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Abstract

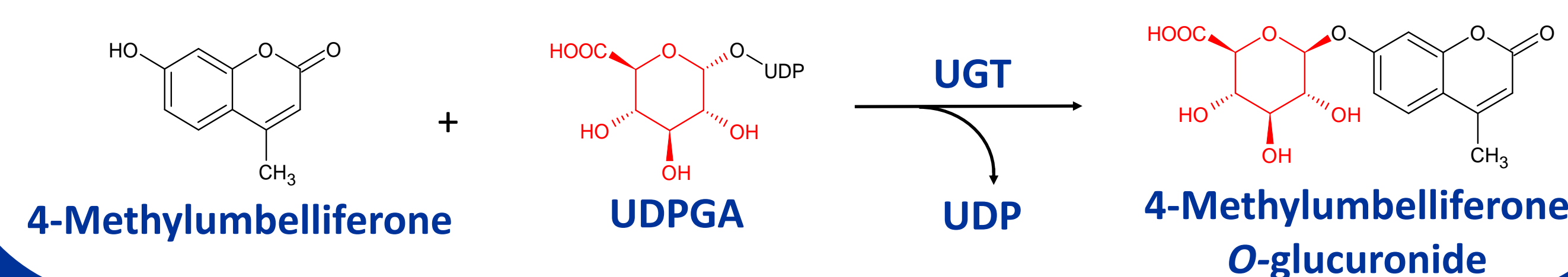
PolyUGTs[®] are novel UDP-glucuronosyltransferases cloned from selected bacteria in Hypha's biotransformation panel and developed into the PolyUGTs metabolite kit. The PolyUGTs screening kit currently contains 11 isoforms and hit reactions can be replicated using scale-up vials to obtain more material for structure elucidation and further studies. This poster illustrates application of the kit to make *O*-, *N*-, acyl and *N*-carbamoyl glucuronides of small molecule drugs.

UDP-glucuronosyltransferases

UDP-glucuronosyltransferases (UGTs) are key enzymes involved in Phase II metabolism and catalyse the transfer of glucuronic acid from the UDP-glucuronic acid (UDPGA) donor to drugs, agrochemicals and endogenous substances.

Several human UGTs are responsible for glucuronidation of small molecule drugs. Despite glucuronides rarely possessing pharmacological activity, pure material of major glucuronides are often needed for bioanalytical sampling handling studies, and for *in vitro* drug-drug interaction testing. Hypha's microbially-derived PolyUGTs are able to make glucuronides formed by various human UGTs.

Figure 1 - Glucuronidation of 4-methylumbelliferone by UGT enzymes requires sugar donor, UDPGA



About PolyUGTs enzymes

PolyUGT[®] enzymes are novel UDP-glucuronosyltransferases cloned from selected bacteria in Hypha's strain collection (Table 1). Through genome mining analysis, a total of 702 putative glycosyltransferases (GTs) were identified, including 47 putative UGTs from 11 bacterial strains.

Table 1—UGT genome mining summary

Strain No.	Putative GTs	Putative UGTs
A	65	4
B	83	6
C	62	5
D	50	1
E	73	2
F	58	2
G	58	2
H	55	5
I	65	8
K	60	5
L	73	7
Total	702	47

