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WHITEPAPER

Developing a Convenient *In Vitro* Method for Predicting Metabolism and Disposition of Acrylamide Covalent Drugs in Humans

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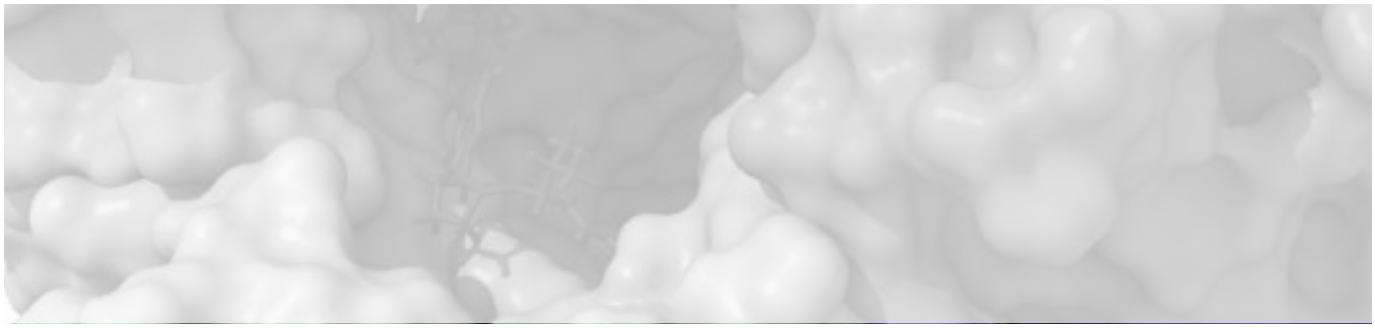
INTRODUCTION



A EXPLANATION OF TARGETED COVALENT INHIBITORS

Covalent drugs have been used for more than a century but concerns over reactivity and toxicity have given pause to developers in the past. Since the 2010s, this category of drug has made a comeback, leading to the emergence of relatively new types of drug modality such as targeted covalent inhibitors.

Targeted covalent inhibitors bind tightly to a very specific part of a protein—i.e., an enzyme or a receptor—that is related to a disease. When introduced to the body, targeted covalent inhibitors are transformed by oxidation and conjugation with Glutathione (GSH), a molecule crucial in the body's detoxification processes.

Because of their unique properties, targeted covalent inhibitors offer drug developers some significant advantages, including:

- **Selectivity:** Targeted covalent inhibitors are designed to interact with a specified target protein by either exploiting a unique binding site or a cysteine residue within the protein. This makes them extremely selective and can reduce off-target effects in the body and increase the effectiveness of treatments.
- **Prolonged activity:** Because the bond formed with the target protein is stable, the inhibition of activity can be long-lasting, even after the drug leaves the body.
- **Potency:** Covalent bonding allows for potencies that are extremely high and, in some cases, for irreversible interactions.
- **Reduced drug resistance:** Targeted covalent inhibitors are less susceptible to the resistance of some pathogens and cancer cells because the bond formation is less easily disrupted by mutations.

B CHALLENGES

Because of their complex nature, targeted covalent inhibitors represent challenges to researchers and developers attempting to bring new drugs to the market. Some of these challenges arise from the complexity inherent in the development and testing of these products. Targeted covalent inhibitors are challenging to design and require a deep understanding of the structure and reactivity of the target protein. It can also be challenging to find suitable binding sites. Another major issue for developers is that covalent drugs can sometimes be irreversible, which creates extra pressure to develop them precisely.

One of the most significant developmental challenges is the lack of systematic and efficient *in vitro* methods to investigate and predict metabolism and disposition of these types of drugs in humans. Without a proper and thorough *in vitro* testing method, it's difficult to predict how these drugs will react inside the human body.

Absorption, distribution, metabolism and excretion (ADME) all need to be carefully studied and optimized as covalent bonding can affect how a drug affects and exits the body. Targeted covalent inhibitors, and all covalent drugs, are subject to rigorous regulatory scrutiny. That makes it even more important to be able to conduct proper *in vitro* testing to avoid adding expense and time to an already burdensome process.

