Superior drug derivatives *via* late stage hydroxylation

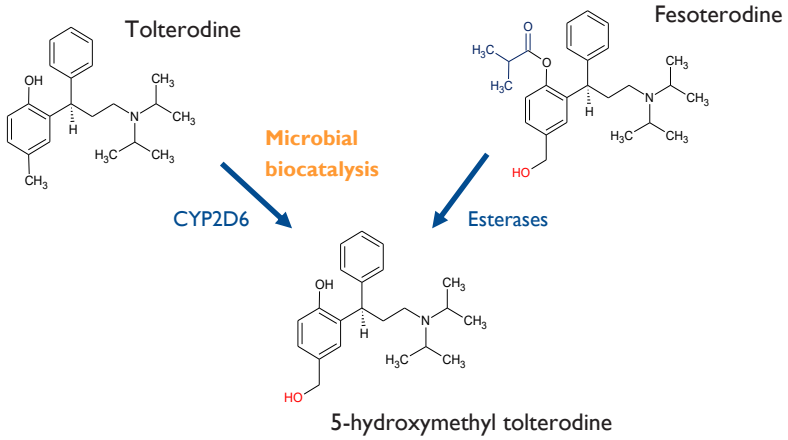
Microsomal and microbial biotransformation of drugs

There is increasing focus on thorough investigation of drug metabolites, exemplified by Pfizer's routine biocatalytic approach in which lead compounds are screened in microsomal systems and key derivatives scaled up via microbial biotransformation and/or chemical synthesis[1]. Similarly, microbial biocatalysis can also be applied at the screening stage to identify and readily scale-up hydroxylated metabolites and derivatives that may have superior properties to the original drug.

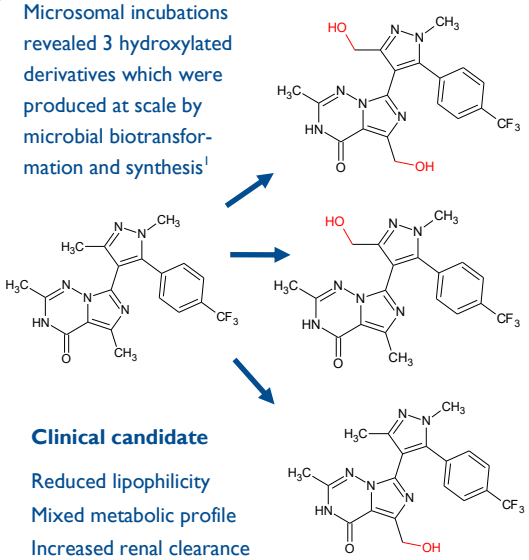
An interesting case study using knowledge of active metabolites was the development of fesoterodine as a superior drug to tolterodine, based on knowledge of the metabolism of the latter compound. Tolterodine is primarily metabolized via *N*-dealkylation or oxidation of the 5-methyl group to yield 5-hydroxymethyl tolterodine.[2] Fesoterodine is a pro-drug that is rapidly hydrolysed by plasma esterases to the active metabolite 5-hydroxymethyl tolterodine, thereby circumventing dosing issues arising from primary metabolism of tolterodine *via* CYP2D6, at the same time as removing undesirable CNS side effects.

Screening of tolterodine against Hypha's microbial panel revealed several hydroxylated derivatives. Scale-up fermentation of one strain producing the main active metabolite resulted in multi mg amounts of purified 5-hydroxymethyl tolterodine. Routine screening of lead compounds coupled with a cost-effective scalable system can thus deliver active metabolites in sufficient quantities for evaluation of properties compared to the parent compound.

Hypha is able to offer a flexible service in which oxidation products of drug leads are generated, often *via* challenging modifications of unactivated C-H bonds, either through a microsomal or microbial route, with the latter providing an immediate scale-up strategy. The activity of these derivatives can thus be evaluated as part of a wider strategy to improve metabolic stability and solubility.



Microsomal incubations revealed 3 hydroxylated derivatives which were produced at scale by microbial biotransformation and synthesis[1]



Clinical candidate

Reduced lipophilicity
Mixed metabolic profile
Increased renal clearance

Pfizer exemplified their routine late stage lead diversification approach (see summary above) with screening of a lead compound in microsomal systems, following which, microbial biotransformation was employed to produce 100s of milligrams of a hydroxylated derivative for evaluation. Through this process, a superior clinical candidate was identified with reduced lipophilicity and CYP liability and increased renal clearance. The derivative was synthesized for *in vivo* studies.[1]

Scott Obach, has recently published a further paper on this strategy at Pfizer[3] and encourages others to form multi-disciplinary collaborations to give the approach a go as, "We usually find surprises."[4]

For more information contact us at mail@hyphadiscovery.co.uk

[1]Stepan *et al.,* 2018. ACS Med. Chem. Lett. 9, 68-72
[2]Andersson *et al.,* 1998. DMD 26(6), 528-535
[3]Obach *et al.,* 2018. J.Med.Chem. 61, 3626-3640
[4]Drahl 2018. Chemical and Engineering News 96 (17)

About Hypha Discovery

Hypha Discovery Ltd is a UK-based microbial biotechnology company providing solutions to pharmaceutical and agrochemical R&D partners through the production of mammalian and microbial metabolites, as well as specialising in microbially-derived chemicals. We have an extensive client base and work with many of the top pharma and agrochemical companies worldwide.