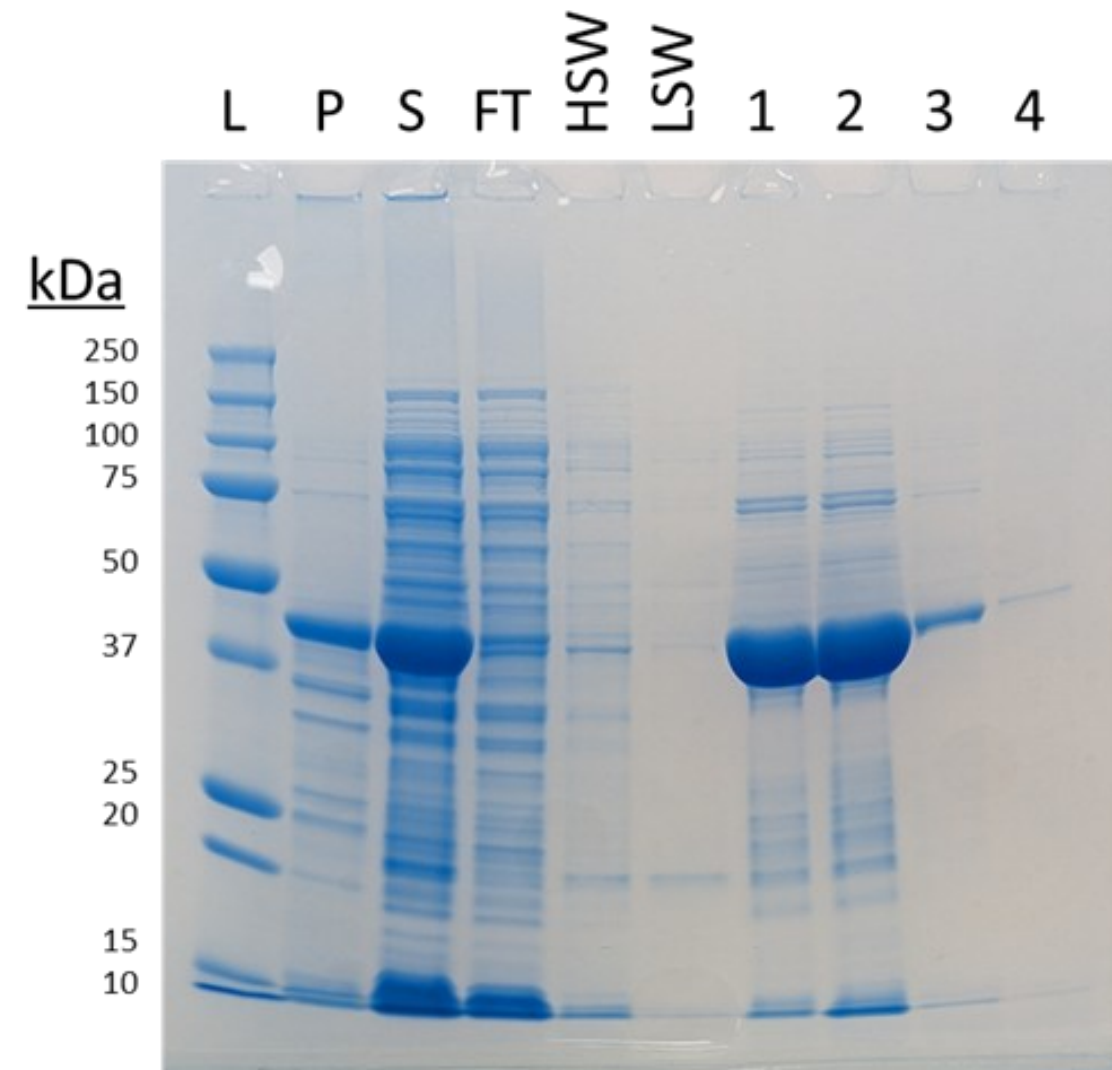


Figure 2 - PolyUGTs were purified from *E. coli* by affinity chromatography.

The purified enzymes were found to be incredibly potent and some PolyUGTs remained highly active in the micromolar range and capable of fully glucuronidating drug compounds (Fig. 3).

The PolyUGT enzymes were assessed against a substrate panel known to produce glucuronide products. The best performing enzymes were further developed and incorporated into a PolyUGT metabolite screening kit (Fig. 4).