It is also difficult to predict this type of drug's metabolism and disposition. This is again partially due to the complexity of targeted covalent inhibitors, but also because there is little precedent for developers to use when testing.

1. OBJECTIVES

A INTRODUCE NEW *IN VITRO* METHOD

This study set out to develop an efficient and comprehensive *in vitro* approach for predicting *in vivo* metabolism and disposition pathways of acrylamide drugs. There is a clear gap in the capability to properly predict metabolism and disposition in these drugs. An improved *in vitro* approach to this testing will allow more accurate prediction of how targeted covalent inhibitors will perform in the human body.

B USE OF MODEL COMPOUNDS (ABIVERTINIB AND OSIMERTINIB)

Two acrylamide covalent drugs, abivertinib and osimertinib, were used as model compounds. Compared to similar drugs, the human metabolism and disposition data of abivertinib and osimertinib were easier to use as data on the drugs was published and complete. These two drugs can also undergo oxidative metabolism and conjugation with GSH and protein, but the conjugation extents are different, making them suitable as model compounds in this study.

2. METHODOLOGY

A DESCRIPTION OF *IN VITRO* METHODOLOGY

A combination of multiple *in vitro* metabolism experiments, including metabolite profiling in human liver microsomes (HLM) with and without GSH, and metabolic stability analysis in human albumin with and without GSH, were employed to study the oxidative metabolism, protein binding and GSH conjugations of the two acrylamide covalent drugs.

Using this method, the main metabolic pathways of irreversible covalent drugs were efficiently evaluated and predicted, with the oxidative metabolism mediated by CYP enzyme and covalent binding with GSH or protein. This method is low cost and easy to execute. The sample analysis was performed by Thermo Q-Exactive™ Plus with Waters® ACQUITY UPLC® System.

B EXPERIMENTATION WITH ACRYLAMIDE COVALENT DRUGS

An *in vitro* methodology was designed and utilized for investigating oxidative metabolism, protein binding and GSH conjugations of two acrylamide covalent drugs: abivertinib and osimertinib.

C INTEGRATION OF METABOLITE PROFILING

In human liver microsomes, which are used to mimic the liver's ability to metabolize drugs, the two drugs were incubated at 10 μM with and without GSH for 0.5h. This allowed researchers to determine the effects of GSH over those 30 minutes. By running the test with and without GSH, the resulting data determined the effect of GSH on the metabolism of the drugs.

D EXPLORATION OF POTENTIAL COVALENT BINDING

Human serum albumin (HSA) is commonly used to study how drugs bind to proteins in the blood, so was an important tool to establish potential covalent binding. In this study, the two covalent drugs were incubated at 1 μM in human serum albumin with and without GSH for 0, 2 and 6 hours. Again, the study was conducted with and without GSH to determine its effects on covalent binding in these two drugs.

3. RESULTS

A METABOLISM AND DISPOSITION PREDICTIONS

Like the metabolism and disposition observed in many conventional covalent drugs, the two acrylamide covalent drugs underwent oxidative metabolism, formed GSH conjugates and protein adducts *in vitro*. The covalent binding of the two drugs to albumin can be estimated indirectly by comparing the remaining percentage and GSH adducts in HSA, HSA with GSH and PB with GSH.

B EFFECTIVENESS OF THE DEVELOPED METHOD

The metabolic pathways and protein binding for Abiverinib and Osimertinib, as determined through the newly established *in vitro* approach, were consistent with those observed in humans. Furthermore, the same conclusion was obtained from the other three acrylamide covalent drugs evaluated in the WuXi lab.

The binding trend of the two drugs in the *in vitro* test was consistent with *in vivo* results reported by Yiqian Liu. et al. [1] and Paul A. Dickinson et al. [2]

3. RESULTS

C DETAILED ANALYSIS

Data analyses were performed using the Xcalibur and Compound discoverer software (Thermo Scientific), the metabolite profiling and metabolic pathway of abivertinib and osimertinib were determined by LC-UV-HRMSn (n = 1~2) in human liver microsomes with and without GSH after incubation at 37 °C for 30 min. The metabolite profiling of abivertinib and osimertinib in human liver microsomes are shown in Figure 1-1 and Figure 1-2, respectively. The proposed metabolic pathways of abivertinib and osimertinib in human liver microsomes are shown in Figure 2-1 and Figure 2-2, respectively.

