ISSN 2706-6819 Vol.6, Issue 9: 20-28, DOI: 10.25236/IJFM.2024.060904

# Phenotyping of VT204 metabolic enzymes in human liver microsomes

**Liang Tan[1,2,3,a], Shu Zhang[3,b], Xiaochuan Li[3,c], Lihua Qin[1,2,3,d], Xiaoqun Duan[1,2,e,*]**

*[1]College of Pharmacy, Guilin Medical University, Guilin, Guangxi, 541004, China*
*[2]College of Biomedical Industry, Guilin Medical University, Guilin, Guangxi, 541004, China*
*[3]Suzhou Xuhui Analysis Co., Ltd., No. 168 Yuanfeng Road, Kunshan, Jiangsu, 215300, China*
*[a]1652265682@qq.com, [b]wyzhangshu@163.com, [c]xlirick@163.com, [d]1686251443@qq.com, [e]robortduan@163.com*
*[*]Corresponding author*

***Abstract:*** *VT204 is a KRASG12C inhibitor for the treatment of non-small cell lung cancer, which has good structural characteristics and pharmacological effects. It has become an essential part of preclinical metabolic research to determine the main subtypes of metabolic enzymes by in vitro study of metabolic enzyme phenotypes.In this study, the main CYP450 isoenzymes involved in VT204 metabolism were investigated by chemical inhibition method and recombinant enzyme method. Two incubation systems were established respectively, and LC-MS/MS technology was used to detect the peak area ratio of VT204 and CYP450 enzyme-specific metabolites and substrates in the incubation solution to internal standard terphenadine, respectively. According to the calculation, ketoconazole (CYP3A4 inhibitor) showed a high inhibition of VT204 by chemical inhibition method (85.7%), followed by Montelukast (CYP2C8 inhibitor, 49.6%) and sulfameprazole (CYP2C9 inhibitor, 17.6%).The residual rate was 19.9% for CYP3A4 and 80.4% for CYP2C8 by recombinant enzyme method. Results Preliminary identification of CYP3A4 as the main metabolic enzyme of VT204, CYP2C8 and CYP2C9 may be involved in the metabolic process of VT204, the results can provide a reference for further study of the metabolism of VT204 in vivo and reasonable clinical compatibility.*

***Keywords:*** *VT204, chemical inhibition, recombinant enzymatic method, LC-MS/MS*

## 1. Introduction

Lung cancer is a global health problem, with non-small cell lung cancer (NSCLC) accounting for 80%-85% of the total[1]. According to Global Cancer Statistics, it is estimated that more than 2 million people are newly diagnosed with lung cancer each year[2]. Unfortunately, about half of all new lung cancer cases occur in Asia[3]. VT204 is a KRASG12C inhibitor used in the treatment of non-small cell lung cancer[4], synthesized by Suzhou Wentian Pharmaceutical Technology Co., Ltd. The molecular structure is shown in Figure 1, the quinazolin parent nucleus in VT204 occupies the SWII pocket of KRAS, which increases its binding rate, and the parent nuclear side chain is replaced by acrylamine, which can covalently bind to the 12C structure, indicating that the drug has good structural characteristics[5]. In vitro drug metabolism studies can exclude in vivo interference factors and provide a reliable theoretical basis for the overall experiment, For drugs with low metabolic conversion rate, high toxicity and lack of sensitive detection methods in vivo, in vitro metabolism study is a good research method[6].There are more than 400 isoforms of the hepatocytochrome P450 family (CYP450), and the main isomers involved in hepatic drug metabolism are CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and CYP3A5[7]. Enzyme activity can be inhibited or induced by many drugs and may be altered by co-administration through drug interactions (DDIs) [8] . Therefore, the evaluation of potential DDI is important for the development of safe and effective new drugs.Determining the main metabolic enzyme isoforms of this compound through in vitro study of metabolic enzyme phenotypes has become an indispensable part of drug preclinical metabolism research.In this study, the main CYP450 isozyme isoforms involved in VT204 metabolism were investigated by chemical inhibition and recombinant enzyme methods. This study provides a reference for further study of VT204's in vivo metabolism and clinical compatibility.

