Gene polymorphisms and drug–drug interactions determine the metabolic profile of blonanserin

Feng Ye¹, Xinyue Li¹, Jinhuan Ni¹, Xiaoyu Xu¹, Jianchao Luo¹, Yunshan Zhong¹, Yahui Wang¹, Shiyu Wang¹, Yuqing Zhang¹, Guoxin Hu¹*, Jianchang Qian¹*

¹ Institute of Molecular Toxicology and Pharmacology, School of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou 325035, Zhejiang, China

Corresponding authors:

Jianchang Qian, Email address: qianjc@wmu.edu.cn

AND

Guoxin Hu, Email address: hgx@wmu.edu.cn
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Abstract

This study aimed to evaluate the effects of cytochrome P450 3A4 (CYP3A4) gene polymorphism and drug interaction on the metabolism of blonanserin. Human recombinant CYP3A4 was prepared using the Bac-to-Bac baculovirus expression system. A microsomal enzyme reaction system was established, and drug–drug interactions were evaluated using Sprague–Dawley rats. Ultra-performance liquid chromatography–tandem mass spectrometry was used to detect the concentrations of blonanserin and its metabolite. Compared with wild-type CYP34A, the relative clearance of blonanserin by CYP3A4.29 significantly increased to 251.3%, while it decreased notably with CYP3A4.4, 5, 7, 8, 9, 10, 12, 13, 14, 16, 17, 18, 23, 24, 28, 31, 33, and 34, ranging from 6.09% to 63.34%. Among 153 tested drugs, nimodipine, felodipine, and amlodipine were found to potently inhibit the metabolism of blonanserin. Moreover, the inhibitory potency of nimodipine, felodipine, and amlodipine varied with different CYP3A4 variants. The half-maximal inhibitory concentration and enzymatic kinetics assay demonstrated that the metabolism of blonanserin was noncompetitively inhibited by nimodipine in rat liver microsomes, and was inhibited in a mixed manner by felodipine and amlodipine in both rat liver microsomes and human liver microsomes. When nimodipine and felodipine were co-administered with blonanserin, the AUC(0–t), AUC(0–∞), and Cmax of blonanserin increased. When amlodipine and blonanserin were combined, the Cmax of blonanserin C increased remarkably. The vast majority of CYP3A4 variants have a low ability to catalyze blonanserin. With combined administration of nimodipine, felodipine, and amlodipine, the elimination of blonanserin was inhibited. This study provides the basis for individualized clinical use of blonanserin.

Keywords: CYP3A4; blonanserin; calcium-channel blocker; drug–drug interaction
Abbreviations: ACN, acetonitrile; AUC, area under the blood concentration–time curve; CCB, calcium channel blocker; CL\textsubscript{int}, intrinsic clearance; CL\textsubscript{z/F}, blood clearance; C\textsubscript{max}, maximum blood concentration; CYP450, cytochrome P450; CYP3A4, cytochrome P450 3A4; DAS, drug and statistics; DDIs, drug–drug interactions; HLM, human liver microsome; IC\textsubscript{50}, half-maximal inhibitory concentration; Ki, inhibition constant; Km, Michaelis–Menten constant; NADPH, nicotinamide adenine dinucleotide phosphate; PBS, phosphate-buffered saline; RLM, rat liver microsome; SPSS, Statistical Package for Social Sciences; t\textsubscript{1/2z}, elimination half-life; T\textsubscript{max}, peak time; UPLC-MS/MS, ultra-performance liquid chromatography–tandem mass spectrometry; V\textsubscript{max}, maximum velocity of the reaction; V\textsubscript{z/F}, apparent volume of distribution.
Significance statement

The enzyme kinetics of novel CYP3A4 enzymes for metabolizing blonanserin were investigated. Clearance of blonanserin by CYP3A4.4, 5, 7–10, 12–14, 16–18, 23–24, 28, 31, 33, and 34 decreased notably, but increased with CYP3A4.29. Additionally, we established a drug interaction spectrum for blonanserin, in which nimodipine, felodipine, and amlodipine kinetics exhibited mixed inhibition. Moreover, their inhibitory potencies decreased with CYP3A4.4 and 5 compared to CYP3A4.1. This study provides essential data for personalized clinical use of blonanserin.
1. Introduction

Blonanserin is a second-generation antipsychotic drug used to treat schizophrenia (Murasaki et al., 2021). It has a high affinity and selective antagonism to dopamine D2/D3 and serotonin 5-HT2A receptors (Kitamura et al., 2021). Owing to its limited influence on lipidemia and blood glucose, blonanserin is widely used for long-term treatment of schizophrenia patients who have anxiety, hyperlipidemia, and hyperglycemia (Takeuchi et al., 2015; Sawagashira et al., 2022). However, its adverse reactions, including extrapyramidal side effects, heart-related abnormalities, akathisia, and elevated serum prolactin levels, negatively affect patient quality of life (Furuse and Hashimoto, 2010; Suzuki and Gen, 2012; Li et al., 2015). In the view of pharmacokinetics, an increase in blood concentrations is a major cause of adverse reactions, and therefore identifying potential influencing factors can provide reference for its individualized clinical application.

Blonanserin is rapidly absorbed orally, with a peak blood concentration reached within two hours (Deeks and Keating, 2010; Chen et al., 2014). Its plasma protein binding rate is exceptionally high (∼99.7%). The main routes of excretion for blonanserin are urine (59%) and feces (30%). In urine, the drug is primarily found in the form of metabolites, whereas in feces, only 5% is in its original drug form (Deeks and Keating, 2010). CYP3A4 is its predominant metabolic enzyme, which catalyzes the formation of blonanserin C (N-desethyl blonanserin) (Deeks and Keating, 2010; Wen et al., 2012). Importantly, CYP3A4 exhibits significant genetic polymorphisms, which affect the metabolic characteristics of clinical drugs (Zhou and Lauschke, 2022). Studies on this include the association of CYP3A4*1B with tacrolimus (Luo et al., 2016), midazolam (He et al., 2005), and omeprazole (Favela-Mendoza et al., 2018). The association between CYP3A4*20 and the pharmacokinetics profile of irinotecan was also reported (Riera et al., 2016).
2018). Therefore, the genetic polymorphism of CYP3A4 may influence the systemic exposure of blonanserin as well. In addition, drug induction or inhibition is also a key factor influencing the activity of CYP3A4 (Hakkola et al., 2020). Patients with schizophrenia often receive multiple medications and combination therapies, leading to widespread occurrences of CYP3A4 induction and inhibition (Du et al., 2009; Wolff-Menzler et al., 2010). However, there are limited reports on the drug interactions involving blonanserin, and therefore it is essential to establish its drug interaction profile.

