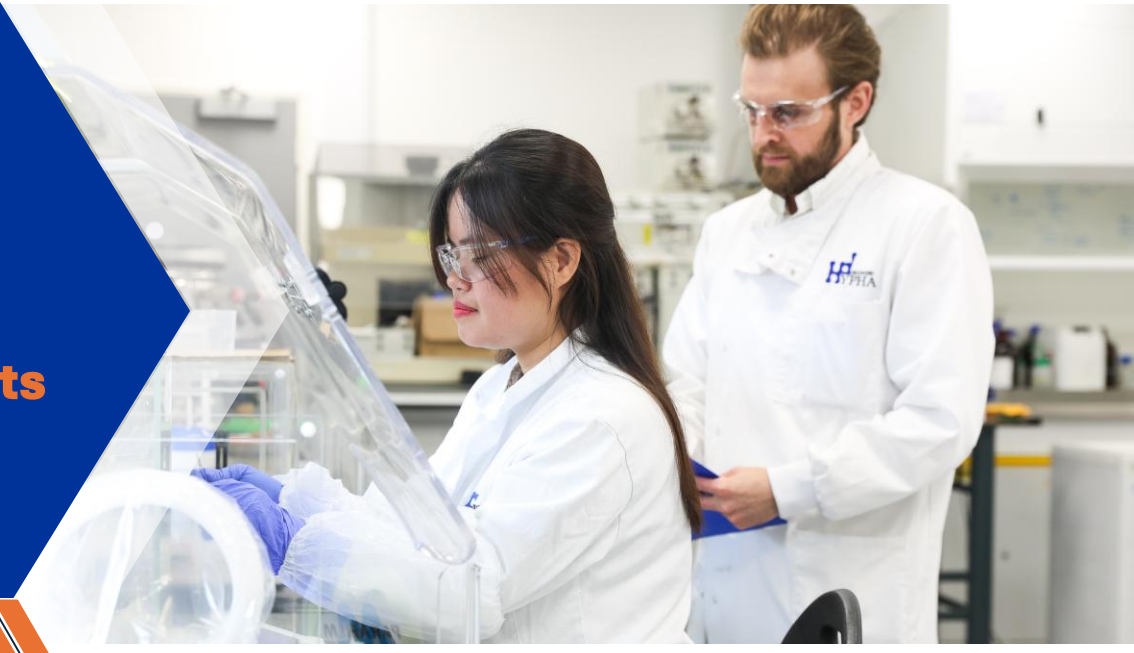
Case Study

HYPHA DISCOVERY

Metabolite Experts



Accessing a cleaved metabolite of Brimarafenib using chemical oxidation

About Brimarafenib

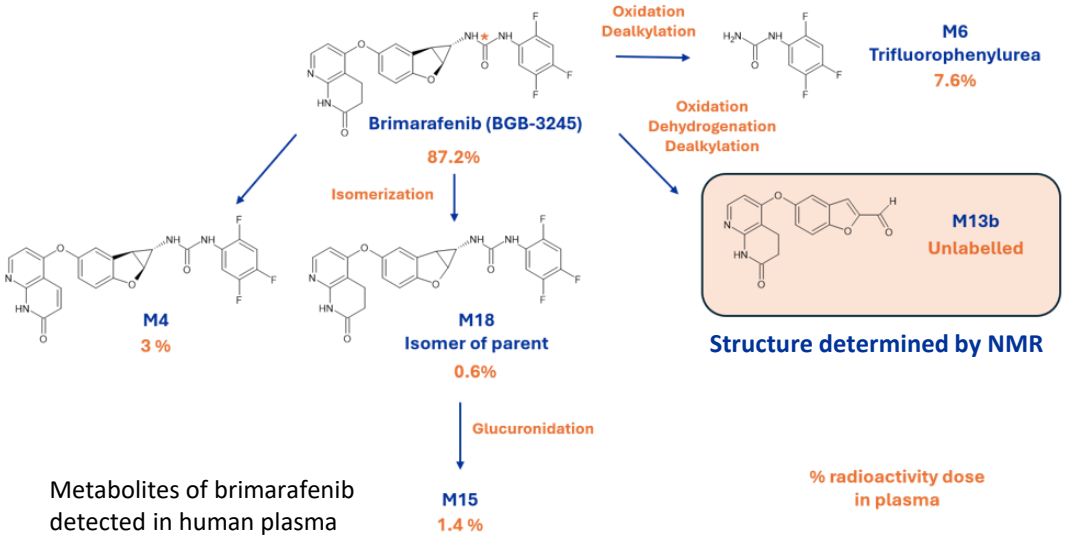
Brimarafenib (BGB-3245) is a second-generation RAF (rapidly accelerated fibrosarcoma) dimer inhibitor with activity across RAF family kinases. The drug was designed to block both the monomer and dimer forms of activating BRAF mutations thereby disrupting the dysregulated mitogen-activated protein kinase (MAPK) / extracellular signal-regulated kinase (ERK) signaling pathway. The MAPK pathway plays a role in the control of cell growth and cell survival. Changes, including mutations, in the MAPK pathway can cause the spread of cancer in patients.