ISSN 2706-6819 Vol.6, Issue 9: 20-28, DOI: 10.25236/IJFM.2024.060904

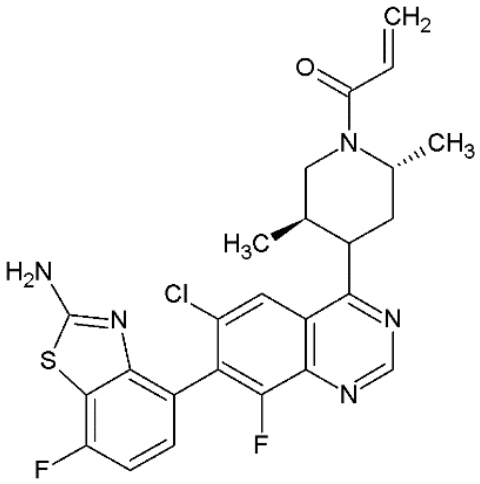


*Figure 1: Structural formula of VT204*

## 2. Experimental materials and reagents

### *2.1 Reagents*

VT204 (mass fraction 98%, Lot No: 190136-197-1), synthesized and provided by Suzhou Wentian Pharmaceutical Technology Co., Ltd.; Terfenadine (99.91%, 50679-08-8) was purchased from the official website of MedChemExpress. Verapamil (99.90, 100223-202103), phenacetin (98.70, 100095-201606), bupropion hydrochloride (99.90, 10067-2018-02), amodiaquine hydrochloride (91.90, lot code: 101217-201401), diclofenac sodium (100.00, 100334-201803), Dextromethorphan Hydrobromide (94.80, 100201-202205), ticlopidine (99.70, 100542-201002), montelukast sodium (98.60, 100893-201902), Ketoconazole (99.80, 100294-201904), acetaminophen (100.00, 100018-202312), all purchased from China Institute for Food and Drug Control; Testosterone (95.90, 0221-RD-0125), dexterphalane (98.80, 0804-RD-0021), both purchased from CATO Corporation in United States; Quinidine (96.99, 12-01-6279), (S)-Mefentoin (98.00, LK20V01), α-Naphthalene flavonoids (98.00%, 604-59-1), sulfabenpyrazole (98.00%, 526-08-9), sodium 4-hydroxydiclofenac (99.30%, 2088-058A7), 4-hydroxymefentoin (99.30%, 1264-068A5), hydroxybupropion (98.10%, 0725-RE-0009), All purchased from TLC Corporation in United States; 6-hydroxytestosterone (98.90%, C15067612), N-deethylamodiaquine (98%, C16837110), Norcarone(97.1, K2202735), Disodium hydrogen phosphate dodecahydrate, dipotassium hydrogen phosphate trihydrate, magnesium chloride hexahydrate, NADPH tetrasodium salt, all purchased from Macklin Company in United States; Sodium dihydrogen phosphate dihydrate and potassium dihydrogen phosphate were purchased from Aladdin in United States; Absolute ethanol (Shanghai Titan Science and Technology Co., Ltd.); acetonitrile, formic acid, ammonium acetate and DMSO (all HPLC grade, Thermo Fisher Scientific); 150 people pooled human liver microsomes (Lot No: DNJ, BioIVT in United States);

### *2.2 Instruments*

API 4000 Qtrap LC-MS System (including DGU-20A3R Degasser, LC-20ADXR Infusion Pump, SIL-20ACXR autosampler and CTO-20A column heater); API 4000 Q trap triple quadrupole tandem mass spectrometer with electrospray ionization (ESI) and Analyst data processing system, AB SCIEX. Water bath thermostatic oscillator (SHZ-YN, Changzhou Yineng Experimental Instrument Factory); High-speed refrigerated centrifuge (Sorvall ST 16R, Thermo Fisher Scientific); Electronic balance (SQP, Sartorius Scientific Instruments (Beijing) Co., Ltd.); GraphPad Prism 9.0 plot analysis software (GraphPad Software, United States);

## 3. Methods

### *3.1 Solution preparation*

VT204 solution preparation: precision weighing a certain amount of VT204 dissolved in dimethyl sulfoxide (DMSO), the stock concentration is 10 $mmol \cdot L^{-1}$.

