Synthesis, Antibacterial and Pharmacokinetic Evaluation of Novel Derivatives of Harmine N\(^9\)-Cinnamic Acid

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Abstract: A series of 16 new derivatives of harmine N\(^9\)-Cinnamic acid were synthesized and fully characterized using NMR and MS. The in vitro antibacterial evaluation revealed that most of the synthesized harmine derivatives displayed better antibacterial activities against Gram-positive strains (S. aureus, S. albus and MRSA) than Gram-negative strains (E. coli and PA). In particular, compound 3c showed the strongest bactericidal activity with a minimum inhibitory concentration of 13.67 µg/mL. MTT assay showed that compound 3c displayed weaker cytotoxicity than harmine with IC\(_{50}\) of 340.30, 94.86 and 161.67 µmol/L against WI-38, MCF-7 and HepG2 cell lines, respectively. The pharmacokinetic study revealed that the distribution and elimination of 3c in vivo were rapid in rats with an oral bioavailability of 6.9%.

Keywords: harmine; synthesis; antibacterial activity; pharmacokinetics

1. Introduction

With the increasing use of antibiotics in humans and agriculture, the resistance has spread worldwide. Meanwhile, the decreasing effectiveness of antibiotics in treating common infections has quickened [1–3]. After the golden age of the antibiotic discovery between 1929 and the 1970s, the development of new antibiotics has stalled. Since then, few new antibiotics have reached the market [4–9]. As a consequence, the development of novel and efficient antibiotics is of great significance.

Harmine (Figure 1), a naturally existing tricyclic \(\beta\)-carboline alkaloid, has been discovered and isolated from the seeds of Peganum harmala L. by Gobel in 1841 [10–12]. Harmine displays a wide range of pharmacological activities, including antimicrobial, antitumor, anti-inflammatory, antidepressant, antioxidant, neuroprotective effects and so on [13,14]. It is reported that the extracts of P. harmala seeds and roots exhibit good antibacterial activities [15,16]. The literature indicated that harmine and its derivatives insert into DNA of microorganism as a highly aromatic planar alkaloid [11]. At present, harmine derivatives are known for their antibacterial activity and researchers are focusing on studying new antibacterial compounds which are more effective than the drugs now in use [17,18].

![Figure 1. Chemical structure of harmine and cinnamic acid.](https://example.com/harmine-cinnamic.png)
Cinnamic acid (Figure 1) is isolated from cinnamon or benzoin resin. Cinnamic acid showed good antibacterial activities in vivo and in vitro [19–22]. It is reported that cinnamic acid exerted its antibacterial activity mainly by an action mode of membrane disruption [21,23]. Because of its low molecular weight and low price, cinnamic acid is often combined with other compounds to exert antibacterial activity synergistically in the design of new antibacterial drugs [21,24,25]. Using the molecular assembly method, a series novel pleuromutilin derivations bearing cinnamic acids moieties were synthesized which showed remarkable antibacterial activity [25]. Narasimhan and coauthor’s research indicated that the antibacterial activity of synthesized ester derivatives of cinnamic acid was stronger than that of amide [26]. Furthermore, it is reported that the different substituents on the benzene ring of cinnamic acid had an important effect on its antibacterial activity [27].

In this work, based on the combination principle, a series of harmine derivatives with diverse length linkers were synthesized by covalent bonding between N9 of harmine and carboxylic acid of cinnamic acids. We further preliminarily investigated the antibacterial activities and cytotoxic activity of the synthesized compounds in vitro. Finally, we performed pharmacokinetics on one of the screened compounds.

2. Result and Discussion

2.1. Chemistry

The synthesis route of target compounds is shown in Scheme 1. Firstly, in order to explore the effect of chain length on biological activity, dibromoalkanes with different carbon chain lengths used as an electrophilic agent, easily attacked the N9 position of harmine (1) to yield intermediates 2a–d. Subsequently, intermediates 2a–d were combined with substituted cinnamic acid by a nucleophilic substitution reaction to obtain 16 new target compounds (3a–d, 4a–d, 5a–d, 6a–d).

Scheme 1. A facile route to the synthesis of target compounds. Reagents and conditions: (i) NaH (1.5 equiv.), Br(CH2)nBr (3 equiv.), dry DMF, dry THF, reflux, 3 h, 66.1–70.5%; (ii) Substituted cinnamic acid (0.33 equiv.), Et3N (5–10 drops), acetone, rt, 3 h, 21.1–52.1%.

The structure information of all synthesis compounds were confirmed by nuclear magnetic resonance spectra (NMR), including 13C and 1H-NMR, and electrospray ionization high-resolution mass spectrometry (ESI-HRMS) ([13C and 1H-NMR spectra of compounds 3a–d, 4a–d, 5a–d, 6a–d had been added in Supplementary Material, Figures S1–S16). Fur-
thermore, a colorless crystal of compound 4a was obtained by slow evaporation of a mixed solution (ethyl acetate:petroleum ether = 1:4). The crystal structures of compound 4a are shown in Figure 2. Crystal data for compound 4a were collected in Tables S1–S3.

Figure 2. ORTEP diagram for compound 4a with ellipsoids set at 75% probability.

2.2. Evaluation of the Antibacterial Activity

According to microplate dilution method, all the prepared compounds were screened for their in vitro antibacterial activity. The investigation included three representative Gram-positive strains, including Staphylococcus aureus CICC 10384 (S. aureus), Staphylococcus albus CICC 20237 (S. albus) and methicillin-resistant Staphylococcus aureus ATCC 43300 (MRSA), and two representative Gram-negative strains, including Escherichia coli CICC 10389 (E. coli) and Pseudomonas aeruginosa CVCC 3359 (PA). Harmine and cinnamic acid were used as reference drugs. The results of minimum inhibitory concentration (MICs) are presented in Table 1. It showed that most of the synthesized harmine derivatives displayed better antibacterial activities against Gram-positive strains (S. aureus, S. albus and MRSA) than Gram-negative strains (E. coli and PA). Notably, Compounds 3c and 5a displayed attractive antibacterial activities than other derivatives.