In this study, we investigated the enzyme kinetics of multiple CYP3A4 variants in metabolizing blonanserin and obtained corresponding characteristic parameters. These variants include previously reported $CYP3A4^*1$, *3, *18, as well as novel alleles discovered by our research group, such as $CYP3A4^*28$–*34 (Hu et al., 2017). Additionally, we established a drug interaction profile of blonanserin using a liver microsome incubation system, clarified the corresponding inhibition types, and validated them in vivo. These research findings will provide foundational data for the rational clinical application of blonanserin.

2. Materials and methods

2.1 Chemicals and reagents

Blonanserin and blonanserin C were purchased from Shanghai Macklin Biotech Co., Ltd. Midazolam was purchased from Jiangsu Nhwa Pharmaceutical Co., Ltd. The detailed information on 153 drugs can be found in Table S1. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was obtained from Sigma-Aldrich Company (St. Louis, Missouri, USA). High-performance liquid chromatography (HPLC)-grade organic solvent was from Merck.
Ultrapure water was prepared by a Milli-Q A10 purification system (Billerica, MA, USA). Rat liver microsomes and human liver microsomes were purchased from Corning Life Science Co., Ltd. (New York, USA).

2.2 Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) conditions

A UPLC-MS/MS was equipped with Waters UPLC BEHC 18 columns (2.1 mm × 50 mm, 1.7-μm particle size). The temperature of the column and the automatic sampler rack were maintained at 40°C and 4°C, respectively. The mobile phase consisted of 0.1% formic acid (A) and methanol (B), and the gradient elution was carried out at 0.4 mL/min for 6 min. The following gradual gradient elution procedures were used: 90% A (0–0.2 min), 90%–10% A (0.2–2.0 min), 10% A (2.0–2.5 min), 10%–90% A (2.5–2.7 min), and 90% A (2.7–6.0 min). Quantitative analysis was carried out by a Waters XEVO TQD triple-quadruple mass spectrometer. Multiple-reaction monitoring was selected in the positive mode to detect the analytes. The ion pairs of blonanserin, blonanserin C, and midazolam (internal standard) were m/z 368.3 → 297.3, m/z 340.24 → 297.14, and m/z 326.1 → 291.0, respectively, as shown in Supplementary Figure 1 (Wen et al., 2012; Zheng et al., 2014).

2.3 Recombinant human CYP3A4 enzyme kinetics study

Recombinant CYP3A4 variants were expressed using a baculovirus insect cell expression system as described previously (Fang et al., 2017; Zhou et al., 2019). The volume of the incubation system was 200 μL, which included 1 pmol CYP3A4, 7.5 μg/mL cytochrome b5, 1 mM NADPH, 5–200 μM blonanserin, and phosphate-buffered saline (PBS). The mixture was preincubated without NADPH at 37°C for 5 min, and then 1 mM NADPH was added to start the reaction. After
optimizing the conditions, the reaction time was finally set to 40 min, at which point the response value of the metabolites reached a plateau. After 40 min of incubation, the sample was cooled to −80°C to terminate the reaction. A total of 300 μL of acetonitrile and 20 μL of midazolam (1 μg/mL) were added to the mixture. After melting, the mixture was vortexed for 2 min, and then centrifuged at 13,000 rpm for 10 min. The supernatant was collected for UPLC-MS/MS analysis.

2.4 Screening the drug–drug interaction spectrum of blonanserin

To screen drugs that could inhibit the metabolism of blonanserin, a microsomal enzymatic reaction was used. The system consisted of PBS buffer, 0.2 mg/mL RLMs, 100 μM of each drug (Supplementary Table 1), 1 mM NADPH, and blonanserin, at a final volume of 200 μL. The concentration of blonanserin used in the reaction system was determined by the Kₘ in the RLMs and HLMs (Supplementary Figure 2). After incubation, the samples were prepared and subjected to UPLC-MS/MS determination.

2.5 Evaluation of the inhibitory effects of nimodipine, felodipine, and amlodipine on the metabolism of blonanserin

To evaluate the IC₅₀ of nimodipine, felodipine, and amlodipine inhibition of blonanserin metabolism, an enzymatic reaction system was prepared consisting of blonanserin; an inhibitor (nimodipine, felodipine, or amlodipine); PBS buffer; 0.2 mg/mL RLMs, HLMs, or 1 pmol CYP3A4; 7.5 μg/mL cytochrome b5; and 1 mM NADPH. The concentrations of nimodipine, amlodipine, and felodipine were 0, 0.01, 0.1, 1, 10, 25, 50, and 100 μM. The concentrations of blonanserin were 45 μM in the RLMs, 15 μM in the HLMs, 15 μM in CYP3A4.1, 25 μM in CYP3A4.4, and 20 μM in CYP3A4.5. Samples were processed as described in Section 2.3.

2.6 Kinetics of inhibition
To clarify the type of interaction, we first conducted IC\textsubscript{50} shift experiments to determine whether the inhibition was time-dependent (Xu et al., 2023). The results showed that the IC\textsubscript{50} (−NADPH) to IC\textsubscript{50} (+NADPH) ratio of nimodipine, amlodipine, and felodipine in the rat liver was less than 1.5, indicating that the inhibition was not time-dependent (Supplementary Figure 3).

To determine the inhibitory mode, the concentrations of blonanserin were 11.25, 22, 45, and 90 μM in the RLMs, and 4, 8, 16, and 32 μM in the HLMs. In the RLMs, the concentrations of nimodipine were 0, 6, 12, and 24 μM based on the IC\textsubscript{50}; the concentrations of felodipine were 0, 10, 20, and 40 μM; and the concentrations of amlodipine were 0, 10, 20, and 40 μM. In the HLMs, the concentrations of nimodipine were 0, 10, 20, and 40 μM; the concentrations of felodipine were 0, 8, 16, and 24 μM; and the concentrations of amlodipine were 0, 8, 16, and 32 μM. The production of blonanserin C was determined using UPLC-MS/MS. We used GraphPad Prism 6.0 software to plot the Lineweaver–Burk graph for blonanserin (1/v against 1/[S]). Then, secondary replots of slope vs. [inhibitor] and intercept vs. [inhibitor] from the primary Lineweaver–Burk plot were performed to determine the inhibition constant (K\textsubscript{i}) and αK\textsubscript{i}, respectively. The inhibition type was ultimately determined based on the intersection of the Lineweaver–Burk plot and the value of α. The inhibition is considered a mixed type when α\neq 1, and noncompetitive inhibition when α=1.

