Pharmacelsus®
CONTRACT RESEARCH ORGANISATION

In Vitro Evaluation of Aromatase Inhibitors

Purpose

The selective inhibition of estrogen production with aromatase inhibitors has been demonstrated to be an effective and safe strategy for the treatment of hormone-dependent metastatic breast cancer. Anastrozole and letrozole have recently been approved as first-line agents in women with metastatic breast cancer.
The first step in the evaluation process of new aromatase inhibitors is the determination of the inhibitory potency *in vitro.*

Assay protocol

Microsomes derived from fresh human placenta or rat ovaries are used as source of the enzyme. Incubations are performed at 37ºC containing [1β-^{3}H-androstenedione, excess NADPH, protein, and either inhibitors or substrate at different concentrations. Control incubations are also performed without any enzyme. The reaction is started with the addition of protein and stopped after 20min with the addition of organic solvent. Following extraction, the organic layer is removed and the tritiated water formed during the aromatization reaction is measured by liquid scintillation counting. Counts from control incubations without enzyme are subtracted from each incubations counts. Results are plotted in a Dixon plot revealing directly the corresponding IC_{50} values. For enzyme kinetic studies Lineweaver-Burk plots are recorded.

Model validation

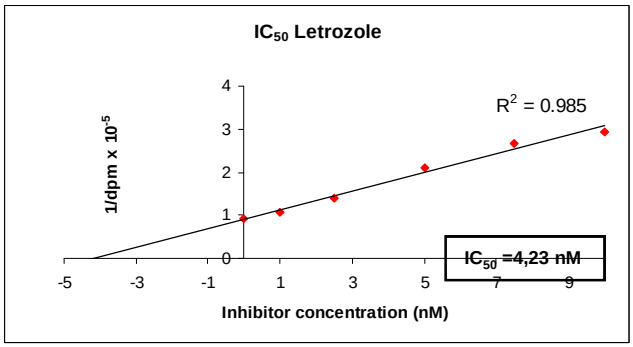

Figure 1: Dixon plot for the IC_{50} value of letrozole

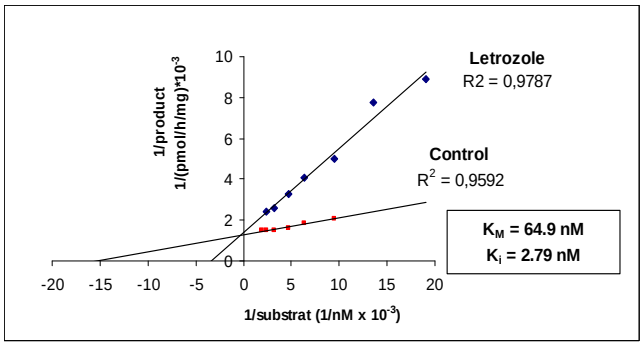

Figure 2: Lineweaver-Burk plot for the k_i value of letrozole

The inhibitory potency of letrozole, a well-known aromatase inhibitor of the third generation was tested by determining the IC_{50} and the k_i value. Both results were in good accordance with data from the literature.

The results of one of our aromatase inhibitor projects has recently published together with our sponsor Novartis Pharma:

Proc AACR **44**:Abstract 539, p123, 2003:
C Batzl-Hartmann, DB Evans and AS Bhatnagar: Comparative aromatase enzyme kinetic studies on fadrozole, formestane, letrozole, anastrozol and exemestane.

Next test step provided

Potent aromatase inhibitors *in vitro* can be further tested ***in vivo*** :

Model: Drug efficacy/potency on the androstenedion -induced uterine hyperplasia

Please don't hesitate to contact us for a customized quotation

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