Because compounds 3c and 5a displayed promising antibacterial activity, we further evaluated their in vitro time-kill assay. The bactericidal properties of 3c and 5a were compared at 1 × MIC and 6 × MIC against S. aureus (the MICs of 3c, 5a and harmine were 13.67, 15.63, 31.25 µg/mL, respectively) (Figure 3). As shown in Figure 3, 3c and 5a showed outstanding antibacterial activity and displayed a concentration-dependent effect. Compound 3c with 1 × MIC concentration achieved a 4-log10 reduction in about 4 h and 1 × MIC of compound 5a showed a 4-log10 reduction in about 24 h. Notably, Compound 3c with 6 × MIC concentration completely killed the bacteria within the first 0.5 h and 6 × MIC of compound 5a completely killed the bacteria within 12 h. However, 1 × MIC of harmine just slowed bacterial propagation and its 6 × MIC achieved a 4-log10 reduction in about 24 h. In general, compared to harmine, 3c and 5a showed more rapid bactericidal kinetics against S. aureus with the same concentration (1 × MIC).
Table 1. Minimum inhibitory concentration of the synthesized harmine derivatives.

| Compound | MIC (µg/mL) | Gram-Positive Bacteria | Gram-Negative Bacteria |
|----------|-------------|------------------------|------------------------|
|          |             | S. aureus | S. albus | MRSA | E. coli | PA |
| 3a       | 325         | 650       | 1300     | 650  | 325    |
| 3b       | 318.75      | 637.5     | 637.5    | 637.5| 318.75 |
| 3c       | 13.67       | 13.67     | 54.69    | 218.75| 218.75 |
| 3d       | 181.25      | 725       | 362.5    | 725  | 362.5  |
| 4a       | 305         | 305       | 610      | 152.5| 305    |
| 4b       | 112.5       | 225       | 450      | 450  | 450    |
| 4c       | 1550        | 1550      | 1550     | 775  | 387.5  |
| 4d       | 26.56       | 53.13     | 106.25   | 212.5| 212.5  |
| 5a       | 15.63       | 15.63     | 62.5     | 250  | 125    |
| 5b       | 1138        | 2275      | 2275     | 1137.5| 1137.5 |
| 5c       | 110         | 110       | 220      | 220  | 220    |
| 5d       | 23.83       | 95.3      | 95.31    | 762.5| 381.25 |
| 6a       | 82.81       | 165.63    | 331.25   | 662.5| 331.25 |
| 6b       | 152.5       | 152.5     | 305.5    | 305  | 305    |
| 6c       | 375         | 750       | 1500     | 750  | 750    |
| 6d       | 53.13       | 53.13     | 212.5    | 212.5| 212.5  |
| Harmine  | 31.25       | 15.63     | 62.5     | 15.63| 15.63  |
| Cinnamic acid | 1125     | 1125     | 1125     | 1125 | 1125   |
| DMSO     | -           | -         | -        | -    | -      |

Figure 3. Time-kill kinetics of compound 3c (A) and 5a (B) at 1 × and 6 × MIC against S. aureus (CICC 10384).

2.3. In Vitro Cytotoxic Activity

In vitro cytotoxic activity of 3c was evaluated using MTT (3-(4,5)-dimethylthiaziolium bromide) assay using WI-38 (Human embryonic lung fibroblasts), MCF-7 (human breast cancer cells) and HepG2 (human liver carcinoma cells). Harmine was used as a control. The calculated IC₅₀ (half maximal inhibitory concentration) values of 3c and harmine are presented in Table 2. It was observed that all the tested compounds showed weaker cytotoxic activity against normal cell than cancer cell lines. Notably, the IC₅₀ of compound 3c was higher than that of harmine against three cell lines, which means the cytotoxic activity of compound 3c was weaker than that of harmine. These combined results suggested that although a nitro group existed in compound 3c, its cytotoxic activity had not been improved, which showed that the further study of 3c is meaningful.
Table 2. Inhibition effect of target compounds in three cell lines. (n = 3; Mean ± SD).

| Compound | IC₅₀ (μmol/L) |
|----------|---------------|
|          | WI-38 | MCF-7 | HepG2 |
| 3c       | 340.30 ± 2.79 | 94.86 ± 3.64 | 161.67 ± 1.31 |
| Harmine  | 103.40 ± 0.96 | 78.88 ± 2.82 | 67.61 ± 1.60 |

2.4. In Vivo Pharmacokinetic Study

Considering the excellent antibacterial activity, we further evaluated the in vivo pharmacokinetics of compound 3c. The mean plasma concentration time curves of 3c after intravenous (i.v.) and intragastric (i.g.) administration are shown in Figure 4. Additionally, the estimated non-compartmental parameters are displayed in Table 3.

![Figure 4](image-url)

**Figure 4.** Mean plasma concentration-time profile after a single intravenous administration of 10 mg/kg 3c solution to rats (A) and after a single oral dose of 40 mg/kg 3c solution (B) (n = 6, mean ± SE).

Table 3. The non-compartmental model pharmacokinetic parameters in rats after a single intravenous injection (10 mg/kg) and intragastric administration (40 mg/kg) of compound 3c. (n = 6; Mean ± SD).

| Pharmacokinetics Parameters | i.v. | i.g. |
|----------------------------|------|------|
| Cmax (ng/mL)               | 836.67 ± 229.45 | 158.73 ± 59.61 |
| Tmax (h)                   | 0.083 ± 0.030 | 0.25 ± 0.033 |
| T1/2 (h)                   | 2.27 ± 0.62 | 1.30 ± 0.47 |
| CL/F (L/h·kg)              | 0.031 ± 0.03 | 0.26 ± 0.10 |
| MRT (h)                    | 2.8585 ± 0.71 | 3.3322 ± 0.83 |
| AUC₀→t (ng·h/mL)           | 1050.59 ± 229.31 | 284.24 ± 54.36 |
| AUC₀→∞ (ng·h/mL)          | 1053.24 ± 231.06 | 290.25 ± 55.17 |

Cmax, maximum concentration; Tmax, time to reach Cmax; T1/2, half-life; CL/F, Clearance rate; MRT, mean resident time; AUC₀→t, Area under the curve from zero to the last measurable plasma concentration; AUC₀→∞, Area under the curve, from the last measurable plasma concentration to infinity.

