OXIDIZED METABOLITES AND UNUSUAL CONJUGATES OF AZD9496 FORMED BY MICROBIAL BIOTRANSFORMATION

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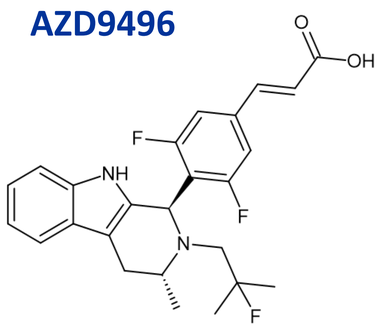
Abstract: AZD9496 is an orally active selective estrogen receptor degrader (SERD) whose clinical development was discontinued by AstraZeneca for treatment of estrogen receptor-positive breast cancer in favor of AZD9833, which is currently in clinical trials. CYP2C8 is largely responsible for biotransformation of AZD9496 to two hydroxylated metabolites observed in human liver microsomes and plasma; M3 (major) and M5 (minor). The two metabolites are diastereoisomers but whose configuration is unknown.[1] Screening of AZD9496 using microbial biotransformation and PolyCYPs® enzyme incubations resulted in the formation of multiple derivatives. A total of 15 derivatives were isolated. Of the oxidized derivatives produced, 2 active metabolites were confirmed as the human metabolites M3 and M5. Analysis of the microbial biotransformation reactions also revealed the formation of a number of conjugates with unusual mass additions, as well as a glucuronide. Three microbial strains were scaled up to generate sufficient material of the unknown conjugates for purification and structure elucidation by NMR. All three strains produced conjugates subsequently identified as amino acid adducts, including glycine, alanine, serine and glutamine conjugates. One of these strains also produced a conjugate with an *N*-acetylcysteine addition to the indole aromatic ring with further oxidation in the same molecule, most likely to an *N*-oxide.

AZD9496



Objectives

- Exploration of susceptibility to microbial biotransformation.
- Creation of oxidized metabolites for exploration of polar SAR.
- Scale-up, purification and structure elucidation of amino acid conjugates.

Process for microbial biotransformation of AZD9496

AZD9496 screened against 23 microbes
↓
Analysis by LC-MS
↓
Scale-up of selected biotransformations
↓
Purification and structure elucidation by NMR