ISSN 2706-6819 Vol.6, Issue 9: 20-28, DOI: 10.25236/IJFM.2024.060904

Internal standard solution preparation: 1.0 mg of terfenadine control substance was accurately weighed and dissolved in 1.0 mL of DMSO solution, and 1.0 mg·mL$^{-1}$ internal standard solution was prepared, and then diluted into 40.0 ng·mL$^{-1}$ with acetonitrile standards.

### *3.2 Establishment of in vitro incubation system*

#### *3.2.1 Chemical inhibition incubation system*

Test group: The total volume of the test group incubation system was 100 uL, including 86 μL human liver microsomes (protein concentration 0.5 mg · mL−1), 2 μL VT204 (0. 5 μmol·L$^{-1}$), 2 μL of each specific inhibitor: α-naphthalene flavonoids (10 μmol·L$^{-1}$, a CYP1A2 inhibitor), ticlopidine (5.0 μmol·L$^{-1}$, CYP2B6 inhibitor), montelukast sodium (50.0 μmol·L$^{-1}$, CYP2C8 inhibitor), sulfabenpyrazole (10.0 μmol·L$^{-1}$, CYP2C9 inhibitor), norcartone (120 μmol·L$^{-1}$, CYP2C19 inhibitor), quinidine (2.0 μmol·L$^{-1}$, CYP2D6 inhibitor), ketoconazole (1.0 μmol·L$^{-1}$, CYP3A4 inhibitor), pre-incubated in a thermomixer at 37°C for 5 min, and 10 μL NADPH regeneration system (including 3.3 mmol·L$^{-1}$MgCl2 , 1.3 mmol·L$^{-1}$ NADP$^{+}$, 3.3 mmol·L$^{-1}$ sodium glucose dehydrogenation of 6-phosphate, 0.4 U·mL$^{-1}$ glucose dehydrogenase of 6-phosphate) to initiate the reaction, in which the organic solvent does not exceed 1%.

Systematic experimental group : The total volume of the incubation system was 100 uL, including 86 μL human liver microsomes (protein concentration 0.5 mg · mL−1), 2 μL specific substrate: phenacetin (40.0 μmol·L$^{-1}$, CYP1A2), bupropion hydrochloride (100 μmol·L$^{-1}$, CYP2B6), amodiaquine hydrochloride (1.0 μmol·L$^{-1}$, CYP2C8), diclofenac sodium (5.0 μmol·L$^{-1}$, CYP2C9), (*S*)-Mefentoin (40.0 μmol·L$^{-1}$, CYP2C19), dextromethorphan hydrobromide (5.0 μmol·L$^{-1}$, CYP2D6), testosterone (50.0 μmol·L$^{-1}$, CYP3A4), 2 μL each specific inhibitor (same as the test group), 10 μL NADPH regeneration system.

Control group: The test group and the system test group were set up as a reaction control group (without inhibitors) and a non-reaction negative control group (without inhibitors and NADPH regeneration system) respectively.

#### *3.2.2 Recombinant enzymatic incubation system*

Test group: The total volume of the incubation system was 100 uL, including 88 μL various types of recombinant enzymes (protein concentration 50 pmol ·L$^{-1}$ ), 2 μL VT204 (0.5 μmol·L$^{-1}$), pre-incubated in a thermostatic shaker in a water bath at 37°C for 5 min, and 10 μL NADPH regeneration system was also pre-incubated for 5 min, in which the organic solvent did not exceed 1%. At the same time, a negative control group (without NADPH regeneration system) was set up.

Systematic experimental group: The total volume of the incubation system was 100 uL, 88 μL various types of recombinant enzymes (protein concentration 50 pmol·L$^{-1}$), 2 μL specific substrate (compounds were the same as above, the concentration was 0.5 μmol·L$^{-1}$), 10 μL NADPH regeneration system. At the same time, a negative control group was set up.

### *3.3 Sample processing*

After the "2.2" incubation system was continued in a 37°C water bath thermostatic shaker for 60 min, 200 μL of acetonitrile (40.0 ng·m L$^{-1}$ terfenadine) protein containing internal standard was added immediately, the reaction was terminated by vortexing for 2 min, centrifugation was carried out at 15400 g at 4℃ for 10 min, 70 μL of supernatant was taken, and 70 L was added Pure water, mix well, and wait for injection.

