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A multi-gram-scale stereoselective synthesis of *Z*-endoxifen

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ABSTRACT

Z-Endoxifen is widely regarded as the most active metabolite of tamoxifen, and has recently demonstrated a 26.3% clinical benefit in a phase I clinical trial to treat metastatic breast cancer after the failure of standard endocrine therapy. Future pharmacological and pre-clinical studies of Z-endoxifen would benefit from reliable and efficient synthetic access to the drug. Here, we describe a short and efficient, stereoselective synthesis of *Z*-endoxifen capable of delivering multi-gram (37 g) quantities of the drug in >97% purity with a *Z/E* ratio >99% after trituration.

Z-4-hydroxy-*N*-desmethyltamoxifen, or *Z*-endoxifen,[1] is an active metabolite of the pioneering anti-breast cancer prodrug, tamoxifen.[2] During phase I metabolism, tamoxifen is oxidized by hepatic membrane-bound cytochrome P450 enzymes (CYP) into at least twenty-two structurally unique metabolites,[3,4] among which, *Z*-4-hydroxytamoxifen and *Z*-endoxifen (Fig. 1) display the strongest antiestrogen effect. *Z*-4-hydroxytamoxifen and *Z*-endoxifen are similar in terms of their biochemical and cellular profiles.[5] For instance, both compounds bind with similarly high affinity to the ligand binding domain of the estrogen receptor (ER) – the molecular target of ER-dependent of breast cancer – thereby inducing a conformational change in the protein that favors co-repressor recruitment and down regulation of gene expression.[6] However, the pharmacological profiles of both compounds are quite different; in one report, the steady-state plasma serum concentration of *Z*-endoxifen in humans was reported to be fivefold higher than for *Z*-4-hydroxytamoxifen,[7] Such findings have led multiple research groups to investigate *Z*-endoxifen as the metabolite most responsible for the therapeutically beneficial effects of tamoxifen in patients.[8,9] For example, the National Cancer Institute (NCI) in the US is currently running a randomized phase II clinical trial of *Z*-Endoxifen Hydrochloride in parallel study with tamoxifen citrate for the treatment of advanced or metastatic, ER+ HER2- breast cancer (ClinicalTrials.gov Identifier: NCT02311933). Furthermore, Atossa Genetics (http://www.atossagenetics.com/) are pursuing clinical studies of oral and topical formulations of endoxifen for the treatment of tamoxifen refractory breast cancer. Recently, a phase I trial demonstrated a 26.3% clinical benefit for *Z*-endoxifen against metastatic breast cancer after the failure of standard endocrine therapy.[10]

The emergence of *Z*-endoxifen as a candidate anti-breast cancer drug is timely given the ongoing global challenge of tamoxifen resistance.[11] A well-documented cause of tamoxifen resistance is the existence of >75 alleles of CYP2D6 with varying metabolizing activity. Some ethnic cohorts carry 'null" alleles that do not express CYP2D6 or express inactive forms of CYP2D6,[12] which has been linked to reduced blood serum concentrations of *Z*-endoxifen and potentially reduced therapeutic benefit in these patients. To complicate matters, selective serotonin uptake inhibitors (SSRIs) such as fluoxetine and paroxetine, co-prescribed with tamoxifen to alleviate hot flashes – a side-effect of tamoxifen therapy – are known to potently inhibit CYP2D6 thereby lowering blood serum levels of *Z*-endoxifen akin to a phenotype observed in the case of poorly metabolizing CYP2D6 alleles. Direct administration of pure

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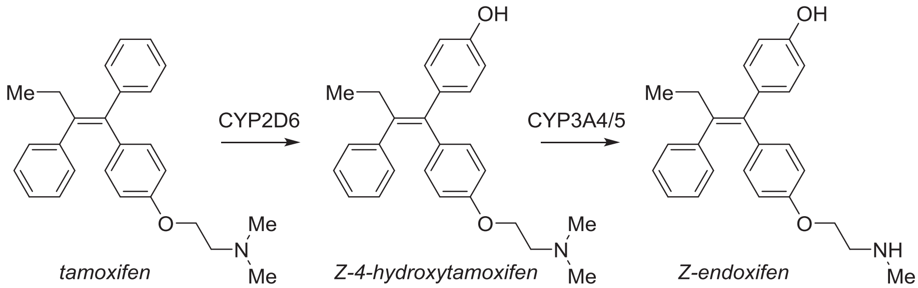

Figure 1. Chemical structures of tamoxifen and metabolites *Z*-4 hydroxytamoxifen and *Z*-endoxifen, and the metabolic relationship between the three compounds.

Z-endoxifen is therefore a promising approach to overcome genotypical tamoxifen resistance and undesirable drug interactions. Besides breast cancer therapy, *Z*-endoxifen is also being investigated as PKC inhibitors for the treatment of biopolar disorder[13,14] and potential bone protective properties in postmenopausal breast cancer patients.[15]