In *in vitro*, the major metabolic pathway of abivertinib and osimertinib were oxygenation and dealkylation in human liver micrsomes with and without GSH. Meanwhile, abivertinib and osimertinib can also conjugated with GSH by chemical conjugation in PB (M3 for abivertinib and M4 for osimertinib), and by GSHT catalyzation and/or chemical conjugation in human liver microsomes (M7-M9 for abivertinib and M2 for osimertinib). In *in vivo*, the major metabolic pathway of abivertinib and osimertinib were oxygenation, dealkylation and hydrolysis of GSH conjugation (such as Cysteine, Acetylcysteine and Glycine-cysteine conjugation) in humans. The metabolic pathways of abivertinib and osimertinib between *in vitro* and *in vivo* human were compared. As showed in Table 1-1 and Table 1-2, their metabolic pathways between *in vitro* and *in vivo* are comparable.

In addition, the protein binding of abivertinib and osimertinib were evaluated indirectly by measuring the remaining of free drug and GSH adducts formation after incubation. The remaining of free drug and GSH adducts formation of abivertinib and osimertinib were showed in Table 2-1 and Table 2-2, the percentage of abivertinib and osimertinib decreased and their GSH adducts increased in human serum albumin with GSH after being incubated for between 2 and 6 hours. Compared with abivertinib, osimertinib binds more readily to HSA in HSA samples with and without GSH. In the phosphate buffer, both drugs were mainly conjugated with GSH and the free drugs were less than 10%.

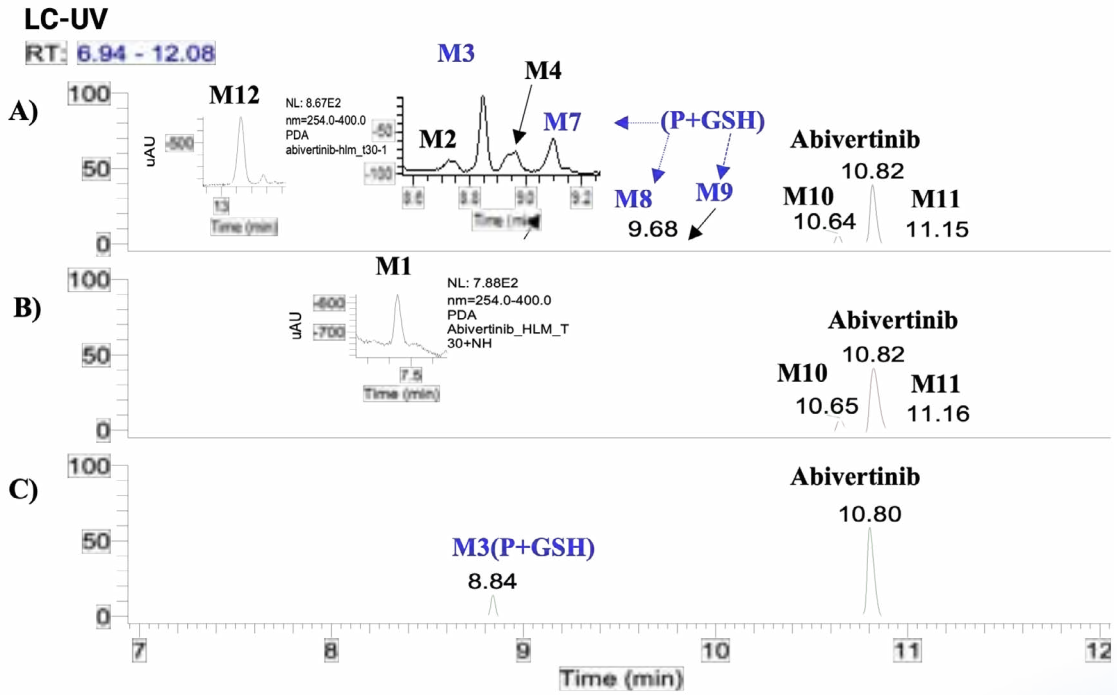


Figure 1-1. LC-UV (λ:254-400 nm) chromatograms (XICs) of abivertinib and its metabolites in human Liver Microsomes after incubation 30 min