### *3.4 HPLC-MS/MS methods*

#### *3.4.1 Determination of VT204*

Chromatographic conditions: ZORBAX Eclipse Plus C18 (2.1*50 mm, 3.5μm, Agilent); Mobile phase: A: 5 mM ammonium acetate in water (with 0.1% formic acid), B: methanol, gradient elution (0.50 ~ 1.00 min, 35%~85% B; 1.00 ~ 2.30min , 85% B ; 2.30 min ~ 2.35 min , 85%~35% B : 2.35 ~ 3.00 min , 35% B) volumetric flow: 0.50 mL/min ; Column temperature 40°C ; The injection volume was 2 μL.

Mass spectrometry conditions: electrospray ionization (ESI), positive ion mode, electrospray voltage 5500 V, ion source temperature 550°C, atomized gas 55 psi, dry gas 55 psi, scanning mode is multiple

ISSN 2706-6819 Vol.6, Issue 9: 20-28, DOI: 10.25236/IJFM.2024.060904

reaction monitoring (MRM) mode. VT204 monitors an ion pair of m/z 5150→m/z 152.2, and the internal standard terfenadine monitored the ion pairs of m/z 472.4 → m/z 436.4.

### *3.4.2 Determination of specific metabolites*

Chromatography: the column is the same as VT204; Mobile phase: A: 0.1% formic acid water, B: 0.1% formate acetonitrile, gradient elution (0.50 ~ 1.50 min, 5%~95% B; 1.50 ~ 2.20min , 95% B ; 2.20 min ~ 2.21 min , 95%~5% B : 2.21 ~ 3.0 min , 5% B) volumetric flow; 0.60 mL/min ; Column temperature 40°C ; The injection volume was 1 μL.

Mass spectrometry: electrospray ionization (ESI), positive ion mode, electrospray voltage 5500 V, ion source temperature 500 °C, atomized gas 55 psi, dry gas 55 psi, scanning mode for multiple reaction monitoring (MRM) mode. Acetaminophen was monitored at m/z 152.1→m/z 110.0, hydroxybupropion was m/z 256.1→ m/z 139.0, N-deethylamodiaquine was m/z 328.2→ m/z 283.1, 4-hydroxydiclofenac sodium was m/z 312.1→m/z 231.3, and 4-hydroxymefentoin was monitored at m/z 235.2→m/z 150.3, dexphthene was monitored as m/z 258.2→m/z 157.0, 6β-hydroxytestosterone was m/z 305.1→m/z 269.1, terfenadine was the same as "2.4.1".

### *3.4.3 Determination of specific substrates*

Chromatography: the conditions are the same as those of "2.4.2".

Mass spectrometry: the conditions of the ion source are the same as those of 2.4.2. Phenacetin monitored ion pairs were m/z 180.2→ m/z 110.1, Bupropion is m/z 240.1→m/z 131.2, amodiaquine hydrochloride is m/z 356.3→m/z 283.2, diclofenac sodium is m/z 296.2→m/z 214.0, (S)-mefentoin is m/z 219.2→ m/z 1341. Dextromethorphan is m/z 272.3→m/z 147.0, testosterone is m/z 289.4→97.1, and terfenadine is the same as "2.4.1". The specific compound parameters are shown in Table 1.

