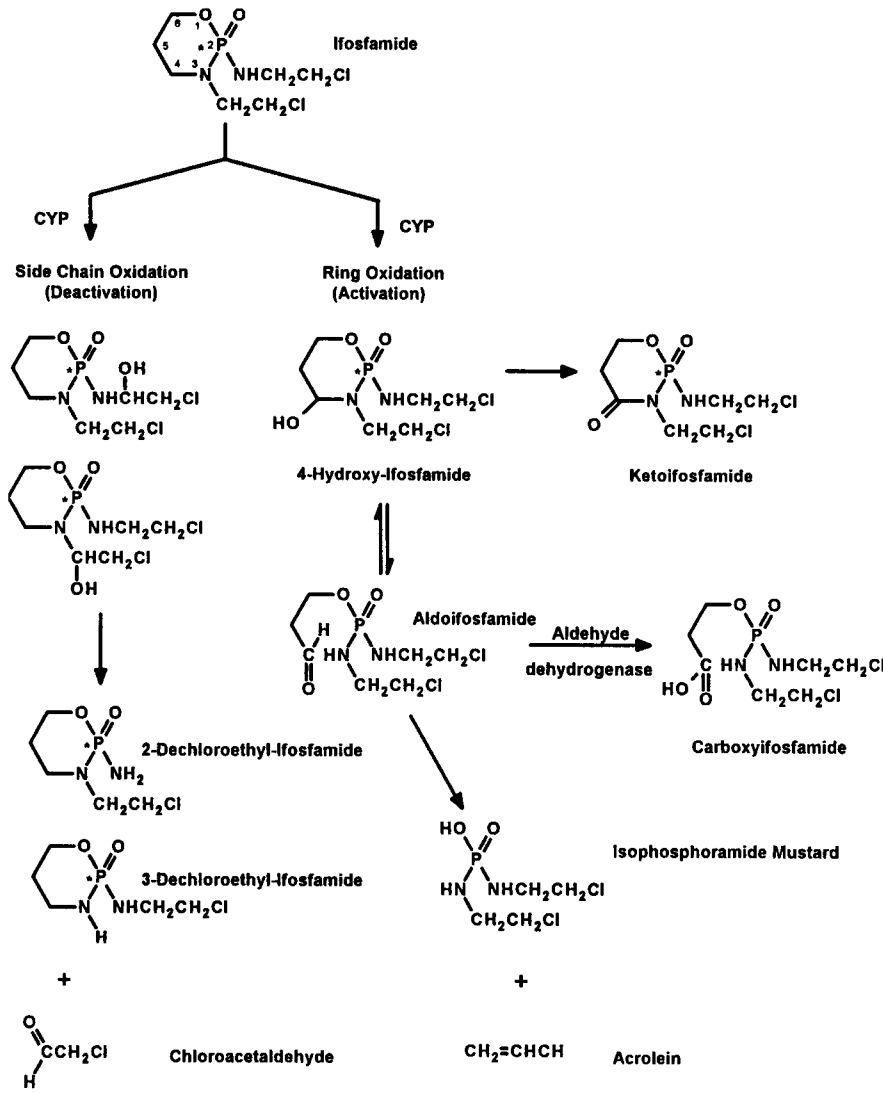


Fig. 1 The metabolic pathways of IFF

levels of (*R*)-IFF than (*S*)-IFF while the concentrations of dechloroethylated metabolites from (*S*)-IFF were higher than those coming from (*R*)-IFF [17]. These results have been confirmed by subsequent studies in humans [3, 19] and animals [12].

The complete pharmacokinetics of (*R*)-IFF and (*S*)-IFF as well as the enantiomers of 2-DCE-IFF and 3-DCE-IFF have not been described in human adults. We report here an investigation of the plasma profiles of these compounds in 14 female patients with recurrent pelvic carcinoma who received (*R*, *S*)-IFF as their sole anticancer agent.

Patients and methods

Patients

The study protocol was approved by the McGill University Ethics Committee. Written informed consent was obtained from each patient before entering the study protocol. A group of 14 female patients suffering from histologically proven recurrent squamous pelvic carcinoma with a life expectancy of at least 16 weeks were entered into the study. Eligibility criteria included non-pregnancy, over 18 years of age, no adjuvant chemotherapy or radiotherapy for at least 4 weeks, measurable disease only, no other previous malignancies except basal cell skin cancer, normal heart, adequate hepatic function (total bilirubin <1.5 mg/100 ml), adequate renal function (serum creatinine >60 cm^3/min), white blood cells >4000/mm^3, neutrophils >1800 mm^3, hemoglobin >100 g/l, platelets $>100,000$/mm^3; no evidence of CNS metastases, and performance status ECOG 0-1-2.