First-in-human pharmacokinetic data indicates that the drug has a long terminal elimination half-life and relies on CYP3A4 for its hepatic biotransformation [1]. The liver clears the drug relatively slowly and multiple consecutive daily oral doses led to significant systemic accumulation before reaching a steady state.

Metabolism of Brimarafenib

The human radiolabelled mass balance study identified unchanged parent drug as the main circulating component at 87.2%; however, several biotransformation pathways resulted in 14 metabolites across plasma, urine and feces. Metabolic routes include oxidation (M1, M2), dehydrogenation (M4, M20), dealkylation (M6), defluorination (M5, M21), and glucuronidation (M15, M16, M17) [2].

Due to the position of the radiolabel in [14C]-brimarafenib and subsequent cleavage of the urea moiety, two of these metabolites (M13a, M13b) were unlabeled. M13a and M13b have the same molecular weight but since only M13b was circulating, a reference standard of M13b was required in order to quantify levels in plasma and for structure elucidation. M13a was detected in urine and feces only and the structure is unknown.



Metabolites of brimarafenib detected in human plasma

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Screening for M13b

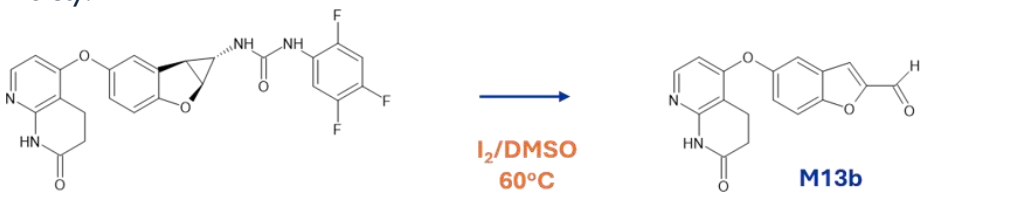
In order to identify a method suitable for scaling the production of M13b, brimarafenib was screened across a panel of biotransformation (microbes and PolyCYPs enzymes) and late-stage chemical oxidation conditions (21 chemical oxidation methods comprising peroxides, other two electron oxidants, transition metal catalysts and radical initiators, as well as three electrochemistry conditions).

Only minor amounts of both isobaric metabolites M13a/M13b with the target m/z 309 were produced in biotransformation screens. However, one of the chemical oxidation conditions screened was identified as tractable for scaling up, with notably good production of the tentative M13b component. LC-MS/MS matching of the tentative M13b produced in this chemical condition was performed at IQVIA Laboratories and confirmed as an exact match to M13b observed in human plasma.

Scale-up & structure elucidation

The chemical oxidation method was scaled up using 100 mg of brimarafenib to generate 13.6 mg M13b after purification (Figure 2). The reaction was performed using a modification of the iodine/dimethyl sulfoxide (I_2/DMSO) oxidative system [3]. The method proceeds through initial iodination of the substrate in the presence of molecular iodine followed by Kornblum oxidation transforming a primary halide to an aldehyde, facilitated by DMSO as a reagent. M13b was isolated from the reaction mixture using a two-step orthogonal purification step to yield 13.6 mg of the metabolite.

NMR spectroscopy identified the structure of M13b as resulting from the loss of N-trifluorophenyl urea and opening of the fused cyclopropane ring with oxidation to form a conjugated aldehyde moiety.



Brimarafenib (BGB-3245)
100 mg

M13b
13.6 mg
97.2% purity by LC-UV
82.9% +/- 1.3% by ERETIC2 qNMR

Significance

Although there were no radiolabeled metabolites circulating at ≥10%, to confirm that unlabeled M13b was not a major circulating metabolite, the standard was evaluated by MS response in plasma and its MS ionization efficiency related to brimarafenib. Based on these results, distribution of M13b in plasma was estimated as a mean (n=11) of 1.8% circulating in the AUC pools and 0.8% in the Cmax samples.

References

[1] Schram AM, et al. AACR Annual Meeting 2023. Presentation slides.

[2] Yi Y, et al. 27th North American ISSX Meeting; 2026. Poster.

[3] Moussa Z, et al. RSC Adv. 2022;12(10):6133–6148. doi:10.1039/d2ra00049k

About Hypha

Hypha Discovery Ltd is a UK-based CRO supporting pharmaceutical and agrochemical R&D partners worldwide. Our core services encompass LC-MS/MS based MetID, synthesis and purification of metabolites, definitive structure elucidation by NMR spectroscopy, impurity isolation, and natural product production.