Note: A): LC-UV chromatograms of abivertinib in human liver microsome with GSH; B) LC-UV chromatograms of abivertinib in human liver microsome without GSH; LC-UV chromatograms of abivertinib in potassium phosphate buffer with GSH, P: Abivertinib.

3. RESULTS

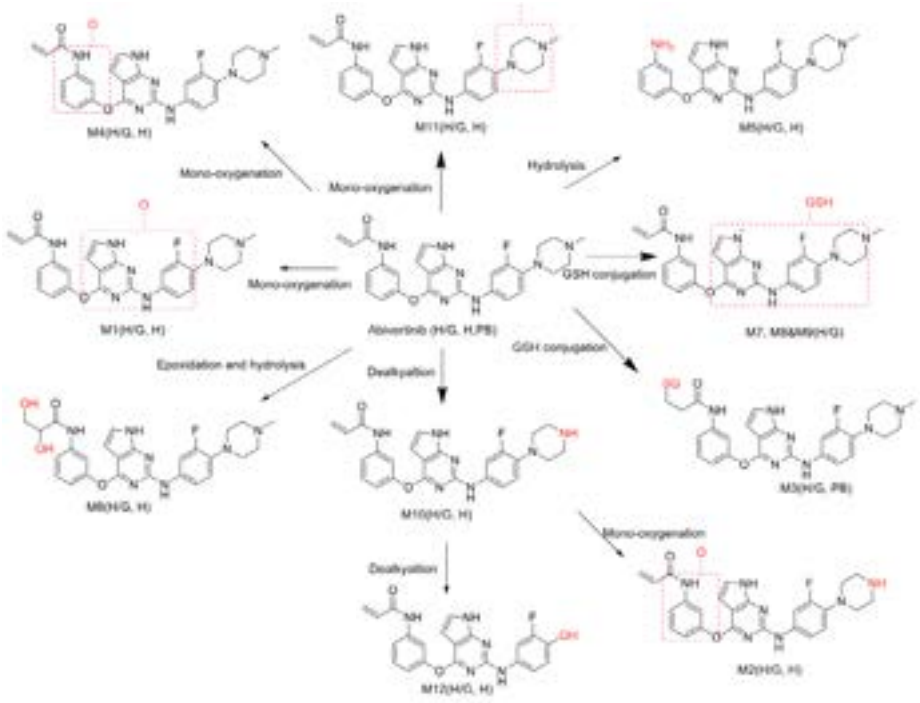


Figure 2-1. The proposed metabolic pathways of abivertinib in human liver microsomes with and without GSH.
Note: H/G: Human liver microsome with GSH **H:** Human liver microsome without GSH **PB:** PB solution with GSH