After a single i.v. and i.g. administration of 3c, the plasma concentration of the drug declined quickly (Figure 4). After 12 h, the compound 3c was almost completely eliminated. These data demonstrated that the distribution and elimination of 3c in vivo were relatively fast. Additionally, compound 3c displayed great individual differences among animals in vivo. Furthermore, the AUC₀→∞ of compound 3c after a single i.g. administration (290.25 ng·h/mL) was only 6.9% of the same dose of i.v. administration (1053.24 ng·h/mL). These results indicated that compound 3c had a significant first pass effect, which need to be paid attention in the further research. In addition, the pharmacokinetic data showed that the plasma concentration of 3c was much lower than its MIC. It reminded us that
we should increase the dosage in the follow-up pharmacodynamics study to achieve an effective plasma concentration.

3. Materials and Methods

3.1. General Information

All chemical reagents were purchased from Energy Reagent Co., Ltd. (Anqing, China) or Shanghai Huangdi Chemical Co., Ltd. (Shanghai, China). Unless otherwise noted, all commercially available reagents were used directly after they were received. In order to monitor the reaction, thin-layer chromatography (TLC) was performed on silica gel plate (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China) and visualized by UV light. All melting points were determined on a digital X4 melting point analyzer (Henan Ruike Medical Instrument Co., Ltd., Xinxiang, China) using open capillary tubes and were uncorrected. HRMS were obtained with an ESI-Brucker APEX II49e mass spectrometer (Bruker, Billerica, MA, USA). $^1$H-NMR and $^{13}$C-NMR spectra were recorded with JNM-ECS (JEOL Co., Ltd., Tokyo, Japan) and Bruke AM-400 spectrometer (Bruker, Billerica, MA, USA), respectively. The solvents used were deuterated reagents (DMSO-$_d$$_6$ and CDCl$_3$). Unless otherwise noted, further purification of products was performed via column chromatography on silica gel (200–300 mesh, Gansu Yihua chemical Glass Instrument Co., Lanzhou, China) and the products were eluted in an appropriate solvent mixture under air pressure. The crystal of the typical compound was obtained by solvent evaporation method and determined by Super Nova single crystal diffractometer (Agilent, Santa Clara, CA, USA).

3.2. Synthesis

General procedure for synthesis of compounds 2a–d. To a round-bottom flask 7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (1) (5 mmol) was added in the mixed solvent of DMF and THF (DMF:17 mL, THF:15 mL), stirred at room temperature until clear. NaH (7.5 mmol) was added in batches, stirred for 10 min at room temperature. Then, dibromoalkanes (15 mmol) were added to the mixture, followed by heating and refluxing. The reaction was monitored by TLC during the whole process. After the mixture fully reacted, the mixture was cooled with ice water, and extracted three times with ethyl acetate. The mixture was added with ether and saturated NaCl solution, after being dried over anhydrous Na$_2$SO$_4$, the mixture was filtered and concentrated. The crude product was purified by silica gel column to obtain 2a–d (dichloromethane:methanol = 10:1, triethylamine 1‰).

9-(3-Bromopropyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2a). Compound 2a was prepared according to the general procedure from 7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (1) and 1,3-dibromopropane. White solid, 68.8% yield. $^1$H-NMR (400 MHz, CDCl$_3$) δ: 8.67 (d, $J = 5.4$ Hz, 1H), 8.02 (d, $J = 8.7$ Hz, 1H), 7.87 (d, $J = 5.4$ Hz, 1H), 6.86–6.79 (m, 2H), 4.36 (t, $J = 7.4$ Hz, 2H), 3.91 (s, 3H), 3.51 (t, $J = 6.4$ Hz, 2H), 2.98 (s, 3H), 1.98–1.87 (m, 2H).

9-(4-Bromobutyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2b). Compound 2b was prepared according to the general procedure from 7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (1) and 1,4-dibromobutane. White solid, 70.1% yield.$^1$H-NMR (400 MHz, CDCl$_3$) δ: 8.21 (d, $J = 5.2$ Hz, 1H), 7.98 (d, $J = 8.6$ Hz, 1H), 7.77 (d, $J = 5.2$ Hz, 1H), 6.97–6.80 (m, 2H), 4.54 (t, $J = 7.5$ Hz, 2H), 3.95 (s, 3H), 3.49 (t, $J = 6.4$ Hz, 2H), 3.01 (s, 3H), 1.96–1.81 (m, 4H).

9-(5-Bromopentyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2c). Compound 2c was prepared according to the general procedure from 7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (1) and 1,5-dibromopentane. White solid, 68.3% yield. $^1$H-NMR (400 MHz, CDCl$_3$) δ: 8.29 (d, $J = 4.8$ Hz, 1H), 7.99 (d, $J = 8.6$ Hz, 1H), 7.74 (d, $J = 5.4$ Hz, 1H), 6.95–6.81 (m, 2H), 4.49 (t, $J = 7.4$ Hz, 2H), 3.96 (s, 3H), 3.41 (t, $J = 6.4$ Hz, 2H), 3.02 (s, 3H), 1.98–1.82 (m, 4H), 1.59–1.44 (m, 2H).
9-(6-Bromohexyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2d). Compound 2d was prepared according to the general procedure from 7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (1) and 1,6-dibromohexane. White solid, 66.1% yield. 1H-NMR (400 MHz, CDCl₃): δ: 8.29 (d, J = 5.4 Hz, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 4.0 Hz, 1H), 6.96–6.79 (m, 2H), 4.48 (t, J = 7.5 Hz, 2H), 3.96 (s, 3H), 3.40 (t, J = 6.4 Hz, 2H), 3.02 (s, 3H), 1.89–1.78 (m, 4H), 1.51–1.39 (m, 4H).