2.7 In vivo experiment

Male Sprague-Dawley rats (250±10 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The animal experiment was approved by the Institution’s Animal Care and Use Committee or local equivalent. On the day before the experiment, the rats were subjected to an overnight fasting period, during which they were deprived of food but allowed free
access to water. Thirty rats were randomly divided into the following five groups (n=6 each group): group A orally received 0.8 mg/kg blonanserin; in group B, both nimodipine (24 mg/kg) and blonanserin (0.8 mg/kg) were given orally; in group C, felodipine (1 mg/kg) and blonanserin (0.8 mg/kg) were administered; group D received single-dose amlodipine (1 mg/kg) and blonanserin; and group E received multiple doses of amlodipine (1 mg/kg) for 1 week, and then blonanserin was given. The drugs were dissolved in oil and administered at a volume of 1 mL/kg. The inhibitor was administered 30 min prior to the dosing of blonanserin. Feeding was resumed 4 h after administration. Appropriate amounts of blood were collected from the caudal vein at 0.33, 0.67, 1.5, 2, 3, 4, 6, 8, 12, and 24 h for each group. Then, 50 μL of plasma was mixed with 150 μL of acetonitrile and 20 μL of midazolam (1 μg/mL). The mixture was vortexed for 2 min, then centrifuged at 13,000 rpm for 10 min. The supernatant was subjected to UPLC-MS/MS determination.

2.8 Statistical analysis

GraphPad Prism 6.0 was used primarily for graphing. Non-compartment model statistical moment parameters were analyzed using Drug and Statistics (DAS) software (Version 3.0, BontzInc, Beijing, China). All data were expressed as mean ± standard deviation. Dunnett’s tests and unpaired t-tests were performed using SPSS 26.0. \( P < 0.05 \) was considered as statistical significance.

3. Results

3.1 Kinetics profile of recombinant human CYP3A4 in catalyzing blonanserin metabolism

We investigated the enzymatic kinetic characteristics of blonanserin with different CYP3A4
variants. Figure 1A–F shows the Michaelis kinetic curves of each enzyme. The $V_{\text{max}}$, $K_m$, and intrinsic clearance ($CL_{\text{int}}$) were determined accordingly (Table 1). Compared to CYP3A4.1, the $V_{\text{max}}$ significantly decreased with CYP3A4.4, 5, 8, 9, 16, 18, 19, 23, 24, 33, and 34, while it markedly increased with CYP3A4.29. In terms of $K_m$ values, significant increases were observed with CYP3A4.4, 9, 19, 31, and 34. Next, the intrinsic clearance and relative clearance were calculated based on $V_{\text{max}}$ and $K_m$. Compared with CYP3A4.1, a vast majority of CYP3A4 variants were deficient in enzymatic activity (Figure 1G). Specifically, CYP3A4.20 showed no enzymatic activity, and the enzymatic activity of CYP3A4.4, 5, 7, 8, 9, 10, 12, 13, 14, 16, 17, 18, 23, 24, 28, 31, 33, and 34 decreased to different extents. In contrast, CYP3A4.29 was more active than CYP3A4.1. The variants CYP3A4.15, 3, and 32 showed no significant differences. Collectively, the data demonstrated that CYP3A4 genetic polymorphisms influenced the metabolism of blonanserin.

3.2 Identifying drugs that inhibit blonanserin metabolism in vitro

Next, we screened drugs that could inhibit the metabolism of blonanserin. A total of 153 drugs were investigated using a microsome enzymatic incubation assay, as shown in Figure 2A, Table S1. The results indicated that amlodipine, nimodipine, felodipine, nisoldipine, apatinib, ketoconazole, isavuconazole, and ritonavir remarkably inhibited the metabolism of blonanserin more than 80% (Figure 2B). Interestingly, there was no specific literature available on the interaction between nimodipine, felodipine, or amlodipine with blonanserin. Therefore, we evaluated their inhibitory capacity of blonanserin in vitro and in SD rats, and explored their inhibitory mechanisms in rat livers. The $IC_{50}$ of nimodipine, felodipine, and amlodipine was $11.99 \pm 0.35 \mu M$, $20.21 \pm 1.3 \mu M$, and $22.74 \pm 2.2 \mu M$, respectively, in the RLMs (Figure 2C), and
19.53 ± 0.30 μM, 16.99 ± 1.1 μM, and 8.33 ± 1.2 μM, respectively, in the HLMs (Figure 2D). The IC₅₀ of nimodipine increased significantly in HLMs (P < 0.001). In contrast, IC₅₀ values decreased in the HLMs for felodipine (P < 0.05) and amlodipine (P < 0.001).

As shown in Figure 2E–G, we also determined the inhibitory potencies of nimodipine, felodipine, and amlodipine with different CYP3A4 variants. The IC₅₀ of nimodipine was 11.78 ± 0.46 μM, 28.37 ± 4.72 μM, and 23.39 ± 1.38 μM with CYP3A4.1, CYP3A4.4, and CYP3A4.5, respectively. Nimodipine had the highest potency for inhibiting blonanserin metabolism with CYP3A4.1 compared with CYP3A4.4 and CYP3A4.5 (P < 0.001), and the trend was the same for the other two drugs. The IC₅₀ values of felodipine in the wild type, CYP3A4.4 and CYP3A4.5 were 7.21 ± 1.34 μM, 19.48 ± 0.31 μM, and 14.81 ± 0.70 μM, respectively, while those of amlodipine were 22.24 ± 2.21 μM, 51.15 ± 1.63 μM, and 50.27 ± 1.81 μM, respectively. These data suggest that the inhibitory potency of nimodipine, felodipine, and amlodipine decreased in CYP3A4.4 and 5 compared to CYP3A4.1.

3.3 Kinetics of nimodipine, felodipine, and amlodipine inhibition of blonanserin metabolism

in vitro

As shown in Figure 3A, the Lineweaver–Burk plot provided an unambiguous indication of non-competitive inhibition by nimodipine with an intersection on the x-axis and α=1. However, felodipine showed a mixed (noncompetitive and competitive) inhibitory type with α≠1 (Figure 3B). Amlodipine inhibited the catalysis reaction with a combination of noncompetitive and anti-competitive inhibition, as shown in Figure 3C. The Kᵢ values of nimodipine, felodipine, and amlodipine in RLMs were 11.09 μM, 3.651 μM, and 50.29 μM, respectively. The corresponding αKᵢ for nimodipine, felodipine, and amlodipine were 11.49, 24.51, and 28.58 μM, respectively.
We further investigated the inhibition type of these three drugs in HLMs. The results demonstrated that nimodipine, felodipine, and amlodipine both noncompetitively and competitively inhibited blonanserin metabolism with the intersection in the second quadrant, $\alpha \neq 1$, Figure 4A–C. The $K_i$ values of nimodipine, felodipine, and amlodipine in HLMs were 9.614 $\mu$M, 1.947 $\mu$M, and 28.32 $\mu$M, respectively. The $\alpha K_i$ values for nimodipine, felodipine, and amlodipine were 44.58, 18.74, and 49.04 $\mu$M, respectively.