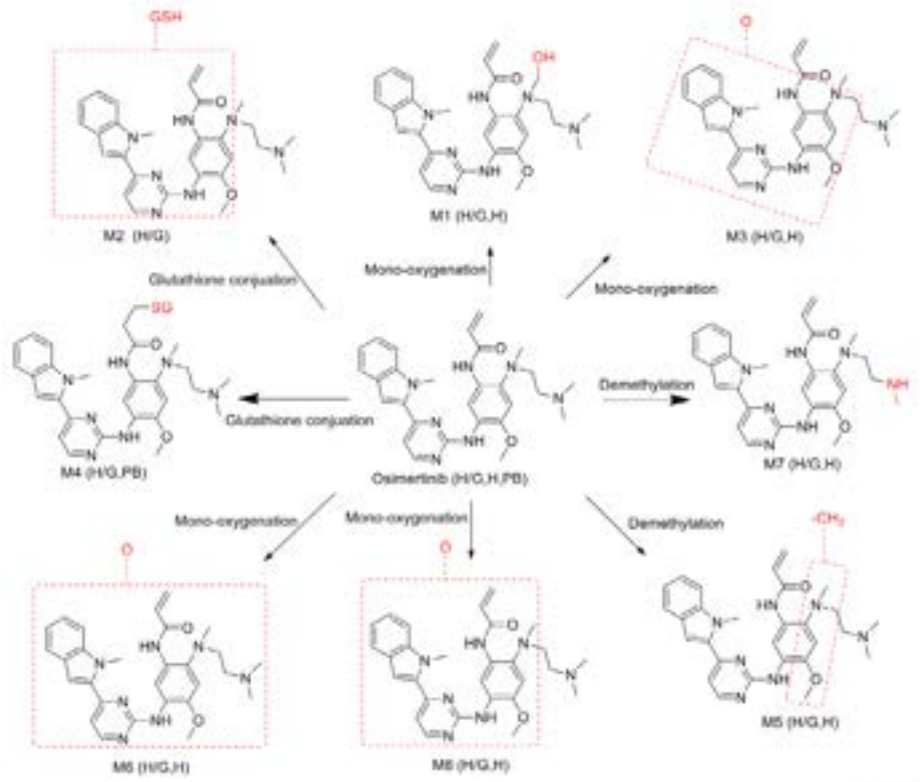


Figure 2-2. The proposed metabolic pathways of osimertinib in human liver microsomes with and without GSH.
Note: H/G: Human liver microsome with GSH **H:** Human liver microsome without GSH **PB:** PB solution with GSH

3. RESULTS

Table 1-1. Comparison of human metabolism pathway between *in vitro* and *in vivo* of abivertinib

#CODE	METABOLIC PATHWAY	*IN VIVO*	*IN VITRO*
Abivertinib	NA	+++	+++
M2	N-oxidation	+	+
M4	Amide hydrolysis	+	+
M5	Oxygenation	+	+
M7	&Reduction	+	ND
MII-1*	Acetylcysteine conjugation	++	++
MII-2*	Cysteine conjugation	+	
MII-6	Acetylcysteine conjugation of M4	+	ND

Note: #: the code from the reported human *in vivo* data *: Further hydrolyzed metabolites of GSH adducts; &: Gut microbiota mediated metabolite; +++: Relative abundance of over 50%; ++: Relative abundance of 10%~50%; +: Relative abundance of less than 10%.

Table 1-2. Comparison of human metabolism pathway between *in vitro* and *in vivo* of osimertinib

#CODE	METABOLIC PATHWAY	*IN VIVO*	*IN VITRO*
Osimertinib	NA	+++	+++
M1	Oxygenation	+	+
M2	Dealkylation	+	+
AZ7550	Demethylation	++	++
AZ5104	Demethylation	++	+
M17	Oxygenation	+	+
M20	Oxygenation	+	+
M23	Oxygenation of M2	+	++
M8*	Glycine-cysteine conjugation	+	++
M21*	Cysteine conjugation	+	
M18	Glucuronidation of M17	+	ND

Note: #: the code from the reported human *in vivo* data *: Further hydrolyzed metabolites of GSH adducts; +++: Relative abundance of over 50%; ++: Relative abundance of 10%~50%; +: Relative abundance of less than 10%.

3. RESULTS

Table 2-1. Remaining% of free drugs of abivertinib and osimertinib in human serum albumin with and without GSH

Incubation time(h)	ABIVERINIB			OSIMERTINIB		
	HSA	HSA+GSH	PB+GSH	HSA	HSA+GSH	PB+GSH
0	100.00	100.00	100.00	100.00	100.00	100.00
2	99.53	89.33	50.28	44.97	40.71	36.33
6	89.33	70.15	8.12	8.19	7.74	2.58

Table 2-2. The GSH adduct formation of abivertinib and osimertinib in human serum albumin with and without GSH