General procedure for synthesis of compounds 3a–d, 4a–d, 5a–d, 6a–d. To a round-bottom flask with a magnetic stirrer substituted cinnamic acid (1 mmol) in acetone (8 mL), triethylamine were added dropwise (5–10 drops), and stirred until completely dissolved. Compound 2a–d (3 mmol) was dissolved and dropped into the above reaction solution, stirred at room temperature for 3 h. The reaction was monitored by TLC during the whole process. After the mixture fully reacted, aqueous HCl was added to the solution to adjust pH to 3–5. The mixture was extracted three times with ethyl acetate. The organic phase was then dried over anhydrous Na₂SO₄, filtered and concentrated. Compounds 3a–d, 4a–d, 5a–d, 6a–d were obtained by further purification using silica gel column chromatography (dichloromethane:methanol = 10:1, triethylamine 1%).

3-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl)propyl(E)-3-(4-methoxyphenyl) acrylate (3a). Compound 3a was prepared according to the general procedure from (E)-3-(4-methoxyphenacyl)acrylic acid and 9-(3-bromopropyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2a). Yellow solid. 52.1% yield; mp 133.2–133.9 °C; 1H-NMR (400 MHz, CDCl₃): 8.29 (d, J = 5.2 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 5.2 Hz, 1H), 7.65 (d, J = 16.0 Hz, 1H), 7.49 (d, J = 8.8 Hz, 2H), 6.94–6.87 (m, 4H), 6.29 (d, J = 16.0 Hz, 1H), 4.65 (t, J = 7.6 Hz, 2H), 4.28 (t, J = 5.8 Hz, 2H), 3.91 (s, 3H), 3.86 (s, 3H), 3.06 (s, 3H), 2.28–2.22 (m, 2H); 13C-NMR (100 MHz, CDCl₃): 166.97, 161.53, 160.89, 144.97, 142.95, 140.47, 138.48, 135.12, 129.76, 129.48, 122.35, 115.21, 114.79, 114.33, 112.22, 108.82, 93.21, 61.42, 55.58, 55.34, 41.79, 29.86, 23.35; ESI-MS: calcd. for C₂₆H₂₆NO₄ [M + H]⁺ 431.1965; found 431.1955.

3-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl)propyl (E)-3-(3,4,5-trimethoxyphenyl) acrylate (3b). Compound 3b was prepared according to the general procedure from (E)-3-(3,4,5-trimethoxyphenacyl)acrylic acid and 9-(3-bromopropyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2a). White solid. 49.2% yield; m.p.: 102.6–104.2 °C; 1H-NMR (400 MHz, CDCl₃): 8.29 (d, J = 5.2 Hz, 1H), 7.96 (d, J = 9.2 Hz, 1H), 7.73 (d, J = 5.2 Hz, 1H), 7.58 (d, J = 16.0 Hz, 1H), 6.90 (s, 2H), 6.76 (s, 2H), 6.31 (d, J = 16.0 Hz, 1H), 4.64 (t, J = 7.4 Hz, 2H), 4.29 (t, J = 5.8 Hz, 2H), 3.92 (s, 9H), 3.90 (s, 3H), 3.05 (s, 3H), 2.28–2.22 (m, 2H); 13C-NMR (100 MHz, CDCl₃): 166.75, 161.05, 153.56, 145.42, 143.12, 140.54, 140.44, 138.59, 135.28, 129.73, 122.54, 116.68, 115.41, 112.40, 108.90, 105.41, 93.48, 61.80, 61.08, 56.28, 55.77, 41.97, 31.82, 29.95, 29.36, 23.45; ESI-MS: calcd. for C₂₆H₃₀N₂O₆ [M + H]⁺ 491.2177; found 491.2171.

4-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl)propyl(E)-3-(2-nitrophenyl) acrylate (3c). Compound 3c was prepared according to the general procedure from (E)-3-(2-nitrophenacyl)acrylic acid and 9-(3-bromopropyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2a). Yellow solid. 44.2% yield; m.p.: 128.6–129.3 °C; 1H-NMR (400 MHz, CDCl₃): 8.29 (d, J = 5.2 Hz, 1H), 8.14 (d, J = 16.0 Hz, 1H), 8.08 (d, J = 7.2 Hz, 1H), 7.97 (d, J = 9.2 Hz, 1H), 7.73–7.64 (m, 3H), 7.60–7.56 (m, 1H), 6.90–6.87 (m, 2H), 6.33 (d, J = 16.0 Hz, 1H), 4.67 (t, J = 7.4 Hz, 2H), 4.33–4.29 (m, 2H), 3.92 (s, 3H), 3.05 (s, 3H), 2.30–2.24 (m, 2H); 13C-NMR (100 MHz, CDCl₃): 165.67, 160.75, 148.18, 142.94, 140.40, 139.88, 138.10, 138.15, 135.16, 130.41, 130.19, 129.26, 128.98, 124.79, 123.03, 122.25, 115.09, 112.14, 108.53, 93.31, 64.62, 55.60, 44.66, 28.47, 23.31; ESI-MS: calcd. for C₂₅H₂₂N₂O₅ [M + H]⁺ 446.1710; found 446.1716.
3-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl) propyl (E)-2-(3,4-dimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl) acrylate (3d). Compound 3d was prepared according to the general procedure from (E)-2-(3,4-dimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl) acrylic acid and 9-(3-bromopropyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2a). White solid. 21.14% yield; m.p.: 173.6–175.2 °C; 1H-NMR (400 MHz, CDCl₃) δ: 8.28 (d, J = 5.2 Hz, 1H), 7.95 (d, J = 8.4 Hz, 1H), 7.71 (d, J = 5.2 Hz, 1H), 7.67 (s, 1H), 6.95 (d, J = 8.4 Hz, 1H), 6.89–6.83 (m, 2H), 6.80 (d, J = 9.2 Hz, 2H), 3.63 (s, 2H), 2.45 (t, J = 7.6 Hz, 2H), 4.33 (t, J = 5.8 Hz, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 3.83 (s, 6H), 3.60 (s, 6H), 2.95 (s, 3H), 2.24–2.19 (m, 2H); 13C-NMR (100 MHz, CDCl₃) δ: 167.73, 152.65, 149.46, 148.86, 143.00, 150.64, 140.37, 139.16, 138.38, 135.11, 130.86, 129.40, 122.50, 115.32, 112.92, 112.33, 111.68, 108.62, 108.17, 93.45, 62.51, 60.88, 55.75, 55.69, 41.99, 29.78, 23.24; ESI-MS: calcd. for C₃₆H₃₈N₂O₈ [M + H]+ 627.2701; found 627.2701.