*Table 1: Reaction ion pair parameters monitored for all analytes*

| Analytes | Q1/Q3(*m/z*) | Dwell Time (ms) | DP (V) | EP (V) | CE(V) | CXP(V) |
|---|---|---|---|---|---|---|
| VT204 | 515.0/512.2 | 100 | 130 | 10 | 37 | 10 |
| Terfenadine | 472.4/436.4 | 100 | 66 | 10 | 45 | 14 |
| Acetaminophen | 152.1/110.0 | 100 | 51 | 10 | 23 | 12 |
| Hydroxybupropion | 256.1/139.0 | 100 | 60 | 10 | 30 | 12 |
| N-Desethylamodiaquine | 328.2/283.1 | 100 | 66 | 10 | 50 | 12 |
| 4-Hydroxydiclofenac | 312.1/231.3 | 100 | 46 | 10 | 29 | 12 |
| 4-Hydroxymefentoin | 235.2/150.3 | 100 | 61 | 10 | 23 | 12 |
| Dexthene | 258.2/157.0 | 100 | 61 | 10 | 40 | 12 |
| 6β-hydroxytestosterone | 305.1/269.1 | 100 | 41 | 10 | 22 | 16 |
| Phenacetin | 180.2/110.1 | 100 | 50 | 10 | 22 | 15 |
| Bupropion | 240.1/131.2 | 100 | 37 | 10 | 37 | 7 |
| Amodiaquine | 356.3/283.2 | 100 | 54 | 10 | 24 | 20 |
| Diclofenac sodium | 296.2/214.0 | 100 | 34 | 10 | 48 | 10 |
| S-Mefentoin | 219.2/134.1 | 100 | 66 | 10 | 50 | 12 |
| Dextromethorphan | 272.3/147.0 | 100 | 46 | 10 | 29 | 12 |
| testosterone | 289.4/97.1 | 100 | 61 | 10 | 23 | 12 |

ISSN 2706-6819 Vol.6, Issue 9: 20-28, DOI: 10.25236/IJFM.2024.060904

### *3.5 Calculation formula*

The remaining percentage of the original drug (test group) is calculated by the ratio of the concentration of the test substance in the presence of NADPH and no inhibitor to the absence of NADPH and inhibitor, as shown in Formula (1):

$$\text{Percentage of remaining \%} = \frac{(A_{Mi} / A_{IS})_I}{(A_{Mi} / A_{IS})_O} \times 100 \quad (1)$$

where AMi refers to the peak area of the remaining amount of the test object, and AIS refers to the peak area of the internal standard.

I refers to the presence of NADPH.

O means that NADPH is not present.

The percentage of inhibition of the metabolism of the test substance by the specific inhibitor, that is, the difference between the remaining percentage of the original drug in the presence and absence of specific inhibitors and the ratio of the original drug metabolized in the absence of inhibitors, as shown in Formula (2)

$$\text{Percentage of inhibition \%} = \frac{\text{Percentage of remaining \%}_P\text{-Percentage of remaining \%}_W}{100\text{-Percentage of remaining \%}_W} \times 100 \quad (2)$$

P refers to the presence of inhibitors.

W refers to the absence of inhibitors.

The percentage of remaining activity of the enzyme is calculated by the ratio of the peak area of the specific metabolite produced in the presence of inhibitors to the ratio of the peak area of the internal standard in the presence of inhibitors and in the absence of inhibitors, as shown in Formula (3),

$$\text{Remaining activity of the enzyme \%} = \frac{(A_{Mi} / A_{IS})_P}{(A_{Mi} / A_{IS})_W} \times 100 \quad (3)$$

where AMi refers to the peak area of specific metabolites, and AIS refers to the peak area of internal standards.

P refers to the presence of inhibitors.

W refers to the absence of inhibitors.

## 4. Results

### *4.1 Chemical inhibition*

#### *4.1.1 Systematic experimental group*

The ratio of the specific metabolites corresponding to the isoenzymes of the system experimental group to the peak area of the internal standard in the samples of the incubation system by chemical inhibition method was determined, and the semi-quantitative calculation method was used, and the percentage of enzyme activity in the systematic experimental group was obtained by combining the calculation formula, as shown in Table 2, and the remaining activity of the enzymes in the seven groups was ≤ 50%, indicating that the incubation system was reliable.

*Table 2: Metabolism of substrates by isoenzyme inhibitors in the Systematic experimental group*

| Isoenzymes | Substrate | Specific inhibitors | Enzyme Activity Percentage % | Percentage of inhibition % |
|---|---|---|---|---|
| CYP1A2 | Phenacetin | α-Naphthalene flavonoids | 10.9 | 89.1 |
| CYP2B6 | Bupropion hydrochloride | Ticlopidine | 5.3 | 94.7 |

ISSN 2706-6819 Vol.6, Issue 9: 20-28, DOI: 10.25236/IJFM.2024.060904

| | | | | |
|---|---|---|---|---|
| CYP2C8 | Amodiaquine hydrochloride | Montelukast sodium | 19.3 | 80.7 |
| CYP2C9 | Diclofenac sodium | Sulfabenpyrazole | 34.3 | 65.7 |
| CYP2C19 | (S) - Mefentoin | Norcarone | 40.3 | 59.7 |
| CYP2D6 | Dextromethorphan hydrobromide | quinidine | 11.1 | 88.9 |
| CYP3A4 | testosterone | Ketoconazole | 17.6 | 82.4 |