Drug administration.

(*R*, *S*)-IFF (Ifex ; Bristol-Myers Squibb Canada, Montréal, Québec, Canada) was given by i.v. infusion at a dose 3 g/m^2 over 3 h mixed with 2 g/m^2 of uroprotector mesna (Uromitexan; Bristol-Myers Squibb Canada) followed 3 h after the administration of IFF by 1 g/m^2 mesna over 60 min.

Concomitant medications

Prior to (*R*, *S*)-IFF infusion, antiemetic therapy with either oral prochlorperazine (10–20 mg) and/or i.v. dimenhydrinate (50–75 mg)

Table 1 Mean (± SEM) pharmacokinetic parameters of (*R*)-IFF and (*S*)-IFF following a 3-h infusion in 14 cancer patients treated with 3 g/m² rac-IFF

Parameter	Tot-IFF	(*R*)-IFF	(*S*)-IFF	(*R*/*S*) Ratio	*P*-value[a]
$AUC_{0-\infty}$ (μM.h)	4433 ± 341	2480 ± 199	1955 ± 146	1.27 ± 0.03	< 0.001
MRT(h)	10.36 ± 1.13	11.17 ± 1.10	9.73 ± 0.88	1.20 ± 0.02	< 0.001
$t_{1/2el}$ (h)	6.58 ± 0.83	7.58 ± 0.99	6.03 ± 0.82	1.20 ± 0.02	< 0.001
(V_{dss}) (l/m²)	26.68 ± 0.98	25.68 ± 0.80	27.35 ± 0.89	0.94 ± 0.01	< 0.05
CL_{tot} (ml/min/m²)	46.59 ± 3.25	41.52 ± 2.90	52.37 ± 3.75	0.79 ± 0.02	< 0.001
CL_r (ml/min/m²)	12.78 ± 1.04	12.38 ± 0.84	12.97 ± 0.96	0.96 ± 0.02	NS
CL_{nr} (ml/min/m²)	35.36 ± 3.44	30.20 ± 2.70	41.40 ± 3.55	0.73 ± 0.02	< 0.001

[a] Student's *t*-test for paired data, (*R*)-IFF vs (*S*)-IFF

was used, supplemented with i.v. dexamethasone (20 mg). Lorazepam (1 or 2 mg s.l.) was used as required. After (*R*, *S*)-IFF infusion, patients received two i.v. injections of 50–75 mg dimenhydrinate 4 h apart, with additional injections every 4 h if needed.

Blood collection

Venous blood samples (10 ml) were collected into heparinized tubes at time zero (preinfusion) and at 1.3, 2.5, 3.3, 4, 5, 7, 9, 13, and 27 h after the start of the infusion. Plasma was separated by centrifugation at 1000 *g* for 10 min. Samples were stored frozen at −80° C until analysis.

Urine collection

Total urinary excretion was collected for 6 h intervals up to 27 h after the start of the infusion. The volumes collected were measured and 50-ml aliquots were stored at −80° C until analysis.

Sample preparation

Urine and plasma concentrations of the enantiomers of IFF and its dechloroethylated metabolites were determined using a previously reported enantioselective gas chromatographic-mass spectrometric (GC-MS) method [11]. Briefly, 100 μl plasma or alkalinized urine were spiked with the internal standard (IS) cyclophosphamide and extracted with 3 ml chloroform, vortex-mixed for 1 min and centrifuged at 1000 *g* for 10 min. The organic phase was evaporated to dryness, the residue dissolved in 100 μl methanol and 1 μl injected into the GC-MS system.

Gas chromatography-mass spectrometry

The chromatographic separation was performed with a capillary column (8 m × 0.25 mm ID, 0.25 μm film thickness) coated with heptakis (2, 6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin. The column oven temperature was linearly programmed from 140° C to 180° C at 1° C/min. The mass spectrometer was operated in the electron-impact ionization mode at an ion source temperature of 180° C and an ionization energy of 70 eV using multiple ion monitoring. The designated ions of interest were *m/z* 211 for (*R*)-IFF and (*S*)-IFF, *m/z* 149 for 2- and 3-DCE-IFF and *m/z* 211 for the IS.

