



Cite this: *RSC Adv.*, 2023, **13**, 3204

Received 2nd December 2022  
 Accepted 16th January 2023

DOI: 10.1039/d2ra07676d

[rsc.li/rsc-advances](http://rsc.li/rsc-advances)

## Late-stage functionalization of 5-nitrofurans derivatives and their antibacterial activities†

Geshuyi Chen,<sup>a</sup> Zhe Chang,<sup>b</sup> Pei Yuan,<sup>a</sup> Si Wang,<sup>b</sup> Yongxiu Yang,<sup>b</sup> Xiaolei Liang<sup>\*ac</sup> and Depeng Zhao<sup>b</sup><sup>\*</sup><sup>b</sup>

Structure modification of drugs is a reliable way to optimize lead compounds, among which the most striking and direct method is late-stage functionalization (LSF). Here, we employed the Cu-catalyzed C–H LSF to modify 5-nitrofuran drugs. A series of modifications have been carried out including hydroxylation, methylation, azidination, cyanation, arylation, etc. Antibacterial activities of all compounds *in vitro* were measured. The results showed that compound **1** and compound **18** were the most active among all compounds. Meanwhile, the cell cytotoxicity assays of potent compounds **1**, **3**, **4**, **5** & **18** and the parent drug FZD were conducted.

## Introduction

Structural modification of lead molecules is to modify functional groups based on the molecules' original skeleton,<sup>1–3</sup> which has the following effects: (a) changes the solubility or  $pK_a$  of the drug,<sup>4</sup> (b) enhances the drug's bioavailability,<sup>5</sup> (c) improves the pharmacokinetics and prolongs the action time of the drug,<sup>6</sup> (d) maintains excellent metabolic stability, (e) increases the target selectivity of drugs,<sup>7</sup> and (f) reduces the toxicity and side effects of drugs.<sup>4</sup> Therefore, chemical structure modification of drugs is a reliable method for optimizing lead compounds and is widely used by medicinal chemists all over the world.

C–H late-stage functionalization (LSF) is the most direct approach of structural modification, helping to generate new drugs rapidly.<sup>8–13</sup> So far, there have been many examples of successfully improving activity after modification such as “magic methyl” effect,<sup>14–18</sup> cyanation,<sup>19</sup> azidination<sup>20</sup> (Fig. 1a). Since the improper use of antibiotics<sup>21</sup> and the natural selection of bacteria,<sup>22</sup> more and more diseases are difficult to cure by current antibiotics, new strategies must be developed, such as the discovery of derivatives of known antibacterial agents.

Nitrogen heterocycles (N-heterocycles) are common structures in antibacterial and anti-inflammatory drugs,<sup>23</sup> and their  $N$ – $\alpha$  position can be used as a modification object in antibacterial drug Zolifludacin (Fig. 1b).<sup>24–26</sup> It has been reported<sup>27</sup> that modifying N-heterocycles can improve their protein-binding ability without influencing the acting mechanism. Furazolidone (FZD, 3-(5-nitrofurylideneamino)-2-oxazolidinone) and Nitrofurantoin (NFT, 1-(((5-nitro-2-furanyl) methylene) amino)-2,4-imidazolidinedione) are members of 5-nitrofurans (NFs),<sup>28</sup> which have broad-spectrum antibacterial effect against Gram-negative and Gram-positive bacteria.<sup>29,30</sup> In particular, FZD was proved to be the only successful NFs against *Helicobacter pylori* (*H. pylori*).<sup>30,31</sup> Therefore, modification of  $N$ – $\alpha$  position of these two antibacterial drugs can afford more active compounds without affecting the target.

Herein, we successfully modified the  $N$ – $\alpha$  position of FZD and NFT, and converted the  $N$ – $\alpha$  position C–H bond into C–O, C–C, C–N, and C–S bonds by Cu(i) catalyzed C–H LSF (Fig. 1c) and the reaction mechanism is shown in Fig. 1d. 18 analogues including 16 FZD derivatives and 2 NFT derivatives were synthesized and their antibacterial activities against *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Candida albicans* (*C. albicans*) and *Helicobacter pylori* (*H. pylori*) *in vitro* were measured. Several compounds were superior to parent drug in activity. We hope that this method can provide an idea of LSF at the  $N$ – $\alpha$  position to medicinal chemists and help them obtain desired compounds in a fast and low-cost way.