3.4 Co-administration of nimodipine, felodipine, or amlodipine with blonanserin changes the pharmacokinetic profile of blonanserin in rats

Next, we investigated drug–drug interactions in vivo. We only selected male rats for research purposes on the metabolism of blonanserin in order to eliminate the influence of hormone level changes during the female rat’s physiological cycle. In order to eliminate the influence of food, the rats were subjected to fasting treatment prior to the experiment. The average concentration-time curve showed that after oral administration of nimodipine and blonanserin, the $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, and $C_{max}$ of blonanserin increased more than two times (Figure 5A, Tables 2 and 3). Moreover, the $T_{max}$ increased almost two-fold. In addition, the $V_{z/F}$ and $CL_{z/F}$ decreased significantly. As shown in Figure 5B, Tables 2 and 3, the $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of blonanserin C significantly increased, while the $V_{z/F}$ and $CL_{z/F}$ decreased dramatically. In the case of co-administration of felodipine and blonanserin, similar trends in the pharmacokinetics profile were observed (Figures 5A and B, and Tables 2–3). According to the semi-logarithmic concentration-time profile, we found that the terminal phase of N-desethyl blonanserin is parallel to the parent drug after co-administration of blonanserin with nimodipine and felodipine, as shown in Figure 5C. Furthermore, there is no significant difference in the half-life of blonanserin and blonanserin C between the groups.
Therefore, it’s indicated that the kinetics of the metabolite become elimination rate limited.

Moreover, we investigated the effect of multiple dosing of amlodipine on blonanserin metabolism. As shown in Figure 5D, no obvious changes in the pharmacokinetics parameters were found compared with the prototype drug. However, the $t_{1/2a}$ and $V_z/F$ of the metabolite were significantly reduced, and the $C_{\text{max}}$ significantly increased in both single and multiple-dosing groups (Figure 5E, Tables 2-3). Based on the semi-logarithmic concentration-time curve of the metabolite, it was observed that the slope of the elimination phase closely resembles that of the parent drug after multiple administrations of amlodipine and a single ingestion, Figure 5F. This indicates that the kinetics of the metabolite are primarily governed by the elimination rate, suggesting an elimination rate-limited metabolic process.

Additionally, when comparing the metabolic ratios, most of the parameters did not change significantly when co-administering nimodipine and felodipine, except for $T_{\text{max}}$ (Table 4). In the amlodipine group, there were differences in the changes of pharmacokinetic parameters between single and multiple doses. The $T_{\text{max}}$ significantly decreased in the single-dose group, while the half-life of blonanserin decreased significantly in the multiple-dose group, and $V_z/F$ also decreased more than three-fold.

4. Discussion

Many factors determine the pharmacokinetic characteristics of blonanserin, such as food, genetics, and drug–drug interactions (Shang et al., 2018). Among them, there is ample evidence in the literature that food can increase blonanserin systemic exposure, so it is recommended to take blonanserin after meals (Saruwatari et al., 2010; Chen et al., 2014; Shang et al., 2018). However, there are few reports on the influence of genetic factors and the drug interaction spectra. Herein,
we conducted research on these two factors. With the application of clinical pharmacogenetics research results, we can predict metabolic phenotypes based on genotypes, and then promote individual medical care (O'Shea et al., 2022). Nevertheless, there are clear gaps in the data chain. The association between the CYP3A4 genotype and metabolic phenotype is still insufficient (Waring, 2020). Herein, we determined the kinetic profile of CYP3A4 variants using blonanserin as the substrate. Similar to previous reports, the relative clearance of most CYP3A4 variants decreased significantly compared with the wild type (Yuan et al., 2023). In particular, CYP3A4.12, 13, 17, and 20 lost almost all enzymatic function (Han et al., 2021; Hu et al., 2022). Patients who carry these alleles are deficient in CYP3A4 activity. In contrast, the function of CYP3A4.29 significantly increased the metabolism of blonanserin. This is a newly discovered variant in the Chinese population, with a distribution frequency of 0.04% (Hu et al., 2017). This data provides fundamental information about the association between CYP3A4 genotypes and metabolic phenotypes.

In addition to genetic factors, drug–drug interaction leads to therapeutic stratification (Malki and Pearson, 2020). In this study, we screened drugs that inhibited the metabolism of blonanserin in vitro. Interestingly, calcium-channel blockers, including nimodipine, felodipine, amlodipine, were found to potently inhibit the disposition of blonanserin. In particular, nimodipine, and felodipine remarkably changed the pharmacokinetics profile of blonanserin in vivo. The comorbidity of schizophrenia and hypertension exists in clinical practice; therefore, the combined use of these two classes of medications is possible. We translated the clinically recommended doses into dosages suitable for rats and conducted in vivo studies.

Interestingly, when co-administered with nimodipine or felodipine, both the plasma exposure
of blonanserin and blonanserin C increased. We believe that improved bioavailability by inhibiting CYP3A4 activity in the gastrointestinal tract, inhibition of hepatic metabolism leading to decreased first-pass elimination, and a decrease in clearance (CL\textsubscript{z/F}) with the combination treatment were important contributing factors. Furthermore, the research findings suggest that metabolic drug-drug interactions do not have an impact on the volume of distribution (Sodhi et al., 2021). Therefore, the increase in bioavailability (F) resulted in a decrease in the pharmacokinetic parameter V\textsubscript{z/F}. This hypothesis is consistent with the results of this study, as we observed a significant reduction in V\textsubscript{z/F} with the drug combination. Moreover, there were no significant changes observed in the t\textsubscript{1/2} of blonanserin and its metabolites after co-administration of blonanserin with nimodipine or felodipine. Because CYP3A4 is predominantly distributed in the liver and intestines, the t\textsubscript{1/2} remains unchanged, suggesting that the interaction between blonanserin and nimodipine or felodipine may primarily occur in the gut. If the interaction only occurred in the liver, the t\textsubscript{1/2} of a drug would be prolonged (Bidstrup et al., 2006; Thummel, 2007). However, when metabolic enzymes are inhibited in the gut, the systemic exposure of a drug increased, but the inherent characteristic t\textsubscript{1/2} of a drug did not change significantly (Grenier et al., 2006).