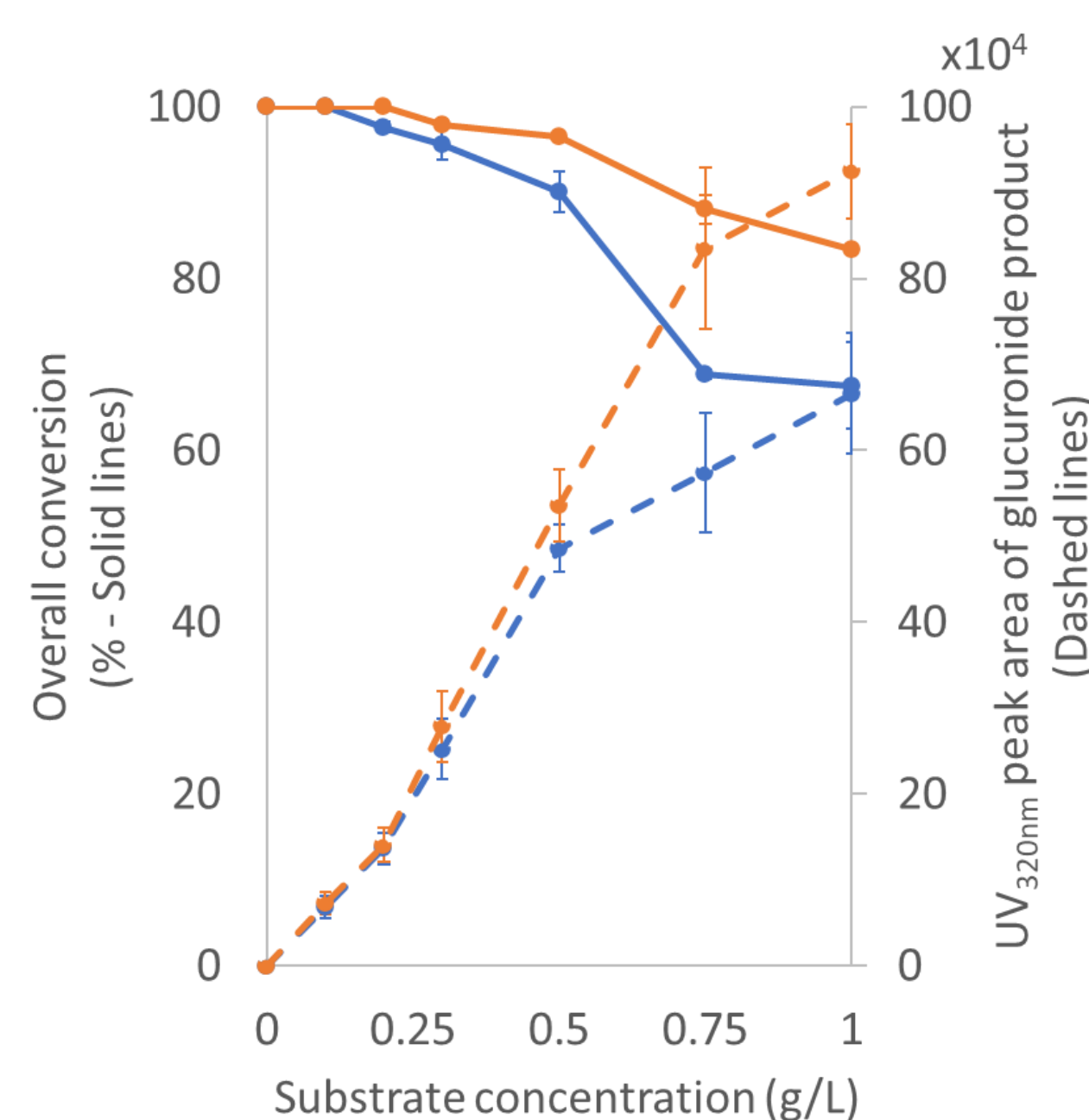


Figure 3 - Dose escalation of 4-methylumbelliferone up to 1 g/L substrate supplemented with either 5 mM (blue) or 10 mM (orange) UDPGA with 1 μ M PolyUGT 216. Overall conversion (solid lines) remained high with improved glucuronide product yields (dashed lines).