Incubation time(h)	ABIVERINIB			OSIMERTINIB		
	HSA	HSA+GSH	PB+GSH	HSA	HSA+GSH	PB+GSH
0	ND	0.00	1.53	ND	0.00	0.00
2	ND	8.78	72.61	ND	19.92	88.73
6	ND	16.07	95.98	ND	67.97	90.51

4. DISCUSSION

A INTERPRETATION OF RESULTS

The results clearly show that the *in vitro* approach explained above is useful for predicting metabolic pathways and protein binding of acrylamide covalent drugs in humans. This new method will make the development of future targeted covalent inhibitors easier, as predictions of *in vivo* metabolism and disposition will improve. This will help develop new covalent drugs which due to their unique properties can improve treatments.

B ADVANTAGES OF THE NEW *IN VITRO* METHOD

A more accurate and efficient *in vitro* method to investigate and predict metabolism and disposition of targeted covalent inhibitors brings several advantages, including:

- Efficiency enables faster drug development, increasing the potential to save lives.
- A robust *in vitro* methodology reduces the need for animal testing for early-stage assessment.
- Increases confidence in covalent drugs and reduces association of risk.
- Ease the steps required for regulatory compliance with such drugs.

5. CONCLUSION

A SUMMARY OF MAJOR FINDINGS

The testing found that the two drugs used in the study, abivertinib and osimertinib, both underwent oxidative metabolism, formed GSH conjugates and protein adducts *in vitro*. The metabolic pathways and protein binding established with the new *in vitro* method were consistent with those observed in humans, and with other covalent drugs tested.

The *in vitro* approach established in this research is useful for predicting metabolic pathways and protein binding of acrylamide covalent drugs in humans.

B IMPACT ON THE FIELD

There are several potential contributions this study could make to the DMPK field. The most impactful is improving the predictability and efficiency of covalent drug development. Although covalent drugs have been around for a long time, the latest wave of target covalent inhibitors are relatively new and hold great potential. The successful introduction of a new *in vitro* method to test them will only hasten their arrival and with it the benefits they bring.

C FINAL THOUGHTS

The benefits of targeted covalent inhibitors are clear to see. This type of drug, because of its ability to bind tightly to just one part of a protein, can target and attack disease in a manner which drug developers consider noteworthy. The development of these drugs will increase in the coming years, but there will be challenges, as with any other relatively new drug type.

The introduction of a new *in vitro* method of testing could be crucial in the evolution of this drug category, as it improves efficiency, safety and efficacy of drugs. These advantages have the potential to significantly impact patient outcomes.

ABOUT US

As a global company with operations across Asia, Europe, and North America, WuXi AppTec provides a broad portfolio of R&D and manufacturing services that enable the pharmaceutical and healthcare industry around the world to advance discoveries and deliver groundbreaking treatments to patients.

Through its unique business models, WuXi AppTec's integrated, end-to-end services include chemistry drug CRDMO (Contract Research, Development and Manufacturing Organization), biology discovery, preclinical testing and clinical research services, and cell and gene therapies CTDMO (Contract Testing, Development and Manufacturing Organization), helping customers improve the productivity of advancing healthcare products through cost-effective and efficient solutions. WuXi AppTec received AA ESG rating from MSCI in 2023 and its open-access platform is enabling more than 6,000 customers from over 30 countries to improve the health of those in need – and to realize the vision that "every drug can be made and every disease can be treated."

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https://labtesting.wuxiapptec.com/dmpk-services/

REFERENCES:

1. Yiqian Liu, Lianke Liu, Lingxiang Liu, et al. A phase I study investigation of metabolism, and disposition of [14C]-anlotinib after an oral administration in patients with advanced refractory solid tumors. Cancer Chemotherapy and Pharmacology.2020, 85, 907–915.
2. Paul A. Dickinson, Mireille V Cantarini, Jo Collier, et al. Metabolic Disposition of Osimertinib in Rats, Dogs, and Humans: Insights into a Drug Designed to Bind Covalently to a Cysteine Residue of Epidermal Growth Factor Receptor. Drug Metab Dispos. 2016,44,1201-1212.