Looking at the concentration-time curve of blonanserin C, the peak concentration was reached at 6 h and was almost completely eliminated after 24 h. The behavior of the N-desethyl metabolite parallels that of blonanserin, indicating that elimination is limited by the rate of production (Finkelstein, 1992; Sgaragli et al., 1995; Cho and Yoon, 2018). Furthermore, nimodipine, felodipine, and amlodipine do not alter the rate-limited elimination metabolic process. Because the t\textsubscript{1/2} of amlodipine is long, we evaluated the influence of single and multiple doses on
blonanserin metabolism. However, no obvious change was observed. *In vitro* IC₅₀ experiments showed that amlodipine had an inhibitory effect on substrates in both rat liver and human liver microsomes, with an inhibition rate of approximately 80%. However, further exploration of the inhibition mechanism revealed a large Ki value. This may be the reason why the *in vivo* inhibitory effect was not strong and there were no significant differences in pharmacokinetic parameters.

The inhibitory modes of nimodipine, felodipine, and amlodipine on blonanserin metabolism were also investigated. Although there were differences in RLMs and HLMs, a noncompetitive inhibitory mode was found among the three calcium-channel blockers. Additionally, the difference in inhibitory potency among three CYP3A4 variants, CYP3A4.1, CYP3A4.4, and CYP3A4.5, were studied. CYP3A4.4 and CYP3A4.5 are two variants with high distribution frequencies in the Asian population (Werk and Cascorbi, 2014). The data demonstrated that the inhibitory potency of CYP3A4.4 and CYP3A4.5 decreased compared with the wild type. Therefore, CYP3A4 genetic polymorphisms also influence the potency of drug–drug interactions. We only selected three genotypes for investigation, which cannot fully represent all genotypes. More research is still needed to provide substantial evidence.

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Data availability statement

The authors declare that all the data supporting the findings of this study are available within the paper and its Supplemental Data.

Authorship contributions.

Participated in research design: Guoxin Hu, Jianchang Qian.

Conducted experiments: Feng Ye, Xinyue Li, Jinhuan Ni, Xiaoyu Xu, Jianchao Luo, Yunshan Zhong, Yahui Wang, Shiyu Wang, Yuqing Zhang.

Performed data analysis: Feng Ye, Xinyue Li, Jinhuan Ni

Contributed to the writing of the manuscript: Guoxin Hu, Jianchang Qian, Feng Ye.

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Conflict of Interest: No conflict of interest needs to be declared.
Figure 1. Michaelis–Menten curves of CYP3A4 metabolism of blonanserin and relative clearance among CYP3A4 variants. A–F Enzymatic reactions were performed as indicated in the Methods section. Michaelis–Menten curves were plotted and nonlinear fitting was performed using Prism 5, with concentrations of blonanserin (5, 10, 20, 50, 100, 200 μM) as the x-axis and velocity (V, normalized by molar mass of CYP) as the y-axis. G V max and K m were obtained from Michaelis–Menten curves. Subsequently, the intrinsic clearance CL int was determined. The relative clearance rates of various variants were calculated and compared to CYP3A4.1 and plotted. Data are presented as mean ± SD, n = 3. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 2. The inhibitory effects of drugs on the metabolism of blonanserin were evaluated. A Rat liver microsomal enzymatic reactions were performed to determine drug–drug interactions with blonanserin. The production of blonanserin C was detected using UPLC-MS/MS, and % of control was determined and plotted. B Drugs with an inhibition rate > 80% were replotted. C and D The inhibition curves of nimodipine, felodipine, and amlodipine showing suppression of blonanserin metabolism in RLMs and HLMs. The enzymatic reaction was performed as indicated in Section 2.5. The data was plotted using Prism 5. Then, the IC 50 was determined using the equation Y=100/(1+10^(X−Log(IC 50))). E–G The inhibition of nimodipine, felodipine, and amlodipine in CYP3A4 microsomes on the metabolism of blonanserin. The IC 50 values of nimodipine, felodipine, and amlodipine on CYP3A4.1, 4, and 5 were determined. Data are presented as mean ± SD, n = 3. ***P < 0.001.

Figure 3. The inhibitory type in RLMs was determined. The kinetic inhibition of blonanserin by nimodipine (A), felodipine (B), and amlodipine (C) was evaluated as indicated in Section 2.6 of the Methods. The data are shown in a Lineweaver–Burk plot, the secondary replot of K i, and the
secondary plot of $\alpha K_i$. The $R^2$ values for the 0, 6, 12, and 24 μM nimodipine lines were 0.961, 0.963, 0.967, and 0.996, respectively. For the secondary replots of the slope and intercept, $R^2$ was 0.997 and 0.996, respectively. B $R^2$ values for the 0, 10, 20, and 40 μM felodipine lines were 0.901, 0.951, 0.961, and 0.978, respectively. For the secondary replots of the slope and intercepts, $R^2$ was 0.982 and 0.993, respectively. C $R^2$ values for the 0, 10, 20, and 40 μM amlodipine lines were 0.942, 0.961, 0.955, and 0.960, respectively. The secondary replots of the slope and intercept $R^2$ values were 0.989 and 0.998, respectively. Data are presented as mean ± SD, n = 3.

Figure 4. The inhibitory type in HLMs was determined. The Lineweaver–Burk plot, secondary plot for $K_i$, and secondary plot for $\alpha K_i$ for the inhibition of blonanserin metabolism by (A) nimodipine, (B) felodipine, and (C) amlodipine at various concentrations. Experimental procedures, sample preparation, and data analysis can be found in Section 2.6 of the Methods. The $R^2$ values for the 0, 10, 20, and 40 μM nimodipine lines were 0.982, 0.985, 0.997, and 0.999, respectively. The secondary replots of the slope and intercept $R^2$ values were 0.981 and 0.961, respectively. B $R^2$ for the 0, 8, 16, and 24 μM felodipine lines were 0.917, 0.985, 0.961, and 0.984, respectively. Secondary replots of the slope and intercept $R^2$ values were 0.966 and 0.961, respectively. C $R^2$ values for the 0, 8, 16, and 32 μM amlodipine lines were 1.000, 0.994, 0.995, and 0.978, respectively. The secondary replots of the slope and intercept $R^2$ values were 0.958 and 0.983, respectively. Data are presented as mean ± SD, n = 3.

Figure 5. Pharmacokinetic profile of blonanserin and blonanserin C in rats. Prior to the experiment, rats were subjected to fasting treatment, followed by drug administration, blood collection, and sample processing and analysis as described in Section 2.7 of the Methods. A graph was plotted with a blood collection time as the x-axis and blood drug concentration as the y-axis. A and B The
curves of blonanserin and blonanserin C when administered alone or in combination with nimodipine or felodipine. C Semi-logarithmic concentration-time curve of blonanserin and blonanserin C according to A and B. D and E The curves of blonanserin and blonanserin C when administered alone or in combination with amlodipine, either as a single dose or multiple doses. F Semi-logarithmic concentration-time curve of blonanserin and blonanserin C based on D and E.