4-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl) butyl (E)-3-(4-methoxyphenyl) acrylate (4a). Compound 4a was prepared according to the general procedure from (E)-3-(4-methoxyphenyl) acrylic acid and 9-(4-bromobutyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2b). White solid. 43.6% yield; m.p.: 118.7–120.2 °C; 1H-NMR (400 MHz, CDCl₃) δ: 8.29 (d, J = 5.2 Hz, 1H), 7.98 (d, J = 9.6 Hz, 1H), 7.74 (d, J = 5.2 Hz, 1H), 7.63 (d, J = 16.0 Hz, 1H), 7.46 (d, J = 8.8 Hz, 2H), 6.91–6.88 (m, 4H), 6.29 (d, J = 16.0 Hz, 1H), 4.54 (t, J = 7.6 Hz, 2H), 4.25 (t, J = 6.4 Hz, 2H), 3.94 (s, 3H), 3.84 (s, 3H), 3.03 (s, 3H), 2.01–1.94 (m, 2H), 1.867–1.80 (m, 2H); 13C-NMR (100 MHz, CDCl₃) δ: 167.21, 161.44, 160.88, 144.76, 142.95, 140.44, 138.38, 135.21, 129.75, 129.42, 126.95, 122.39, 115.23, 115.12, 114.30, 112.26, 108.66, 93.37, 63.47, 55.68, 55.67, 44.38, 27.18, 26.13, 23.41; ESI-MS: calcd. for C₂₇H₂₉N₂O₄ [M + H]+ 445.2111; found 445.2116.

4-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl) butyl (E)-3-(3,4,5-trimethoxyphenyl) acrylate (4b). Compound 4b was prepared according to the general procedure from (E)-3-(3,4,5-trimethoxyphenyl) acrylic acid and 9-(4-bromobutyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2b). Yellow solid. 49.0% yield; m.p.: 95.7–96.3 °C; 1H-NMR (400 MHz, CDCl₃) δ: 8.29 (d, J = 5.2 Hz, 1H), 7.99 (d, J = 9.2 Hz, 1H), 7.75 (d, J = 5.2 Hz, 1H), 7.59 (d, J = 16.0 Hz, 1H), 6.91–6.89 (m, 2H), 6.74 (s, 2H), 6.31 (d, J = 16.0 Hz, 1H), 4.55 (t, J = 7.8 Hz, 2H), 4.26 (t, J = 6.2 Hz, 2H), 3.94 (s, 3H), 3.89 (s, 9H), 3.03 (s, 3H), 2.02–1.95 (m, 2H), 1.87–1.80 (m, 2H); 13C-NMR (100 MHz, CDCl₃) δ: 166.83, 160.87, 153.40, 145.08, 142.95, 140.41, 140.19, 138.40, 135.21, 129.69, 129.44, 122.42, 116.87, 115.26, 112.28, 108.61, 105.23, 93.43, 63.67, 60.93, 56.13, 55.69, 44.37, 27.19, 26.12, 23.42; ESI-MS: calcd. for C₂₉H₂₉N₂O₄ [M + H]+ 505.2333; found 505.2339.

4-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl) butyl (E)-3-(2-nitrophenyl) acrylate (4c). Compound 4c was prepared according to the general procedure from (E)-3-(2-nitrophenyl) acrylic acid and 9-(4-bromobutyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2b). White solid. 43.6% yield; m.p.: 130.9–132.2 °C; 1H-NMR (400 MHz, CDCl₃) δ: 8.28 (d, J = 5.2 Hz, 1H), 8.11 (d, J = 15.6 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 9.2 Hz, 1H), 7.73 (d, J = 5.2 Hz, 1H), 7.67–7.60 (m, 2H), 7.56–7.52 (m, 1H), 6.90–6.87 (m, 2H), 6.34 (d, J = 16.0 Hz, 1H), 4.54 (t, J = 7.8 Hz, 2H), 4.27 (t, J = 6.4 Hz, 2H), 3.94 (s, 3H), 3.03 (s, 3H), 2.01–1.93 (m, 2H), 1.87–1.80 (m, 2H); 13C-NMR (100 MHz, CDCl₃) δ: 165.62, 160.88, 148.22, 142.94, 140.41, 140.38, 138.36, 135.17, 133.49, 130.40, 130.33, 129.43, 129.08, 124.88, 122.73, 122.37, 115.20, 112.24, 108.69, 93.34, 64.07, 55.67, 44.32, 27.16, 26.03, 23.38; ESI-MS: calcd. for C₂₆H₂₅N₃O₅ [M + H]+ 460.1867; found 460.1865.
5-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl) amyl (E)-3-(4-methoxyphenyl) acrylate (5a). Compound 5a was prepared according to the general procedure from (E)-3-(4-methoxyphenyl) acrylic acid and 9-(5-bromopentyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2c). White solid. 46.2% yield; m.p.: 91.1–92.3 °C; 1H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ: 8.17 (d, J = 5.2 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 5.2 Hz, 1H), 7.66 (d, J = 8.8 Hz, 2H), 7.57 (d, J = 16.0 Hz, 1H), 7.20 (s, 1H), 6.98 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.6 Hz, 1H), 6.44 (d, J = 16.0 Hz, 1H), 4.57 (t, J = 7.6 Hz, 2H), 4.13 (t, J = 6.6 Hz, 2H), 3.91 (s, 3H), 3.81 (s, 3H), 2.96 (s, 3H), 1.82–1.74 (m, 2H), 1.72–1.69 (m, 2H); 13C-NMR (100 MHz, CDCl<sub>3</sub>) δ: 167.84, 161.86, 152.62, 149.30, 148.72, 144.00, 140.48, 139.07, 135.45, 134.70, 131.03, 129.65, 128.42, 122.97, 122.18, 119.25, 114.68, 112.86, 111.54, 109.93, 108.11, 105.32, 93.29, 64.26, 60.86, 55.78, 55.72, 44.52, 27.42, 26.18, 21.46; ESI-MS: calcd. for C<sub>27</sub>H<sub>34</sub>N<sub>2</sub>O<sub>7</sub> [M + H]<sup>+</sup> 641.2857; found 641.2844.