***4.1.2 Test group***

*Table 3: Effects of isoenzyme inhibitors on VT204 metabolism*

| Isoenzymes | Compond | Mean % of Percentage of Remaining | Percentage of inhibition % |
|---|---|---|---|
| CYP1A2 | VT204 | 15.3 | 2.6 |
| CYP2B6 | | 19.9 | 7.6 |
| CYP2C8 | | 56.5 | 49.6 |
| CYP2C9 | | 28.9 | 17.6 |
| CYP2C19 | | 21.0 | 8.5 |
| CYP2D6 | | 19.3 | 6.5 |
| CYP3A4 | | 87.7 | 85.7 |

As shown in Table 3 and Figure 2, VT204 was inhibited to a certain extent, with ketoconazole (CYP3A4 inhibitor) being the main inhibitor, with an inhibition percentage of 85.7%, followed by montelukstat (CYP2C8 inhibitor, 49.6%), Sulfadioxazole (CYP2C9 inhibitor, 17.6%).

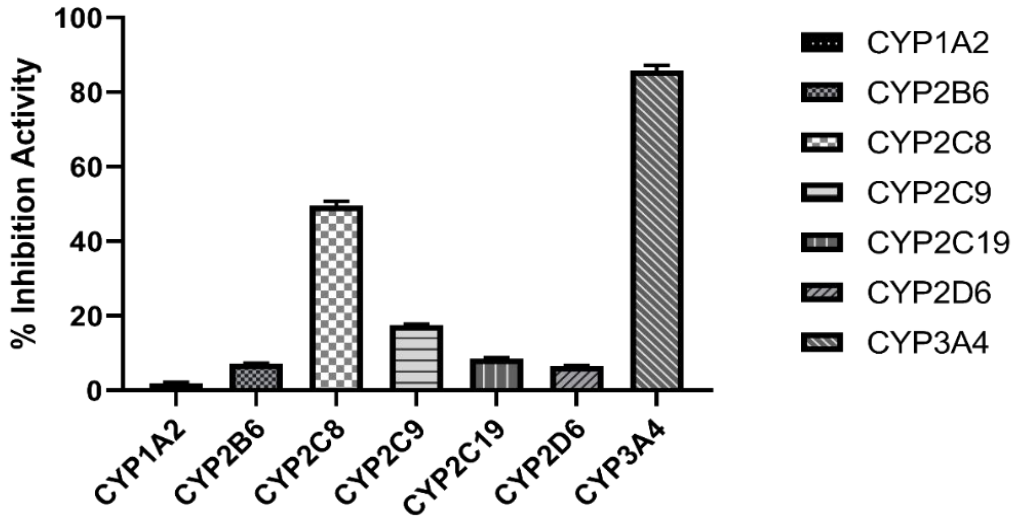


*Figure 2: Effect of isoenzyme inhibitors on VT204 metabolism*

ISSN 2706-6819 Vol.6, Issue 9: 20-28, DOI: 10.25236/IJFM.2024.060904

### *4.2 Recombinant enzymes*

#### *4.2.1 Systematic experimental group*

After the specific substrate was incubated with a recombinant enzyme for 60 minutes, the remaining percentage of the substrate in each group was shown in the table 4, which were rhCYP1A2 46.9%, rhCYP2B6 83.3%, rhCYP2C8 0.31%, rhCYP2C9 3.44% , rhCYP2C19 42.5% , rhCYP2D6 0.33% , rhCYP3A4 29.6 % . The results showed that the incubation system was reliable.