Data are presented as mean ± SD, n = 6

Table 1 Enzymatic kinetic parameters of the metabolism of blonanserin by CYP3A4.

<table>
<thead>
<tr>
<th>variants</th>
<th>$V_{\text{max}}$ (pmol/min/pmol CYP)</th>
<th>$K_m$ (μM)</th>
<th>$\text{CL}<em>{\text{int}}$ ($V</em>{\text{max}}$/$K_m$) (μL/min/pmol CYP)</th>
<th>Relative clearance (% of wild type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1</td>
<td>1.663±0.037</td>
<td>15.78±0.74</td>
<td>0.105±0.005</td>
<td>100.0±4.9</td>
</tr>
<tr>
<td>*3</td>
<td>1.509±0.083</td>
<td>15.47±1.8</td>
<td>0.098±0.006</td>
<td>92.93±1.9</td>
</tr>
<tr>
<td>*4</td>
<td>1.181±0.037</td>
<td>23.93±0.54</td>
<td>0.049±0.001</td>
<td>46.78±0.46</td>
</tr>
<tr>
<td>*5</td>
<td>1.118±0.078</td>
<td>18.31±2.7</td>
<td>0.061±0.005</td>
<td>58.29±1.5</td>
</tr>
<tr>
<td>*7</td>
<td>1.087±0.11</td>
<td>39.23±9.4</td>
<td>0.028±0.004</td>
<td>26.83±1.7</td>
</tr>
<tr>
<td>*8</td>
<td>1.157±0.058</td>
<td>53.84±7.9</td>
<td>0.022±0.002</td>
<td>20.57±0.94</td>
</tr>
<tr>
<td>*9</td>
<td>1.371±0.047</td>
<td>31.92±2.3</td>
<td>0.043±0.002</td>
<td>40.82±1.8</td>
</tr>
<tr>
<td>*10</td>
<td>1.366±0.099</td>
<td>46.12±7.3</td>
<td>0.030±0.002</td>
<td>28.31±2.3</td>
</tr>
<tr>
<td>*12</td>
<td>2.144±0.59</td>
<td>308.9±91</td>
<td>0.007±0.000</td>
<td>6.615±0.31</td>
</tr>
<tr>
<td>*13</td>
<td>1.979±0.44</td>
<td>214.6±70</td>
<td>0.010±0.001</td>
<td>9.019±0.44</td>
</tr>
<tr>
<td>*14</td>
<td>1.516±0.24</td>
<td>55.27±20</td>
<td>0.029±0.005</td>
<td>27.13±4.8</td>
</tr>
<tr>
<td>*15</td>
<td>1.645±0.032</td>
<td>15.45±1.4</td>
<td>0.107±0.008</td>
<td>101.5±7.1</td>
</tr>
<tr>
<td>*16</td>
<td>1.208±0.014</td>
<td>29.79±3.7</td>
<td>0.041±0.004</td>
<td>38.77±2.6</td>
</tr>
<tr>
<td>*17</td>
<td>1.620±0.32</td>
<td>258.5±79</td>
<td>0.006±0.001</td>
<td>6.088±0.49</td>
</tr>
<tr>
<td>*18</td>
<td>1.023±0.086</td>
<td>42.69±5.6</td>
<td>0.024±0.001</td>
<td>22.81±0.84</td>
</tr>
<tr>
<td>*19</td>
<td>1.188±0.021</td>
<td>26.40±0.54</td>
<td>0.045±0.002</td>
<td>42.66±0.59</td>
</tr>
<tr>
<td>*20</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>*23</td>
<td>1.142±0.054</td>
<td>21.73±2.6</td>
<td>0.053±0.004</td>
<td>50.08±1.8</td>
</tr>
<tr>
<td>*24</td>
<td>1.105±0.022</td>
<td>32.87±2.8</td>
<td>0.034±0.002</td>
<td>32.00±2.2</td>
</tr>
<tr>
<td>*28</td>
<td>1.261±0.086</td>
<td>28.22±4.8</td>
<td>0.045±0.006</td>
<td>42.93±4.3</td>
</tr>
<tr>
<td>*29</td>
<td>2.773±0.080</td>
<td>10.51±1.1</td>
<td>0.265±0.019</td>
<td>251.3±18</td>
</tr>
<tr>
<td>*31</td>
<td>1.254±0.012</td>
<td>31.25±1.8</td>
<td>0.040±0.002</td>
<td>38.12±1.1</td>
</tr>
<tr>
<td>*32</td>
<td>1.658±0.030</td>
<td>18.19±2.8</td>
<td>0.092±0.013</td>
<td>87.66±7.6</td>
</tr>
<tr>
<td>*33</td>
<td>1.206±0.028</td>
<td>18.20±2.2</td>
<td>0.067±0.007</td>
<td>63.34±4.9</td>
</tr>
<tr>
<td>*34</td>
<td>1.039±0.020</td>
<td>39.49±2.9</td>
<td>0.026±0.002</td>
<td>25.05±0.99</td>
</tr>
</tbody>
</table>