## Results and discussion

### Synthetic procedures

Inspired by our recent advances in copper catalyzed late-stage C–H functionalization of N-heterocycles,<sup>32</sup> which can generate hemiaminal intermediate *via* cross-dehydrogenation coupling

<sup>a</sup>The First Clinical Medical College, Lanzhou University, Lanzhou, China. E-mail: chengshy20@lzu.edu.cn

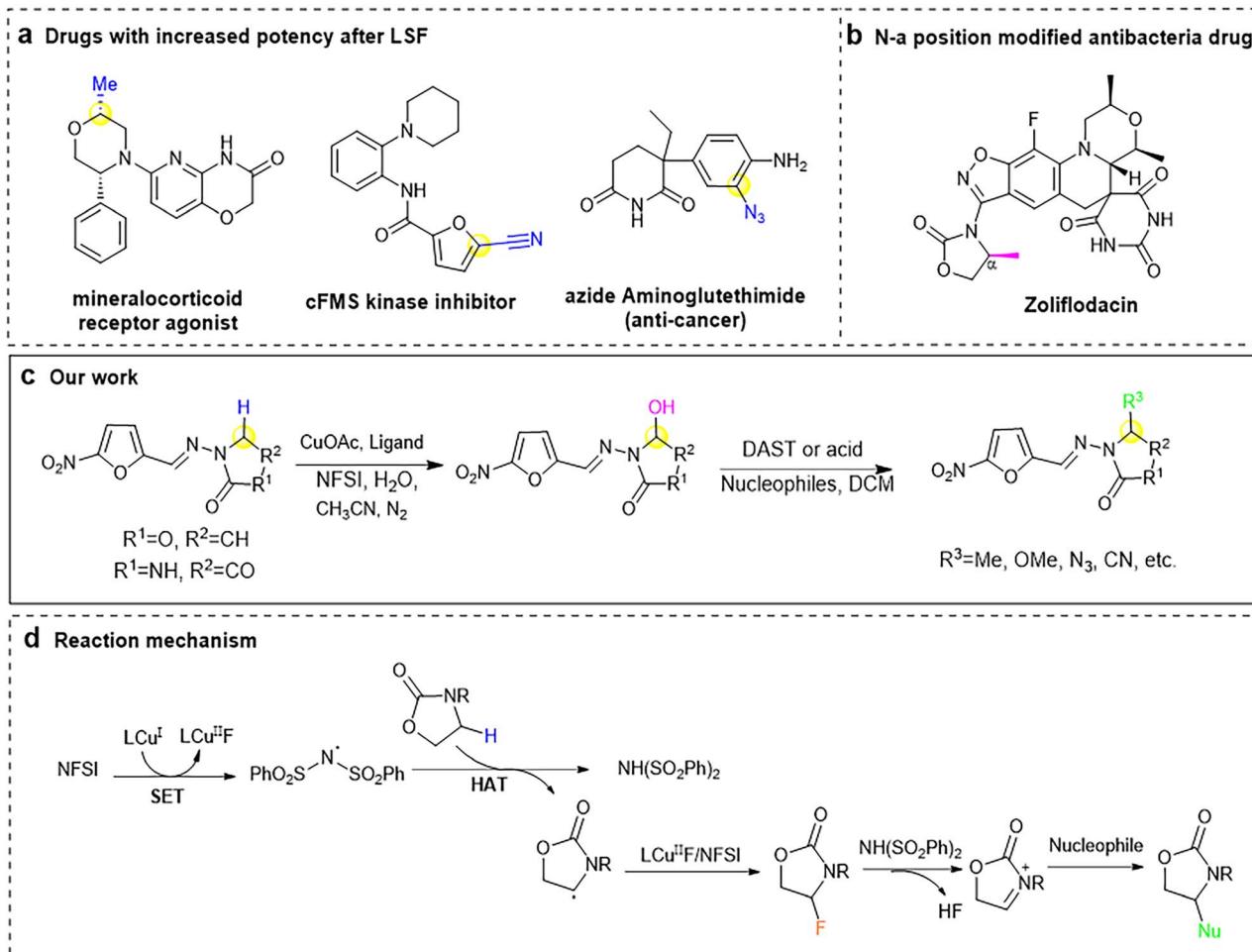
<sup>b</sup>Guangdong Provincial Key Laboratory of Chiral Molecule and Drug Discovery, School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, China. E-mail: zhaodp5@mail.sysu.edu.cn