*Table 4: Metabolism of substrates in each isoform of recombinant enzymes in the systematic test group*

| Recombinase | Substrate | Mean % of Percentage of Remaining |
|---|---|---|
| rhCYP1A2 | Phenacetin | 46.9 |
| rhCYP2B6 | Bupropion hydrochloride | 83.3 |
| rhCYP2C8 | Amodiaquine hydrochloride | 0.31 |
| rhCYP2C9 | Diclofenac sodium | 3.44 |
| rhCYP2C19 | (S) - Mefentoin | 42.5 |
| rhCYP2D6 | Dextromethorphan hydrobromide | 0.33 |
| rhCYP3A4 | testosterone | 29.6 |

#### *4.2.2 Test group*

As shown in Table 5 and Figure 3, VT204 was metabolized to varying degrees after incubation with the addition of each recombinase. Except for rhCYP2C8 and rhCYP3A4, the remaining percentage of the original drug of the other five recombinant enzymes was above 90%, and rhCYP1A2 was as high as 99%, indicating that VT204 was almost not metabolized by these five isozymes, and after rhCYP3A4 incubation, VT204 was only 19.9%, followed by CYP2C8 (80.4%）.

*Table 5: Metabolism of VT204 in various isoforms of recombinant enzymes*

| Recombinase | Compond | Mean % of Remaining Percentage of Active Ingredient |
|---|---|---|
| rhCYP1A2 | VT204 | 99.0 |
| rhCYP2B6 | | 93.5 |
| rhCYP2C8 | | 80.4 |
| rhCYP2C9 | | 95.5 |
| rhCYP2C19 | | 94.5 |
| rhCYP2D6 | | 97.0 |
| rhCYP3A4 | | 19.9 |

ISSN 2706-6819 Vol.6, Issue 9: 20-28, DOI: 10.25236/IJFM.2024.060904

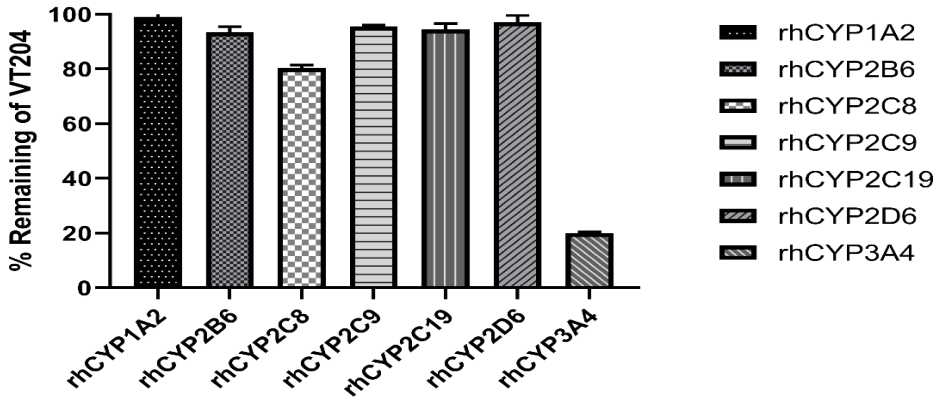


*Figure 3: Percentage of VT204 remaining after 60 groups of incubation in each isoform of recombinase*

## 5. Discussion

The expected effect of the drug depends on its concentration at the site of action, which is affected by the processes of absorption, distribution, metabolism and excretion in the body[9]. In these processes, metabolism plays an important role in drug disposition, with CYP450 representing the most important phase I drug-metabolizing enzyme for the biotransformation of xenodrugs[10].If the drug is likely to be cleared primarily through a metabolic pathway, there is a great risk of drug use, because if the clearance pathway is inhibited or induced by a combination drug, the former will be affected by DDI, and the treatment effect may be affected, thus increasing the failure rate of drug treatment. Therefore, the more enzymes involved in metabolism and the more metabolic pathways, the smaller the drug differences between patients and the less likely DDI will occur. Therefore, phenotypic identification of CYP450 enzyme metabolism of drug candidates is of great significance.

There are four commonly used methods for enzyme phenotype identification, including chemical inhibitor method, antibody inhibitor method, recombinant enzyme method and correlation analysis method.Each of these four methods has its own advantages and disadvantages, and it is recommended to use two or more methods at the same time to identify the results obtained more accurately[11].In this experiment, chemical inhibition was first used, but the selection of chemical inhibitors needs to consider a variety of factors, because most inhibitors not only inhibit one metabolic enzyme subtype; In order to solve the problem of selectivity of chemical inhibitors, recombinant enzymatic method was added for identification.Recombinant enzyme methods can identify whether a particular enzyme can metabolize drug candidates or generate specific metabolites, and is useful for studying allele metabolism candidates[12].