Note: Compared with the wild type, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. ND, not determined. ↑, ↓.
indicates increase; ↓ indicates decrease.
<table>
<thead>
<tr>
<th></th>
<th>Blonanserin</th>
<th>Blonanserin+nimodipine</th>
<th>Blonanserin+felodipine</th>
<th>Blonanserin+amlodipine (single dose)</th>
<th>Blonanserin+amlodipine (multiple dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUC</strong>&lt;sub&gt;(0–t)&lt;/sub&gt; (μg/L·h)</td>
<td>150.66±38.08</td>
<td>313.08±93.81**</td>
<td>245.50±15.61***</td>
<td>193.80±28.41</td>
<td>146.98±25.53</td>
</tr>
<tr>
<td><strong>AUC</strong>&lt;sub&gt;(0–∞)&lt;/sub&gt; (μg/L·h)</td>
<td>152.63±38.04</td>
<td>317.49±95.07**</td>
<td>247.67±16.78***</td>
<td>196.03±26.28</td>
<td>149.07±25.66</td>
</tr>
<tr>
<td><strong>t&lt;sub&gt;1/2z&lt;/sub&gt;</strong> (h)</td>
<td>3.28±1.14</td>
<td>2.95±1.14</td>
<td>2.88±0.68</td>
<td>2.59±1.16</td>
<td>3.58±1.01</td>
</tr>
<tr>
<td><strong>T&lt;sub&gt;max&lt;/sub&gt;</strong> (h)</td>
<td>3.50±1.23</td>
<td>6.00±0.00**</td>
<td>6.00±1.27**</td>
<td>5.17±2.23</td>
<td>3.67±1.37</td>
</tr>
<tr>
<td><strong>V&lt;sub&gt;z/F&lt;/sub&gt;</strong> (L/kg)</td>
<td>26.62±13.68</td>
<td>11.01±4.34**</td>
<td>13.41±2.91</td>
<td>16.04±9.42</td>
<td>28.61±10.83</td>
</tr>
<tr>
<td><strong>CL&lt;sub&gt;z/F&lt;/sub&gt;</strong> (L/h/kg)</td>
<td>5.50±1.27</td>
<td>2.76±1.01**</td>
<td>3.24±0.21**</td>
<td>4.14±0.56</td>
<td>5.51±1.01</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;max&lt;/sub&gt;</strong> (μg/L)</td>
<td>18.60±4.97</td>
<td>40.33±15.81**</td>
<td>32.95±8.92**</td>
<td>28.17±6.58</td>
<td>24.93±9.45</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Blonanserin</th>
<th>Blonanserin+nimodipine</th>
<th>Blonanserin+felodipine</th>
<th>Blonanserin+amlodipine (single dose)</th>
<th>Blonanserin+amlodipine (multiple doses)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUC</strong>&lt;sub&gt;(0–t)&lt;/sub&gt; (μg/L·h)</td>
<td>29.35±7.49</td>
<td>56.80±19.02**</td>
<td>43.58±9.31**</td>
<td>39.27±15.17</td>
<td>36.48±10.16</td>
</tr>
<tr>
<td><strong>AUC</strong>&lt;sub&gt;(0–∞)&lt;/sub&gt; (μg/L·h)</td>
<td>30.75±7.39</td>
<td>58.91±19.39**</td>
<td>45.28±9.38*</td>
<td>40.22±15.54</td>
<td>36.55±10.25</td>
</tr>
<tr>
<td><strong>t&lt;sub&gt;1/2z&lt;/sub&gt;</strong> (h)</td>
<td>4.70±0.63</td>
<td>4.23±0.64</td>
<td>4.32±0.49</td>
<td>3.63±0.22**</td>
<td>1.84±0.61***</td>
</tr>
<tr>
<td><strong>T&lt;sub&gt;max&lt;/sub&gt;</strong> (h)</td>
<td>6.00±1.27</td>
<td>6.00±1.27</td>
<td>6.33±0.82</td>
<td>6.33±0.82</td>
<td>5.50±1.23</td>
</tr>
<tr>
<td><strong>V&lt;sub&gt;z/F&lt;/sub&gt;</strong> (L/kg)</td>
<td>185.72±52.13</td>
<td>92.72±43.08**</td>
<td>115.51±32.90**</td>
<td>117.98±43.22**</td>
<td>58.77±13.51**</td>
</tr>
<tr>
<td><strong>CL&lt;sub&gt;z/F&lt;/sub&gt;</strong> (L/h/kg)</td>
<td>27.02±5.02</td>
<td>14.93±5.16**</td>
<td>18.33±3.86**</td>
<td>22.59±8.75</td>
<td>23.58±7.56</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;max&lt;/sub&gt;</strong> (μg/L)</td>
<td>3.02±0.93</td>
<td>6.30±2.53**</td>
<td>4.72±1.24**</td>
<td>4.97±1.88</td>
<td>4.92±1.42***</td>
</tr>
</tbody>
</table>