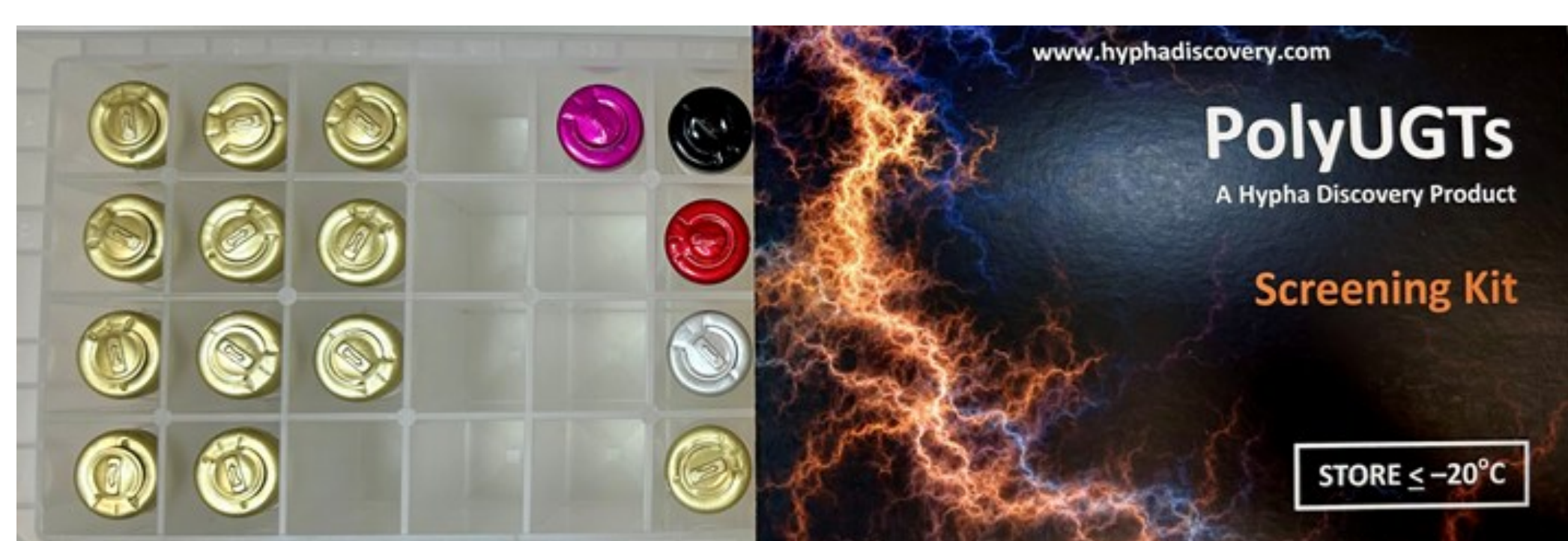
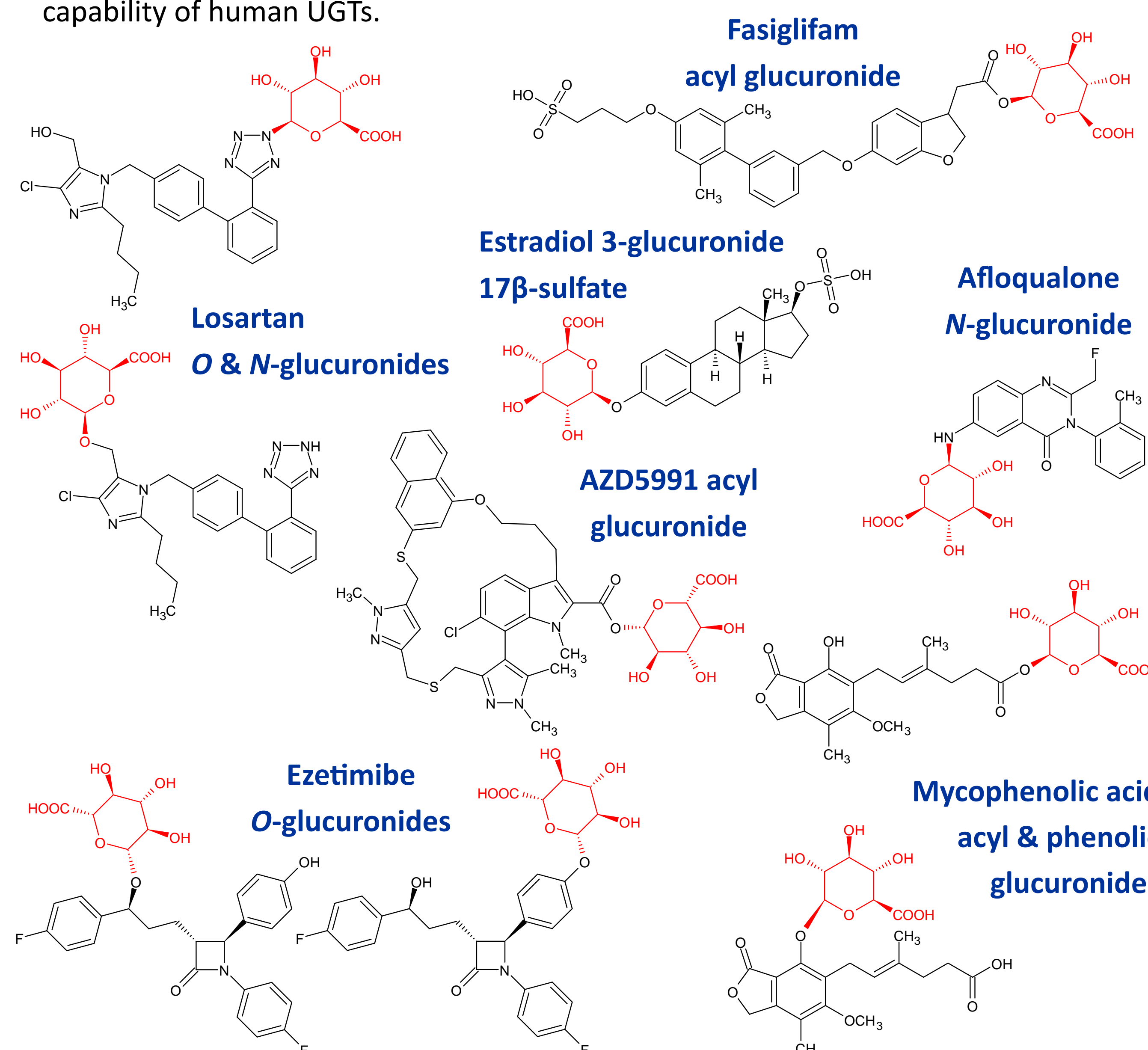