The method was linear for IFF concentrations ranging from 0.48 to 383 μ*M* of each enantiomer and from to 0.63 to 505 μ*M* for each enantiomer of 2-DCE-IFF and 3-DCE-IFF. The recoveries for the enantiomers of IFF and its dechloroethylated metabolites ranged between 80% and 106%. Intra-day and inter-day coefficients of variation were less than 8%. Plasma concentrations of the enantiomers of IFF, 2- and 3-DCE-IFF were expressed in micromoles.

Pharmacokinetic analysis

Plasma concentrations of (*R*)-IFF and (*S*)-IFF were determined using a noncompartmental approach. The area under the plasma concentration vs time curves (AUC) was estimated using the trapezoidal rule [10]. The elimination rate constant (ß) was determined by linear regression of the terminal portion of the concentration vs time curve using at least four data points. The elimination half-life ($t_{1/2el}$) was calculated from 0.693/ß. The mean residence time (MRT) was calculated from AUMC/AUC. The systemic clearance Cl_{tot} was calculated from Dose/AUC. The volume of distribution at steady state Vd_{SS} was calculated from $Cl_{tot} \times MRT$. The renal clearance (Cl_R) was determined by the fraction excreted in the urine over 27 h divided by the corresponding AUC. The nonrenal clearance (Cl_{NR}) was calculated from ($Cl_{tot} - Cl_R$). The extent of IFF dechloroethylation was assessed by calculating the AUC (0–27 h) of the dechloroethylated metabolites.

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Kinetic parameters for each enantiomer were compared using Student's *t*-test for paired data. All *P*-values < 0.05 were considered statistically significant.

Results

The clinical pharmacokinetics of (*R*, *S*)-IFF have been studied after single or multiple doses using nonstereoselective assays [2, 13, 14, 16]. In order to compare the results of this study with those of previous investigations, the plasma concentrations of (*R*)-IFF and (*S*)-IFF at each time point were combined to yield total IFF (Tot-IFF) levels. New plasma concentration vs time curves were constructed for each patient and the pharmacokinetic parameters calculated (Table 1). The half-life of Tot-IFF (6.6 h) was similar to previously published data (2.8–14.2 h) [8, 14, 16] as were Cl_{tot} (47 [this study] vs 31–148 ml/m² per min [14]) and Cl_R (13 [this study] vs 2.54–50.20 ml/m² per min [16]). These results indicate that the findings in this study are consistent with previously reported IFF pharmacokinetic parameters.

Stereoselective disposition of IFF

The mean plasma concentration vs time curves of the enantiomers of IFF until 27 h for the 14 patients

included in the study are presented in Fig. 2. For each patient and at all time points, (*R*)-IFF concentrations were higher than those of (*S*)-IFF. At first, (*R*)-IFF plasma concentrations were only slightly higher, but after 27 h they were twice those of (*S*)-IFF. Indeed, the mean ratio of (*R*)-IFF to (*S*)-IFF was 1.07 ± 0.01 (range 1.01–1.14) at the end of infusion and increased to 2.35 ± 0.19 (range 1.54–3.67) after 27 h. Consequently, the AUCs for (*R*)-IFF were 1.27 ± 0.03 times higher than for (*S*)-IFF (Table 1). Overall, (*R*)-IFF represented 56 ± 0.49% of Tot-IFF AUC compared with only 44 ± 0.5% for (*S*)-IFF.

The mean pharmacokinetic parameters describing the fate of each enantiomer as well as the mean (*R*/*S*) ratios are summarized in the Table 1. (*R*)-IFF had a significantly longer half-life and mean residence time than (*S*)-IFF, approximately 8 vs 6 h and 11 vs 9 h, respectively, partially due to lower (*R*)-IFF total clearance (42 vs 52 ml/m^2 per min). The volume of distribution at steady state of (*R*)-IFF was significantly lower than that of (*S*)-IFF (25.67 ± 0.80 vs 27.35 ± 0.89 l/m^2), although the difference between the enantiomers was only 6 ± 1.4%. For both enantiomers, nonrenal excretion was responsible for most of their elimination from plasma. Renal clearances accounted for only 24% to 30% of the total clearance of (*S*)- and (*R*)-IFF, respectively. In addition, IFF renal clearance was not stereoselective, 12.38 (*R*) vs 12.97 (*S*) ml/m^2/min. Nonrenal clearance, however, was highly stereoselective, (*R*)-IFF being cleared significantly more slowly that (*S*)-IFF, 30.20 vs 41.40 ml/min/m^2.