5-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl) amyl (E)-3-(3,4,5-trimethoxyphenyl) acrylate (5b). Compound 5b was prepared according to the procedure from (E)-3-(3,4,5-trimethoxyphenyl) acrylic acid and 9-(5-bromopentyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2c). White solid. 48.9% yield; m.p.: 103.7–104.7 °C; 1H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ: 8.16 (d, J = 5.1 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 5.4 Hz, 1H), 7.57 (d, J = 15.9 Hz, 1H), 7.20 (s, 1H), 7.07 (s, 2H), 6.86 (d, J = 8.7 Hz, 1H), 6.64 (d, J = 15.9 Hz, 1H), 4.56 (t, J = 7.4 Hz, 2H), 4.14 (t, J = 6.5 Hz, 2H), 3.91 (s, 3H), 3.83 (s, 3H), 3.70 (s, 3H), 2.95 (s, 3H), 1.81–1.69 (m, 4H), 1.53–1.44 (m, 2H); 13C-NMR (100 MHz, CDCl<sub>3</sub>) δ: 166.88, 160.99, 153.55, 145.16, 143.21, 140.97, 139.93, 138.19, 135.02, 130.08, 128.90, 122.81, 117.76, 114.68, 112.67, 109.54, 106.40, 94.12, 64.19, 60.56, 56.50, 56.04, 44.41, 30.39, 28.47, 23.53, 23.20; ESI-MS: calcd. for C<sub>29</sub>H<sub>36</sub>N<sub>2</sub>O<sub>7</sub> [M + H]<sup>+</sup> 459.2278; found 459.2288.

5-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl) amyl (E)-3-(2-nitrophenyl) acrylate (5c). Compound 5c was prepared according to the procedure from (E)-3-(2-nitrophenyl) acrylic acid and 9-(5-bromopentyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2c). Yellow solid. 44.2% yield; m.p.: 127.4–130.0 °C; 1H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 8.28 (d, J = 5.2 Hz, 1H), 8.11 (d, J = 16.0 Hz, 1H), 8.06 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 9.2 Hz, 1H), 7.73 (d, J = 5.6 Hz, 1H), 7.69–7.62 (m, 2H), 7.54–7.58 (m, 1H), 6.89–8.66 (m, 2H), 6.34 (d, J = 16.0 Hz, 1H), 4.53–4.49 (m, 2H), 4.24 (t, J = 6.4 Hz, 2H), 3.95 (s, 3H), 3.03 (s, 3H), 1.95–1.87 (m, 2H), 1.82–1.75 (m, 2H), 1.59–1.50 (m, 2H); 13C-NMR (100 MHz, CDCl<sub>3</sub>) δ: 165.82, 158.08, 148.23, 144.12, 140.01, 138.76, 136.94, 134.49, 130.51, 130.40, 129.37, 127.31, 124.87, 123.16, 118.24, 115.39, 113.62, 108.74, 104.15, 99.94, 64.73, 62.57, 59.60, 33.71, 32.46, 31.74, 24.40; ESI-MS: calcd. for C<sub>27</sub>H<sub>27</sub>N<sub>2</sub>O<sub>7</sub> [M + H]<sup>+</sup> 519.2490; found 519.2474.

5-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl) pentyl (E)-2-(3,4-dimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl) acrylate (5d). Compound 5d was prepared according to the procedure from (E)-2-(3,4-dimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl) acrylic acid and 9-(5-bromopentyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2c). White solid. 28.2% yield; m.p.: 117.4–119.9 °C; 1H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 8.28 (d, J = 5.2 Hz, 1H), 7.97 (d, J = 8.8 Hz, 1H), 7.73 (d, J = 5.2 Hz, 1H), 7.67 (s, 1H), 6.89 (d, J = 2.0 Hz, 1H), 6.87 (s, 1H), 6.85 (s, 1H), 6.77–6.75 (m, 2H), 6.35 (s, 2H), 4.39 (t, J = 7.8 Hz, 2H), 4.22 (t, J = 6.4 Hz, 2H), 3.94 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.58 (s, 6H), 2.96 (s, 3H), 1.88–1.84 (m, 2H), 1.76–1.73 (m, 2H), 1.51–1.43 (m, 2H); 13C-NMR (100 MHz, CDCl<sub>3</sub>) δ: 167.69, 162.39, 160.81, 152.47, 149.15, 148.54, 142.98, 140.20, 139.91, 138.86, 137.94, 135.10, 135.09, 131.21, 129.69,
6-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl) hexyl (E)-3-(4-methoxyphenyl) acrylate (6a). Compound 6a was prepared according to the general procedure from (E)-3-(4-methoxyphenyl) acrylic acid and 9-(6-bromohexyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2d). Yellow solid. 37.6% yield; m.p.: 88.7–90.4 °C; 1H-NMR (400 MHz, CDCl3) δ: 8.29 (d, J = 5.4 Hz, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 5.1 Hz, 1H), 7.63 (d, J = 16.2 Hz, 1H), 7.46 (d, J = 8.5 Hz, 2H), 6.84–6.90 (m, 4H), 6.29 (d, J = 15.9 Hz, 1H), 4.45 (t, J = 7.8 Hz, 2H), 4.18 (t, J = 6.7 Hz, 2H), 3.94 (s, 3H), 3.82 (s, 3H), 3.02 (s, 3H), 1.90–1.81 (m, 2H), 1.74–1.66 (m, 2H), 1.50–1.45 (m, 4H); 13C-NMR (100 MHz, CDCl3) δ: 167.25, 161.27, 160.70, 144.30, 142.95, 140.37, 138.08, 135.16, 129.60, 129.28, 127.00, 122.88, 115.42, 115.11, 114.21, 112.15, 108.51, 93.31, 64.03, 55.60, 55.26, 44.68, 30.47, 28.60, 26.52, 25.76, 23.30; ESI-MS: calcd. for C38H42N2O8 [M + H]+ 665.3014; found 655.3018.