Figure 4 - PolyUGT screening kit containing 11 PolyUGT isoforms, UDPGA cofactor, positive control substrate, pH reduction vial & formulation reagent.

Examples of glucuronides produced by PolyUGTs

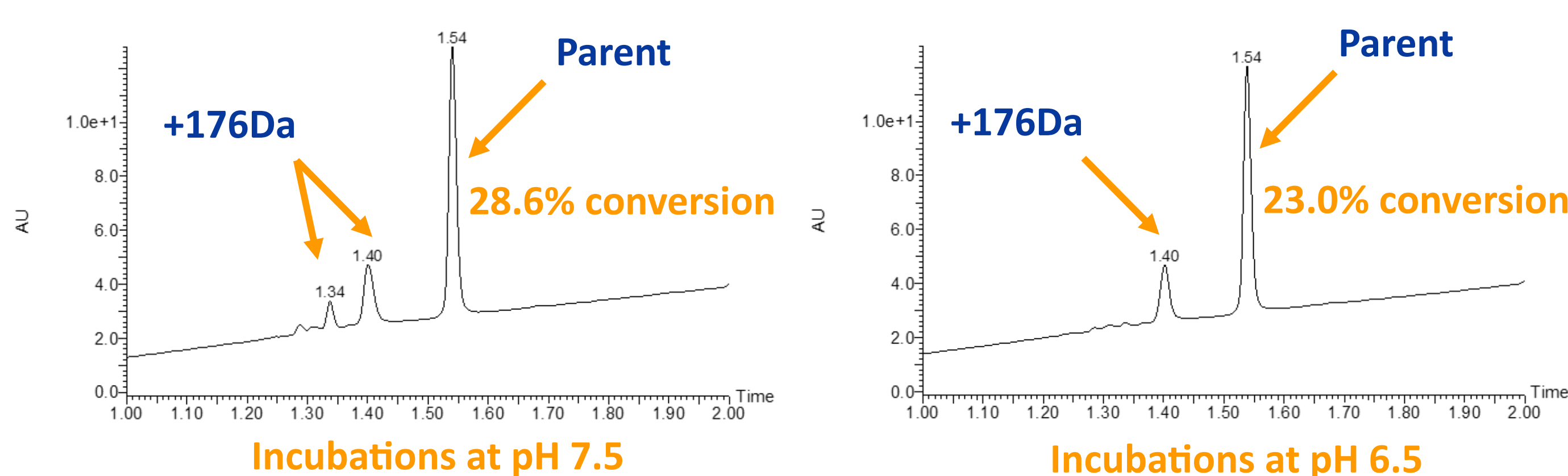
Hit reactions can be scaled-up using PolyUGT scale-up vials or by whole cell biotransformation using *Streptomyces lividans* strains expressing PolyUGT enzymes. The PolyUGTs catalyse a broad range of glucuronidation reactions mimicking the metabolism capability of human UGTs.