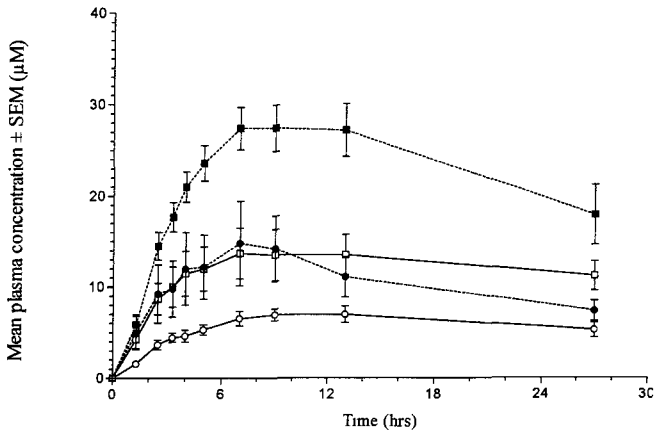
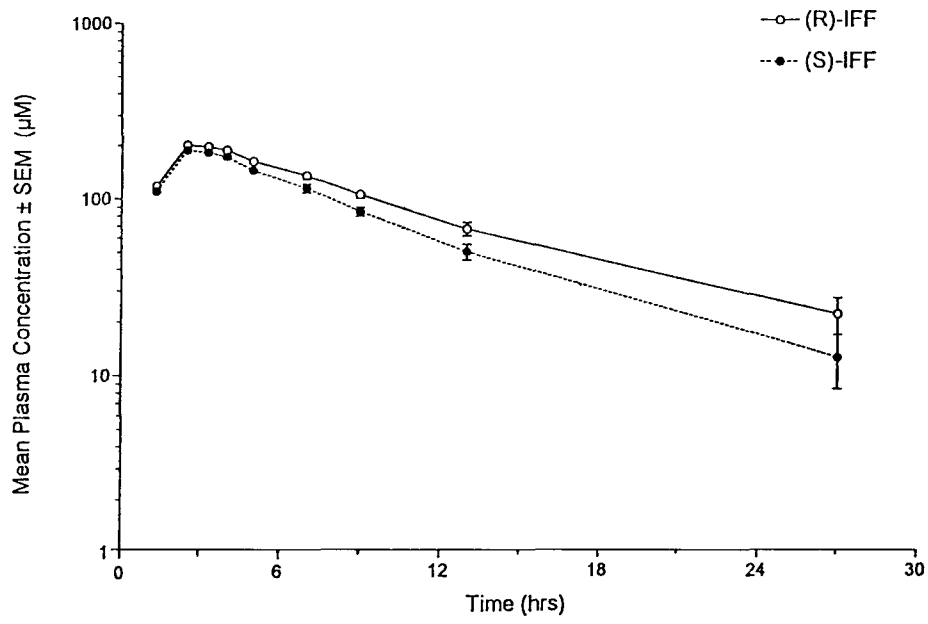


Fig. 3 Mean plasma concentration vs time curves of DCE-IFF metabolites following a 3-h infusion in 14 cancer patients treated with 3 g/m^2 (*R*,*S*)-IFF. *R*-IFF produces (*R*)-2-DCE-IFF (○) and (*S*)-3-DCE-IFF (□) while (*S*)-IFF produces (*S*)-2-DCE-IFF (●) and (*R*)-3-DCE-IFF (■)

Stereoselective disposition of 2-DCE-IFF and 3-DCE-IFF

The mean plasma concentration vs time curves of the enantiomers of 2-DCE-IFF and 3-DCE-IFF are presented in Fig. 3. The results demonstrate that 3-dechloroethylation predominated over 2-dechloroethylation with the combined AUCs of the 3-DCE-IFF metabolites being much higher than those of the 2-DCE-IFF metabolites (Table 2). The data also indicate that the dechloroethylations were stereoselective. The AUC of (*S*)-2-DCE-IFF was significantly greater than that of (*R*)-2-DCE-IFF and a statistically significant difference was also observed between the AUCs of (*R*)-3-DCE-IFF and (*S*)-3-DCE-IFF. In analyzing metabolite data, it is important to recognize that (*S*)-IFF produces (*S*)-2-DCE-IFF and (*R*)-3-DCE-IFF while (*R*)-IFF produces (*R*)-2-DCE-IFF and