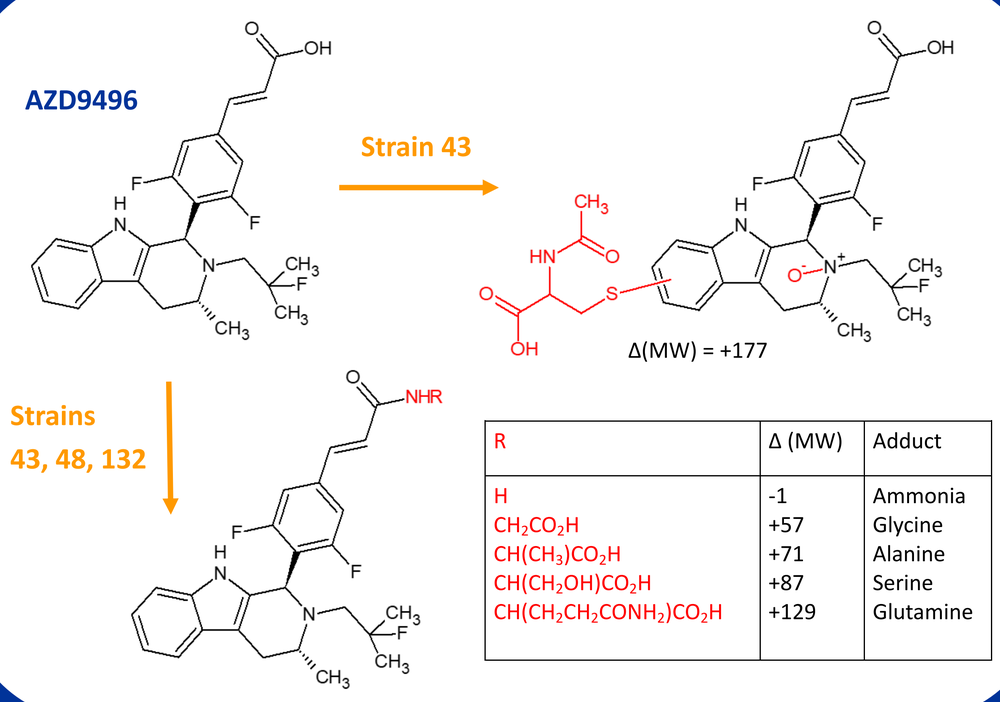
Results

- Six strains were able to biotransform AZD9496 to several oxidised metabolites including 6 hydroxylated metabolites (+16 Da), a hydrolysis product (+18 Da), and 2 dihydroxylated products (+32 Da). Several unexpected conjugated products were also produced.
- Scale-up, purification and analysis of the 2 main hydroxylated products revealed that these were active. Subsequently matching against synthesized standards at AZ confirmed identity with M3 and M5, the main metabolites of AZD9496 formed in humans by CYP2C8.
- Three strains were scaled up to provide enough material for structure elucidation of the 6 conjugates. Amounts isolated ranged from 0.8 mg to 10.6 mg of each conjugate at 91% to 98% purity (LC-UV). Most were amino acid based conjugations at the carboxyl group. A glucuronide was also detected (+176) but the structure was not elucidated. A conjugate with an *N*-acetylcysteine (NAC) addition to the indole aromatic ring in conjunction with likely *N*-oxidation (+177) was also produced at 81% purity.
- The only AZD9496 amino acid conjugate observed in hepatocyte incubations was with taurine in dog at < 1%. A glutathione conjugate was also observed in human and mouse hepatocytes incubations at < 1%.

Scale-up of AZD9496 conjugates

Broth and biomass were extracted followed by 2 rounds of purification by RP-HPLC

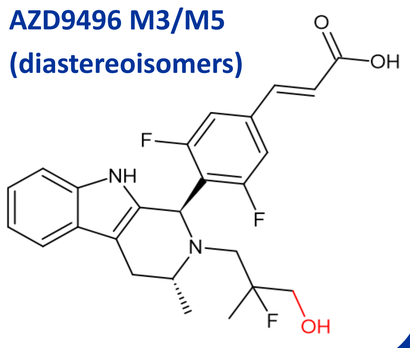
- 1L scale-up of strain 43: Produced ammonia (2.3 mg) and NAC (6.0 mg) adducts.
- 2L scale-up of strain 132: Produced glycine (10.6 mg), serine (5.1 mg) and alanine (0.8 mg) conjugates.
- 0.5L scale-up of strain 48: Produced glutamine conjugate (2.9 mg).



R	Δ (MW)	Adduct
H	-1	Ammonia
CH_2CO_2H	+57	Glycine
$CH(CH_3)CO_2H$	+71	Alanine
$CH(CH_2OH)CO_2H$	+87	Serine
$CH(CH_2CH_2CONH_2)CO_2H$	+129	Glutamine

Structures determined from interpretation of COSY and HMBC NMR spectra

AZD9496 M3/M5 (diastereoisomers)



Activity testing	ERα down regulation	ERα antagonism	ERα agonism
AZD9496	0.14 nM	0.28 nM	>3125 nM
M3 (major)	4.88 nM	8.8 nM	>3125 nM
M5 (minor)	0.38 nM	0.8 nM	>3125 nM

Conclusions / Discussion

- AZD9496 is biotransformed by microbes into several oxidized and conjugated metabolites, including the human active hydroxylated metabolites M3 and M5. M5 is more active than M3.
- The low-level susceptibility of AZD9496 to conjugation with amino acids that was observed in mammalian hepatocyte incubations (< 1% production of glutathione and taurine conjugates), was more pronounced in microbial biotransformation reactions, highlighting the potential utility of the latter for scaling up production for definitive identification by NMR.
- Subsequently, PolyCYPs enzymes 349, 353 and 359 were shown to produce a major and minor hydroxylated metabolite of AZD9496 at conversions of up to 59.6%. PolyCYPs kits can offer a quicker way to screen for oxidized metabolites compared to whole cell microbial biotransformation.

Reference

[1] Complete Substrate Inhibition of CYP2C8 by AZD9496. Tashinga E. Bapiro, Andy Sykes, Scott Martin, Michael Davies, James W. T. Yates, Matthias Hoch, Helen E. Rollison and Barry Jones. Drug Metabolism and Disposition, 2018, 46 (9) 1268-1276.

About Hypha: Hypha Discovery is a specialist CRO with expertise in the scalable synthesis, purification and identification of drug metabolites. Using our biocatalytic and organic chemistry capabilities, we also make oxidized derivatives of lead compounds and API degradation products. Hypha also has a wealth of experience in the production, purification and structure elucidation of natural products. We work with pharmaceutical and agrochemical companies globally.

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