PolyUGTs are inverting UGT enzymes & produced the same human glucuronide product in β -configuration

Reducing migration isomers of acyl glucuronides

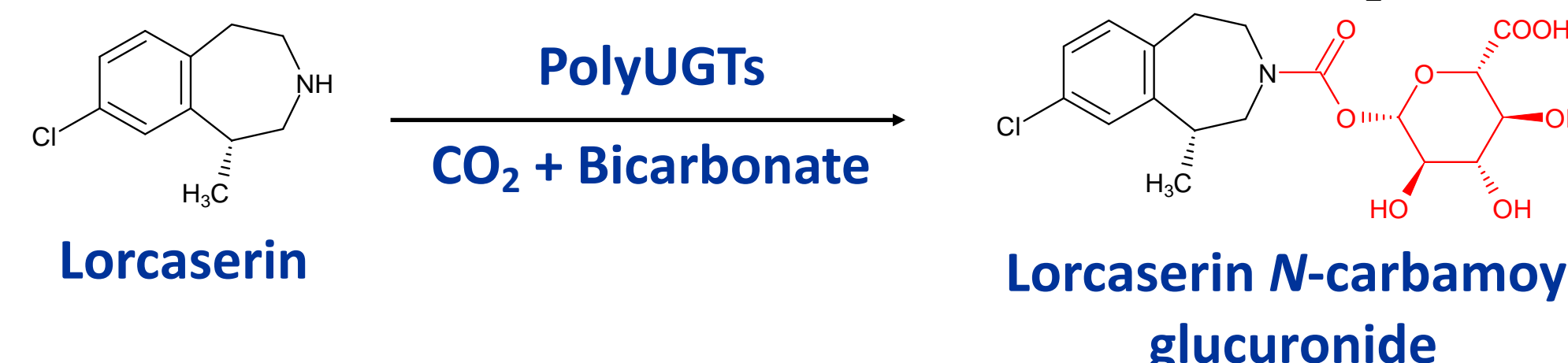
Zomepirac is a nonsteroidal anti-inflammatory drug that was withdrawn from the market due to formation of a reactive unstable acyl-glucuronide responsible for the adverse effects of drug through irreversible protein binding and immune-mediated toxicity¹.



An optional pH reduction buffer is included for when an acyl-glucuronide is anticipated. This will reduce the reaction pH from 7.4 to around pH 6.5 to help reduce formation of acyl migration isomers.

N-carbamoyl glucuronidation

Lorcaserin is a weight loss drug that activates serotonin receptors to reduce appetite, however it was withdrawn from the market due to increased occurrence of cancer. Lorcaserin *N*-carbamoyl glucuronide was successfully produced by several PolyUGT enzymes by recreating conditions described by Gunduz *et al*². Five PolyUGTs could form the *N*-carbamoyl glucuronide in the presence of bicarbonate buffer alone, achieving 15% conversion, compared to a maximum of 18% observed with bicarbonate buffer and CO₂.



Incorporation of CO₂ from bicarbonate buffer, in equilibrium with exogenous CO₂, into a carbamoyl moiety then glucuronidation to an *N*-carbamoyl glucuronide