Fig. 2 Mean plasma concentration vs time profiles of (*R*)-IFF and (*S*)-IFF following a 3-h infusion of 3 g/m^2 (*R*, *S*)-IFF in 14 cancer patients



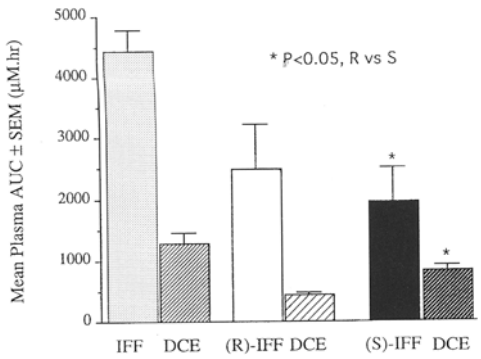


Fig. 4 Mean (AUC) of IFF and its DCE metabolites following a 3-h infusion of 3 g/m^2 (*R*, *S*)-IFF in 14 cancer patients

(*S*)-3-DCE-IFF, the apparent change in stereochemical configuration being a result of the Cahn–Ingold–Prelog nomenclature system.

The faster clearance of (*S*)-IFF was evidenced by the higher levels of dechloroethylated metabolites originating from this enantiomer. The plasma concentrations of (*R*)-3-DCE-IFF were greater than (*S*)-3-DCE-IFF and the same trend was observed for (*S*)-2-DCE-IFF compared to (*R*)-2-DCE-IFF (Fig. 3). The magnitude of the difference is demonstrated in Fig. 4. The combined AUCs of the metabolites stemming from (*S*)-IFF were equivalent to 47% of the AUC of (*S*)-IFF itself, while the combined AUCs of the metabolites arising from (*R*)-IFF accounted for only 20% of the AUC of (*R*)-IFF.

Discussion

The findings of the present study together with previously published data [3, 8, 9, 17–19] demonstrate stereoselective differences in the disposition of the enantiomers of IFF. The mechanism of this stereoselectivity between (*R*)-IFF and (*S*)-IFF can be explained by different rates of the metabolism, through side-chain oxidation or ring hydroxylation. The data from the present study clearly indicate that the dechloroethylation pathway is enantioselective and is a major factor in the observed pharmacokinetic differences between the two IFF enantiomers. This can be deduced from the increase in the mean (*R*)-IFF/(*S*)-IFF plasma concentration ratios from 1.07 to 2.35 (Fig. 2) which is coupled with the predominance of dechloroethylated metabolites from (*S*)-IFF (Figs. 3 and 4). However, this study did not address the pathway leading to the 4-hydroxylated metabolite. Studies are now in progress to determine the enantioselectivity of ring hydroxylation and to evaluate the balance between the two pathways in the disposition of each isomer.

In this study, a large interpatient variability was observed in the AUC values of both enantiomers of 2- and 3-DCE-IFF. Coefficients of variation were particularly high, at 82% and 42% for (*S*)-2-DCE-IFF and (*R*)-3-DCE-IFF, respectively, and 42% and 64% for (*R*)-2-DCE-IFF and (*S*)-3-DCE-IFF, respectively. These variations are much higher that those expected from the analytical method (8%). The same variability was observed in urine with 72%, 46%, 50% and 78% for (*S*)-2-DCE-IFF, (*R*)-3-DCE-IFF, (*R*)-2-DCE-IFF, and (*S*)-3-DCE-IFF, respectively [19]. Other authors have reported similar variability in the urinary levels of the enantiomers of the dechloroethylated metabolites [3, 17, 18]. Wide interpatient variability in the levels of dechloroethylated metabolites both in plasma and urine suggests that the CYP isoforms responsible for their formation may have different levels of expression or activity in different patients. This question has been addressed elsewhere [19].

The elucidation of the stereochemical aspects of the metabolism and disposition of chiral drugs is the key to the effective use of these agents. In the case of IFF, the results from this and related studies [19] indicate that the CYP-mediated *N*-dechloroethylation is a primary source of the observed enantioselective differences between (*R*)-IFF and (*S*)-IFF and may also be an important pathway to consider to try and avoid or reduce treatment-associated CNS toxicities. The verification of this approach awaits a complete description of this pathway.

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