6-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl) hexyl (E)-3-(4,5,trimethoxyphenyl) acrylate (6b). Compound 6b was prepared according to the general procedure from (E)-3-(4,5,trimethoxyphenyl) acrylic acid and 9-(6-bromohexyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2d). Yellow solid. 38.1% yield; m.p.: 73.7–73.9 °C; 1H-NMR (400 MHz, CDCl3) δ: 8.29 (d, J = 5.2 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 5.2 Hz, 1H), 7.59 (d, J = 15.6 Hz, 1H), 6.91–6.86 (m, 2H), 6.75 (s, 2H), 6.33 (d, J = 16.0 Hz, 1H), 4.48 (t, J = 7.8 Hz, 2H), 4.20 (t, J = 6.6 Hz, 2H), 3.95 (s, 3H), 3.88 (s, 3H), 3.02 (s, 3H), 1.90–1.83 (m, 2H), 1.74–1.69 (m, 2H), 1.50–1.47 (m, 4H); 13C-NMR (100 MHz, CDCl3) δ: 166.95, 160.86, 153.36, 144.70, 143.08, 140.30, 140.05, 137.94, 135.19, 129.81, 129.46, 122.40, 117.21, 115.15, 112.26, 108.57, 105.15, 93.44, 64.24, 60.91, 56.10, 55.68, 47.46, 30.50, 28.61, 26.55, 25.77, 23.19; ESI-MS: calcd. for C39H39N2O8 [M + H]+ 533.2646; found 533.2648.

6-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl) hexyl(E)-3-(2-nitrophenyl) acrylate (6c). Compound 6c was prepared according to the general procedure from (E)-3-(2-nitrophenyl) acrylic acid and 9-(6-bromohexyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2d). White solid. 33.9% yield; m.p.: 117.3–119.9 °C; 1H-NMR (400 MHz, DMSO-d6) δ: 8.16 (d, J = 5.1 Hz, 1H), 8.08 (d, J = 8.7 Hz, 2H), 7.95–7.90 (m, 2H), 7.87 (t, J = 7.5 Hz, 1H), 7.78 (t, J = 7.5 Hz, 1H), 7.68 (t, J = 7.7 Hz, 1H), 7.18 (s, 1H), 6.86 (d, J = 8.7 Hz, 1H), 6.62 (d, J = 15.9 Hz, 1H), 4.55 (t, J = 7.4 Hz, 2H), 4.16 (t, J = 6.5 Hz, 2H), 3.90 (s, 3H), 2.95 (s, 3H), 1.78–1.71 (m, 2H), 1.66–1.60 (m, 2H), 1.45–1.40 (m, 4H); 13C-NMR (100 MHz, CDCl3) δ: 165.67, 160.75, 148.18, 142.94, 140.40, 139.88, 138.10, 135.16, 133.40, 130.41, 130.19, 129.26, 128.98, 124.79, 123.03, 122.25, 115.09, 112.14, 108.53, 93.31, 64.62, 55.60, 44.66, 30.48, 28.47, 26.51, 25.76, 23.31; ESI-MS: calcd. for C29H29N2O5 [M + H]+ 488.2180; found 488.2201.

6-(7-Methoxy-1-methyl-9H-pyridine [3,4-b] indole-9-yl) hexyl(E)-2-(3,4-dimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl) acrylate (6d). Compound 6d was prepared according to the general procedure from (E)-2-(3,4-dimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl) acrylic acid and 9-(6-bromohexyl)-7-methoxy-1-methyl-9H-pyridine [3,4-b] indole (2d). White solid. 27.3% yield; m.p.: 113.1–115.0 °C; 1H-NMR (400 MHz, CDCl3) δ: 8.27 (d, J = 5.6 Hz, 1H), 7.99 (d, J = 8.8 Hz, 1H), 7.82 (d, J = 5.2 Hz, 1H), 7.68 (s, 1H), 6.93 (d, J = 8.8 Hz, 1H), 6.84 (s, 2H), 6.79–6.74 (m, 2H), 6.34 (s, 2H), 4.43 (t, J = 7.6 Hz, 2H), 4.18 (t, J = 6.6 Hz, 2H), 3.95 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 3.55 (s, 6H), 3.11 (s, 3H), 1.83–1.78 (m, 2H), 1.70–1.65 (m, 2H), 1.45–1.36 (m, 4H); 13C-NMR (100 MHz, CDCl3) δ: 167.90, 161.62, 153.13, 152.62, 149.25, 148.67, 143.88, 140.03, 138.95, 134.89, 131.39, 129.83, 128.61, 122.86, 114.83, 113.03, 112.62, 111.47, 109.55, 108.04, 105.24, 93.42, 64.84, 60.86, 55.95, 55.72, 44.85, 30.50, 28.50, 26.46, 25.67; ESI-MS: calcd. for C39H44N2O8 [M + H]+ 669.3170; found 669.3196.
3.3. Evaluation of the Antibacterial Activity

3.3.1. MIC Testing

The antibacterial efficiency of all prepared compounds was determined using the microplate dilution method [28,29]. All compounds were prepared into different concentrations of stock solution according to their solubility. The compounds were added to the 96 well plate and serially diluted in Mueller-Hinton broth. Three Gram-positive strains (S. aureus, S. albus and MRSA) and two Gram-negative strains (E. coli and PA) were cultivated and added to the 96 well plate. The initial concentration of bacteria should be higher than $10^5$ CFU/mL. The samples were incubated at 37 °C for 18–24 h. The MIC was determined for compounds by observing change of clarity in the broth. The same procedure was repeated in triplicate.

