In Vitro Aromatase (CYP19A1) Assay

Need

Endocrine disruption is of regulatory importance globally and is a primary consideration in determining the safety and/or efficacy of a dermatological drugs, cosmetics, chemicals, and agrochemicals. The aromatase enzyme (CYP19A1) catalyzes the conversion of androstenedione and testosterone to the hormones estrone and estrogen, respectively. Compound or chemical perturbation of normal aromatase activity may have adverse effects on normal development, reproductive health, and/or the integrity of the reproductive system. For new and existing drugs, cosmetics, chemicals, and agrochemicals, the cost and time necessary to assess disruption of aromatase *in vivo* is prohibitory to efficient product development and safety assessment.

Solution

LifeNet Health LifeSciences offers the Aromatase assay for the assessment of potential interference of aromatase activity by test compounds or chemicals. In the Aromatase assay, the CypExpress™ Aromatase model is used. This model is a permeabilized and stabilized dried yeast powder preparation containing full length, unmodified, human CYP19A1 and recombinant human NADPH oxidoreductase. CypExpress™ Aromatase retains the cellular mechanisms to provide the aromatase enzyme with the energy and cofactors to continue to function for long term experiments and can generate larger amounts of metabolite than mammalian microsomes or other genetically engineered expression systems. Compounds are prepared in assay buffer with the necessary cofactors for aromatase activity (i.e., NADPH) and are added to the CypExpress™ Aromatase model (containing enzyme and androstenedione). The activity of aromatase is then determined by assessing the production of estrone via LC-MS/MS at the conclusion of the assay.

Accurate & reliable data

Fast turnaround times

Unsurpassed expertise

Collaborative approach

LifeNet Health® LifeSciences

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Standard Protocol

ASSAY PARAMETERS	PROTOCOL
Model	CypExpress™ Aromatase (CYP19A1)
Replicates	3
Solvent of Choice	DMSO (preferred), sterile water, or assay buffer
Test Article Formulation	1000, 100, 10, 1, 0.1, 0.01, and 0.001 µM (depending on solubility)
Solvent Controls	DMSO, sterile water, or assay buffer
Positive Control	4-hydroxyandrostenedione
Background Control	No enzyme
Exposure Time	1 hour
Estrogen Concentration	LC-MS/MS
Time to Complete	4-6 weeks from test article receipt
Regulatory	Non-GLP or GLP
Deliverables	Full Report including: IC_{50} (if possible) and categorization (positive, negative, or equivocal for aromatase inhibition).

Key References

Khan et al. (2011) Potential utility of natural products as regulators of breast cancer-associated aromatase promoters. Reproductive Biology and Endocrinology 9(91).

Figure 1. The aromatase pathway
(from Khan et al. 2011)

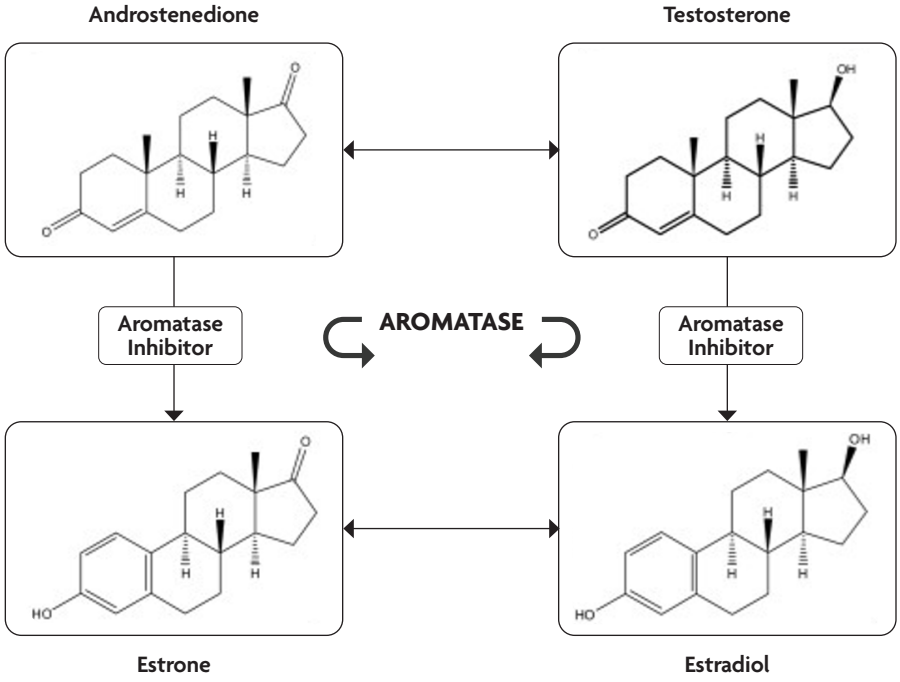

Figure 1. The steroidogenesis pathway

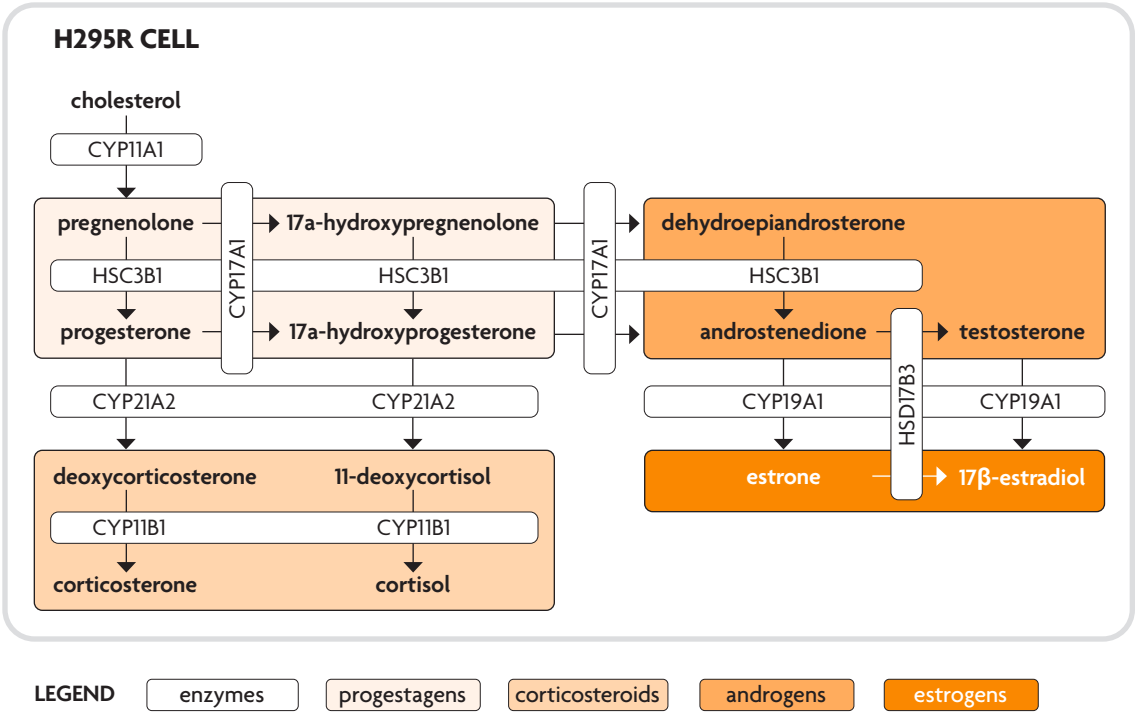

Figure 2. Positive and Negative decision criteria