This trial examined CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6,There were seven CYP450 subtypes of CYP3A4 enzymes, and the results of chemical inhibition and recombinant enzymatic assay showed that VT204 may be mainly metabolized by CYP3A4. The results suggest that VT204 may interact with the two isoenzyme inducers and inhibitors in clinical combination, and the experimental results can provide a reference for the clinical use of VT204.

## 6. Conclusion

In this study, the main metabolic enzyme of VT204 in human liver microsomes was determined by chemical inhibition and recombinant enzyme method, and CYP2C8 and CYP2C9 may be involved in the metabolic process of VT204.

## Author's contribution

Liang Tan and Shu Zhang designed and performed experiments. Xiaochuan Li is responsible for supervising the experiment. Liang Tan and LiHua Qin jointly wrote the first draft of the article. Xiaoqun Duan supervised the study, interpreted the data and provided funds for the study. All the authors strictly

ISSN 2706-6819 Vol.6, Issue 9: 20-28, DOI: 10.25236/IJFM.2024.060904

reviewed the research plan and manuscript, and approved the final manuscript.

**References**

*[1] SIEGEL R L, MILLER K D. Cancer statistics, 2022 [J]. CA: a cancer journal for clinicians, 2022, 72(1): 7-33.*
*[2] SUNG H, FERLAY J, SIEGEL R L, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries [J]. Ca-a Cancer Journal for Clinicians, 2021, 71(3): 209-49.*
*[3] CHEN P X, LIU Y H, WEN Y K, et al. Non-small cell lung cancer in China [J]. Cancer Communications, 2022, 42(10): 937-70.*
*[4] YANG X, ZHANG S, YANG Y, et al. VT204: A Potential Small Molecule Inhibitor Targeting KRASG12C Mutation for Therapeutic Intervention in Non-Small Cell Lung Cancer [J]. Technology in cancer research & treatment, 2024, 23: 15330338241264853.*
*[5] LI X, CHEN N, JIANG C. Progress in Research on Targeted KRAS Protein Inhibitors [J]. Journal of China Pharmaceutical University, 2024, 55(02): 257-69.*
*[6] LU S, JIA C Y, YANG J S. Future therapeutic implications of new molecular mechanism of colorectal cancer [J]. World Journal of Gastroenterology, 2023, 29(16): 2359-68.*
*[7] KOZAKAI K, YAMADA Y, OSHIKATA M, et al. Cocktail-substrate Approach-based High-throughput Assay for Evaluation of Direct and Time-dependent Inhibition of Multiple Cytochrome P450 Isoforms [J]. Drug Metabolism and Pharmacokinetics, 2014, 29(2): 198-207.*
*[8] BACHMANN K A, LEWIS J D. Predicting inhibitory drug-drug interactions and evaluating drug interaction reports using inhibition constants [J]. The Annals of pharmacotherapy, 2005,39(6): 1064-72.*
*[9] DA SILVA R M, CARRAO D B, HABENSCHUS M D, et al. Prediction of seriniquinone-drug interactions by in vitro inhibition of human cytochrome P450 enzymes [J]. Toxicology in Vitro, 2020, 65.*
*[10] ALAGGA A A, PELLEGRINI M V, GUPTA V. Drug Absorption [M]. StatPearls. Treasure Island (FL) ineligible companies. Disclosure: Mark Pellegrini declares no relevant financial relationships with ineligible companies.; StatPearls PublishingCopyright © 2024, StatPearls Publishing LLC. 2024.*
*[11] LIU H. Qualitative and quantitative methods for identifying the liver cytochrome P450 enzyme phenotype that mediates the metabolism of candidate drugs. [J]. Chinese Journal of Pharmacology and Toxicology, 2013, 27(04): 760-5.*
*[12] OGILVIE B W. In Vitro Approaches for Studying the Inhibition of Drug-Metabolizing Enzymes and Identifying the Drug-Metabolizing Enzymes Responsible for the Metabolism of Drugs (Reaction Phenotyping) with Emphasis on Cytochrome P450 [J]. Drug Drug Interactions, 2008.*