3.3.2. Bactericidal Time–Kill Kinetics

The bacteria S. aureus was prepared in Mueller-Hinton broth at 37 °C for 6 h with shaking. The solution of 3c (1 × MIC and 6 × MIC), 5a (1 × MIC and 6 × MIC), harmine (1 × MIC and 6 × MIC) and saline (served as a control group) were separately added to the bacterial suspension so that the final bacterial concentration were $10^6$–$10^7$ CFU/mL. At the predetermined time point (0, 0.5, 2, 4, 6, 12 and 24 h), 20 mL of aliquots were serially diluted in normal saline, plated on sterile Mueller Hinton agar plates, and incubated at 37 °C for 24 h. After incubation, the viable colonies were counted and represented as log$_{10}$ (CFU/mL). The same procedure was repeated in triplicate.

3.4. In Vitro Cytotoxic Activity

The in vitro cytotoxicity of 3c was determined using MTT assay. WI-38 (Human embryonic lung fibroblasts), MCF-7 (Human breast cancer cells) and HepG2 (human liver carcinoma cells) were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were maintained using Dulbecco’s modified eagle’s medium (DMEM, Sangon Biotech, Shanghai, China) supplemented with 10% fetal bovine serum (FBS, Sijiqing Bioengineering Material Co., Ltd., Hangzhou, China), 100 U/mL penicillin and 100 µg/mL streptomycin in a humidified 37 °C incubator under 5% CO$_2$. 100 µL culture medium at a concentration of $1 \times 10^4$ cells/mL was added to 96-well plate and incubated in a humidified 37 °C incubator under 5% CO$_2$. After 24 h, 10 µL solution of 3c and harmine dissolved in DMSO and PBS and then diluted with DMEM under a concentration gradient, which was added in the wells for final concentrations of 1000, 500, 250, 125, 62.5 µmol/L of compounds in WI-38 and 160, 80, 40, 20, 10 µmol/L of compounds in MCF-7 and Hep-G2. After 48 h incubation, 10 µL MTT solution (5 mg/mL) was added into each well and incubated in a humidified 37 °C incubator under 5% CO$_2$. After 4 h, the supernatant was removed and 150 µL DMSO was added. The absorbance of each well was determined by an enzyme-linked immunosorbent assay reader (Thermo Fisher Scientific Inc., Rochester, NY, USA) at wavelengths of 570 nm. The experiments were performed in triplicate.

3.5. In Vivo Pharmacokinetic Study

3.5.1. Animals and Ethics Statement

Adult males and females Sprague Dawley rats, with average weight of 200–250 g, were purchased from the Experiment Animal Center of Lanzhou University (Lanzhou, China). The rats were housed under an environmentally controlled room for one week before staring the experiment. All procedures were performed according to the guidelines issued by the Ethical Committee of Lanzhou University (Permit Number: SYXK(Gan)-2018-0002).

3.5.2. Pharmacokinetic Experiment

After 7 days acclimatization, rats were randomly divided into two groups with half male and half female in each group ($n = 6$). All animals were fasted for 12 h with access to water prior to dosing. One group was intravenously administered with 3c at dose of 10 mg/kg. Another group was oral administered with 3c at dose of 40 mg/kg.
approximately 0.3 mL of blood samples were collected into heparinized tubes from the orbital vein at 0.083, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12 and 24 h after the administration. The heparinized blood samples were immediately centrifuged at 4000 rpm for 10 min and the upper plasma was separated and immediately stored at −20 °C used for UPLC-MS/MS analysis.

Plasma samples were processed as follows. First, 100 µL of plasma samples and 50 µL of tinidazole (1000 ng/mL, internal standard) were mixed by vortex for 1 min. Next, 250 µL of acetonitrile were added to the mixture and vortexed for 10 min. After centrifugation at 10,000 rpm for 10 min, 200 µL of the upper organic layer were removed into 1.5 mL centrifuge tube and dried with nitrogen. Finally, the residue was reconstituted in 100 µL of 50% acetonitrile, vortexed for 1 min, centrifuged at 10,000 rpm for 10 min, and then the supernatant was used for UPLC-MS/MS analysis.

3.5.3. Statistical Analysis

The obtained data were analyzed using a non-compartment open model with PKsolver Software [30,31]. Pharmacokinetic parameters and the experiment data were expressed as the mean ± standard deviation (SD). A paired student’s t-test was used for statistical analysis, with p < 0.05 considered the minimum level of significance.

4. Conclusions

In summary, 16 novel harmine derivatives were synthesized by covalent bonding between N⁹ of harmine and carboxylic acid of cinnamic acid derivatives. Most of the synthesized harmine derivatives displayed better antibacterial activities against Gram-positive strains (S. aureus, S. albus and MRSA) than Gram-negative strains (E. coli and PA) in vitro. Notably, compound 3c, the most effective compound, showed excellent bactericidal activity against S. aureus and S. albus in time-kill assay. In MTT assay, it showed that compound 3c had weaker cytotoxicity than harmine with IC₅₀ of 340.30, 94.86 and 161.67 µmol/L against WI-38, MCF-7 and HepG2 cell lines, respectively. Pharmacokinetic studies showed that compound 3c showed rapid absorption and excretion with an oral bioavailability of 6.9% in vivo.

Supplementary Materials: The following are available online at, Figures S1–S16: The ¹H-NMR and ¹³C-NMR spectrum of compound 3a–d, 4a–d, 5a–d and 6a–d; Tables S1–S3: Crystal data of compound 4a.

Author Contributions: Experiment design—Y.L. and Z.W.; synthesis of the experiment—Y.L., D.H. and L.T.; antibacterial activity and pharmacokinetics—D.Z. and J.L.; original draft and supervision—Y.L. and D.H. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethical Committee of Lanzhou University (SYXK(Gan)-2018-0002 and 11 January 2018).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article and Supplementary Material, further inquiries can be directed to the corresponding author.

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Sample Availability: Samples of the compounds are available from the authors.
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