*<i>P</i> < 0.05, **<i>P</i> < 0.01, ***<i>P</i> < 0.001 compared with the control group. AUC: area under the blood concentration–time curve; **<i>t</i> <sub>1/2z</sub>: elimination half-life; **<i>T</i> <sub>max</sub>: peak time; **<i>V</i> <sub>z/F</sub>: apparent volume of distribution; **<i>CL</i> <sub>z/F</sub>: blood clearance; **<i>C</i> <sub>max</sub>: maximum blood concentration. †, indicates increase; †† indicates decrease.
### Table 3 Ratio of pharmacokinetic parameters between each combination group and the single-use blonanserin group (with inhibitor/without inhibitor ratio).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Blonanserin</th>
<th>Blonanserin+Nimodipine</th>
<th>Blonanserin+Felodipine</th>
<th>Blonanserin+Amlodipine (Single dose)</th>
<th>Blonanserin+Amlodipine (multiple doses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(0–t) (μg/L·h)</td>
<td>1.00±0.25</td>
<td>2.08±0.62**†</td>
<td>1.63±0.10***†</td>
<td>1.29±0.19</td>
<td>0.98±0.17</td>
</tr>
<tr>
<td>AUC(0–∞) (μg/L·h)</td>
<td>1.00±0.25</td>
<td>2.08±0.62**†</td>
<td>1.62±0.11***†</td>
<td>1.28±0.17*†</td>
<td>0.98±0.17</td>
</tr>
<tr>
<td>t_{1/2z} (h)</td>
<td>1.00±0.35</td>
<td>0.90±0.35</td>
<td>0.88±0.21</td>
<td>0.79±0.35</td>
<td>1.09±0.31</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>1.00±0.35</td>
<td>1.71±0.00**†</td>
<td>1.71±0.36**†</td>
<td>1.48±0.64</td>
<td>1.05±0.39</td>
</tr>
<tr>
<td>V_{z/F} (L/kg)</td>
<td>1.00±0.51</td>
<td>0.41±0.16*</td>
<td>0.50±0.11</td>
<td>0.60±0.35</td>
<td>1.07±0.41</td>
</tr>
<tr>
<td>CL_{z/F} (L/h/kg)</td>
<td>1.00±0.23</td>
<td>0.50±0.18**†</td>
<td>0.59±0.04**†</td>
<td>0.75±0.10*†</td>
<td>1.00±0.18</td>
</tr>
<tr>
<td>C_{max} (μg/L)</td>
<td>1.00±0.27</td>
<td>2.17±0.85**†</td>
<td>1.77±0.48**†</td>
<td>1.51±0.35*†</td>
<td>1.34±0.51</td>
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</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Blonanserin C</th>
<th>Blonanserin+Nimodipine</th>
<th>Blonanserin+Felodipine</th>
<th>Blonanserin+Amlodipine (Single dose)</th>
<th>Blonanserin+Amlodipine (multiple doses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(0–t) (μg/L·h)</td>
<td>1.00±0.26</td>
<td>1.94±0.65*†</td>
<td>1.48±0.32**†</td>
<td>1.34±0.52</td>
<td>1.24±0.35</td>
</tr>
<tr>
<td>AUC(0–∞) (μg/L·h)</td>
<td>1.00±0.24</td>
<td>1.92±0.63**†</td>
<td>1.47±0.31**†</td>
<td>1.31±0.51</td>
<td>1.19±0.33</td>
</tr>
<tr>
<td>t_{1/2z} (h)</td>
<td>1.00±0.13</td>
<td>0.90±0.14</td>
<td>0.92±0.10</td>
<td>0.77±0.05***†</td>
<td>0.39±0.13***†</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>1.00±0.21</td>
<td>1.00±0.21</td>
<td>1.06±0.14</td>
<td>1.06±0.14</td>
<td>0.92±0.20</td>
</tr>
<tr>
<td>V_{z/F} (L/kg)</td>
<td>1.00±0.28</td>
<td>0.50±0.23**†</td>
<td>0.62±0.18*†</td>
<td>0.64±0.23*†</td>
<td>0.32±0.07***†</td>
</tr>
<tr>
<td>CL_{z/F} (L/h/kg)</td>
<td>1.00±0.19</td>
<td>0.55±0.19**†</td>
<td>0.68±0.14**†</td>
<td>0.84±0.32</td>
<td>0.87±0.28</td>
</tr>
<tr>
<td>C_{max} (μg/L)</td>
<td>1.00±0.31</td>
<td>2.09±0.84**†</td>
<td>1.56±0.41*†</td>
<td>1.65±0.62</td>
<td>1.63±0.47*†</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001 in comparison with the control group. AUC: area under the blood concentration–time curve; t_{1/2z}: elimination half-life; T_{max}: peak time; V_{z/F}: apparent volume of distribution; CL_{z/F}: blood clearance; C_{max}: maximum blood concentration. † indicates increase; ‡ indicates decrease.
Table 4 Ratio of the major pharmacokinetic parameters of blonanserin and blonanserin C in each group (metabolite/parent ratio).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Blonanserin</th>
<th>Blonanserin+Nimodipine</th>
<th>Blonanserin+Felodipine</th>
<th>Blonanserin+Amlodipine (Single dose)</th>
<th>Blonanserin+Amlodipine (multiple doses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(0–t) (μg/L·h)</td>
<td>0.19±0.05</td>
<td>0.18±0.06</td>
<td>0.18±0.04</td>
<td>0.20±0.08</td>
<td>0.25±0.07</td>
<td></td>
</tr>
<tr>
<td>AUC(0–∞) (μg/L·h)</td>
<td>0.20±0.05</td>
<td>0.19±0.06</td>
<td>0.18±0.04</td>
<td>0.21±0.08</td>
<td>0.25±0.07</td>
<td></td>
</tr>
<tr>
<td>t1/2z (h)</td>
<td>1.43±0.19</td>
<td>1.43±0.22</td>
<td>1.50±0.17</td>
<td>1.40±0.09</td>
<td>0.51±0.17***</td>
<td></td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.71±0.36</td>
<td>1.00±0.21**</td>
<td>1.06±0.14**</td>
<td>1.23±0.16**</td>
<td>1.50±0.33</td>
<td></td>
</tr>
<tr>
<td>Vz/F (L/kg)</td>
<td>6.98±1.96</td>
<td>8.42±3.91</td>
<td>8.61±2.45</td>
<td>7.36±2.69</td>
<td>2.05±0.47**</td>
<td></td>
</tr>
<tr>
<td>CLz/F (L/h/kg)</td>
<td>4.91±0.91</td>
<td>5.41±1.87</td>
<td>5.65±1.19</td>
<td>5.45±2.11</td>
<td>4.28±1.37</td>
<td></td>
</tr>
<tr>
<td>Cmax (μg/L)</td>
<td>0.16±0.05</td>
<td>0.16±0.06</td>
<td>0.14±0.04</td>
<td>0.18±0.07</td>
<td>0.20±0.06</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001 in comparison with the control group. AUC: area under the blood concentration–time curve; t1/2z: elimination half-life; Tmax: peak time; Vz/F: apparent volume of distribution; CLz/F: blood clearance; Cmax: maximum blood concentration. ↓, indicates decrease.
Figure 1

A, B, C, D, E, F, G graphs showing the concentration of Bionanserin (μM) on the x-axis and the rate of Bionanserin C (pmol/min/pmol CYP) on the y-axis. Each graph contains multiple lines representing different concentrations of Bionanserin for various samples labeled 3A4*1, 3A4*3, 3A4*4, 3A4*5, 3A4*7, 3A4*8, 3A4*9, 3A4*10, 3A4*12, 3A4*13, 3A4*14, 3A4*15, 3A4*16, 3A4*17, 3A4*18, 3A4*19, and 3A4*23.

G graph showing the relative clearance (%) of CYP3A4.1 with asterisks indicating statistical significance.
Figure 2

A drugbank

Production of BNA (\% of control)

A

B

Production of BNA (\% of control)

Control
Amiodarone
Nimodipine
Fenoldopim
Nifedipine
Amodipine
Laranolactone
Kynurenic acid

***

C

RLM

Nimodipine IC_{50}=11.99 \pm 0.35 \mu M
Felodipine IC_{50}=20.21 \pm 1.25 \mu M
Amodipine IC_{50}=22.74 \pm 2.16 \mu M

D

HLM

Nimodipine IC_{50}=19.53 \pm 0.39 \mu M
Felodipine IC_{50}=16.99 \pm 1.14 \mu M
Amodipine IC_{50}=8.33 \pm 1.16 \mu M

E

CYP3A4.1

Nimodipine IC_{50}=11.78 \pm 0.45 \mu M
Felodipine IC_{50}=7.21 \pm 1.34 \mu M
Amodipine IC_{50}=22.24 \pm 2.21 \mu M

F

CYP3A4.4

Nimodipine IC_{50}=28.37 \pm 4.72 \mu M
Felodipine IC_{50}=19.48 \pm 0.31 \mu M
Amodipine IC_{50}=51.15 \pm 1.63 \mu M

G

CYP3A4.5

Nimodipine IC_{50}=23.39 \pm 1.38 \mu M
Felodipine IC_{50}=14.81 \pm 0.70 \mu M
Amodipine IC_{50}=50.27 \pm 1.81 \mu M

Relative activity (% of control)

log (inhibitor)

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Figure 4

A

HLM

1/(μmol/mg protein) vs. 1/[Blonanserin concentration, μM]

B

HLM

1/(μmol/mg protein) vs. 1/[Blonanserin concentration, μM]

C

HLM

1/(μmol/mg protein) vs. 1/[Blonanserin concentration, μM]
Figure 5