Collectively, a short scalable stereoselective synthesis of *Z*-endoxifen would potentially be of benefit to the pharmacology field by improving the tailoring of tamoxifen treatment,[16,17] and enabling wide-scale pharmacological profiling of the drug, ranging from the mg-quantities needed for biochemical and cellular testing to the grams needed for animal studies. Two independent syntheses of Z-endoxifen have been reported.[14,18] During one route, the tetra-substituted olefin is generated as a 1/1 mixture of *E/Z* isomers, which are separable by RP-HPLC.[14] The second route is stereoselective and founded on an earlier synthesis of *Z*-4-hydroxytamoxifen published by Gauthier and Labrie et al. (Fig. 2). Here, the synthesis began with mono-protection of **1** with a pivaloyl (Piv) group to form **2**, followed by an *E*-selective olefination reaction between **2** and propiophenone under reducing metal McMurry conditions to generate **3** in a >100/1 *E/Z* ratio after trituration in MeOH. At this juncture, Fauq et al. efficiently steered the synthesis toward *Z*-endoxifen via first an *O*-alkylation reaction between **3** and alcohol **4** under Mitsunobu conditions to generate the protected precursor of *Z*-endoxifen, **5**, importantly with retention of stereopurity (Fig. 2). The synthesis of *Z*-endoxifen concluded with a one-pot deprotection of the pivaloyl and ethyl carbamate protecting groups using an excess of MeLi at —78 °C. Some stereorandomization of *Z*-endoxifen was reported to occur during these final deprotection and isolation steps resulting in accumulation of 30% of the *E*-isomer, which was nonetheless successfully separated by RP-HPLC. For our purposes, we sought a synthetic entry to isomerically pure *Z*-endoxifen, which was equally short and efficient but not reliant on RP-HPLC, and which ideally circumvent the use of highly reactive MeLi, given the need for gram quantities of the drug. We were also keen to understand more about the adventitious isomerization of *Z*-endoxifen and sought ways to overcome this.

Our synthesis efforts began by following the synthetic route to precursor **5** reported by Fauq et al. (Fig. 2). When we subjected **5** to the reported MeLi-mediated deprotection conditions, we did not detect any significant loss of stereopurity of the crude *Z*-endoxifen immediately after work-up and prior to silica gel column chromatography, as judged by ^{1}H NMR in *d6*-DMSO (circ. 95/5 *Z/E*; Figure S3, top panel). However, when we then purified the crude by silica gel column chromatography using 15% MeOH in CH_2Cl_2 – as described in the supporting information provided by Fauq et al. – and remeasured the ^{1}H NMR in *d6*-DMSO, we did observe some stereorandomization, specifically 22% formation of the *E*-isomer as judged by ^{1}H NMR (Figure S2 and S3, bottom panel) and HPLC analysis (Figure S5, left panel). Our finding suggested that *Z*-endoxifen is unstable to the acidic conditions of the silica gel column chromatography but, in our hands, stable to the basic cleavage and mildly acidic work-up conditions. This finding is supported by an earlier stereoselective synthesis of *Z*-4-hydroxytamoxifen,[19] in which Gauthier and Labrie use similar basic reaction conditions (MeLi) to cleave a pivaloyl protecting group from *Z*-4-hydroxytamoxifen. Here, a 66% yield and *Z/E*-product ratio of >100:1 is reported after near identical work-up conditions used for this present synthesis of Z-endoxifen i.e. quench with saturated

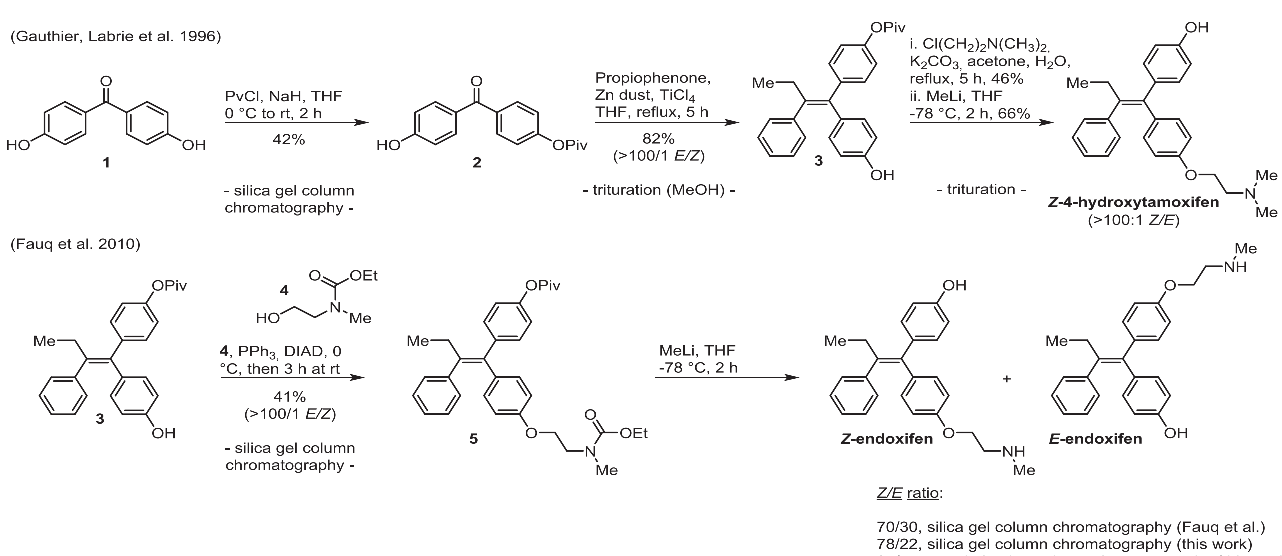

Figure 2. Overview of Gauthier and Labrie's stereoselective synthesis of *Z*-4-hydroxytamoxifen & Fauq's stereoselective synthesis of *Z*-endoxifen.