<sup>c</sup>The First Clinical Medical College, Lanzhou University. Department of Obstetrics and Gynecology, The First Hospital of Lanzhou University, Key Laboratory for Gynecologic Oncology, Lanzhou, 730000, Gansu Province, China. E-mail: yxyanglzu@163.com; liangxl07@lzu.edu.cn

<sup>†</sup>Lead Contact, China

† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d2ra07676d>





**Fig. 1** C–H LSF of drugs and modifications of NFs. (a) Representative examples of drugs with increased potency after C–H late-stage functionalization. (b) *N*- $\alpha$  position modified antibacterial drug Zoliflodacin. (c) This work: C–H late-stage functionalization of FZD and NFT. (d) Reaction mechanism.

reaction after C–H bond oxidation, we attempted to modify the  $\alpha$ -position of N-heterocycle-containing drugs. A variety of functional groups, such as those containing O, N, and S, were projected to be introduced in *N*- $\alpha$  position in order to increase the receptor-binding capacity and activity of the drug. Herein, we successfully converted C–H bonds into C–O and C–C bonds. Since N and S are the most common active atoms in medicinal compounds, we also paid attention to C–N and C–S coupling for the first time. Derivatives of FZD were obtained using copper(I) catalyzed C–H LSF in desired yield (43–95%). A series of groups were added in *N*- $\alpha$  position of NFs containing  $-\text{OH}$ ,  $-\text{OCH}_3$ ,  $-\text{N}_3$ ,  $-\text{CN}$ , *etc.* The analogues are shown in Fig. 2, including 16 FZD derivatives and 2 NFT derivatives.

We initially oxidized FZD with the conditions of  $\text{CuOAc}/\text{Ligand}$ ,  $\text{H}_2\text{O}$  and *N*-fluorobenzenesulfonimide (NFSI) in  $\text{CH}_3\text{CN}$ , obtained hemiaminal **1** in 91% yield. Replacing water with methanol or azidotrimethylsilane in above system, the corresponding FZD derivatives **2** and **3** were formed in one-step with good yields (93% and 83% respectively).

Influenced by the one-step reaction of trimethylsilane, we proceeded with the C–N coupling. Amines were initially added

to our reaction system in order to obtain C–N coupled products in one-step, but the reaction failed. Therefore, a one-pot reaction was performed. After the completion of the reaction of hemiaminal **1**, an amine was added to afford **4** and **5** in excellent yields (89% and 87% respectively). It is worth mentioning that chain amines such as dimethylamine, *n*-propylamine, *N*-methyl-*n*-propylamine and ammonium salts including methylamine hydrochloride and ethylamine hydrochloride could react with hemiaminal **1** giving corresponding C–N coupling products. However, these desired products were all unstable, they partially hydrolyzed during purification, back to hemiaminal **1**, preventing us from obtaining pure products.

Next, we focused on the C–C coupling at the *N*- $\alpha$  position of NFs. Methylation of **1** with diethylaminosulfur trifluoride (DAST) afforded **6** in moderate yields (71%). Treatment of **1** with  $\text{BF}_3 \cdot \text{OEt}_2$  and trimethylsilylcyanide provided products **7** in moderate yield (45%). Moreover, in order to evaluate the effect of active hydrogen, we further synthesized amide **8** with two active hydrogens based on product **7** under the conditions of  $\text{LiOH}$  and  $\text{H}_2\text{O}_2$ , the overall yield of two steps was 43%. Compounds **9–10** were products of hemiaminal **1**,  $\text{BF}_3 \cdot \text{OEt}_2$ ,

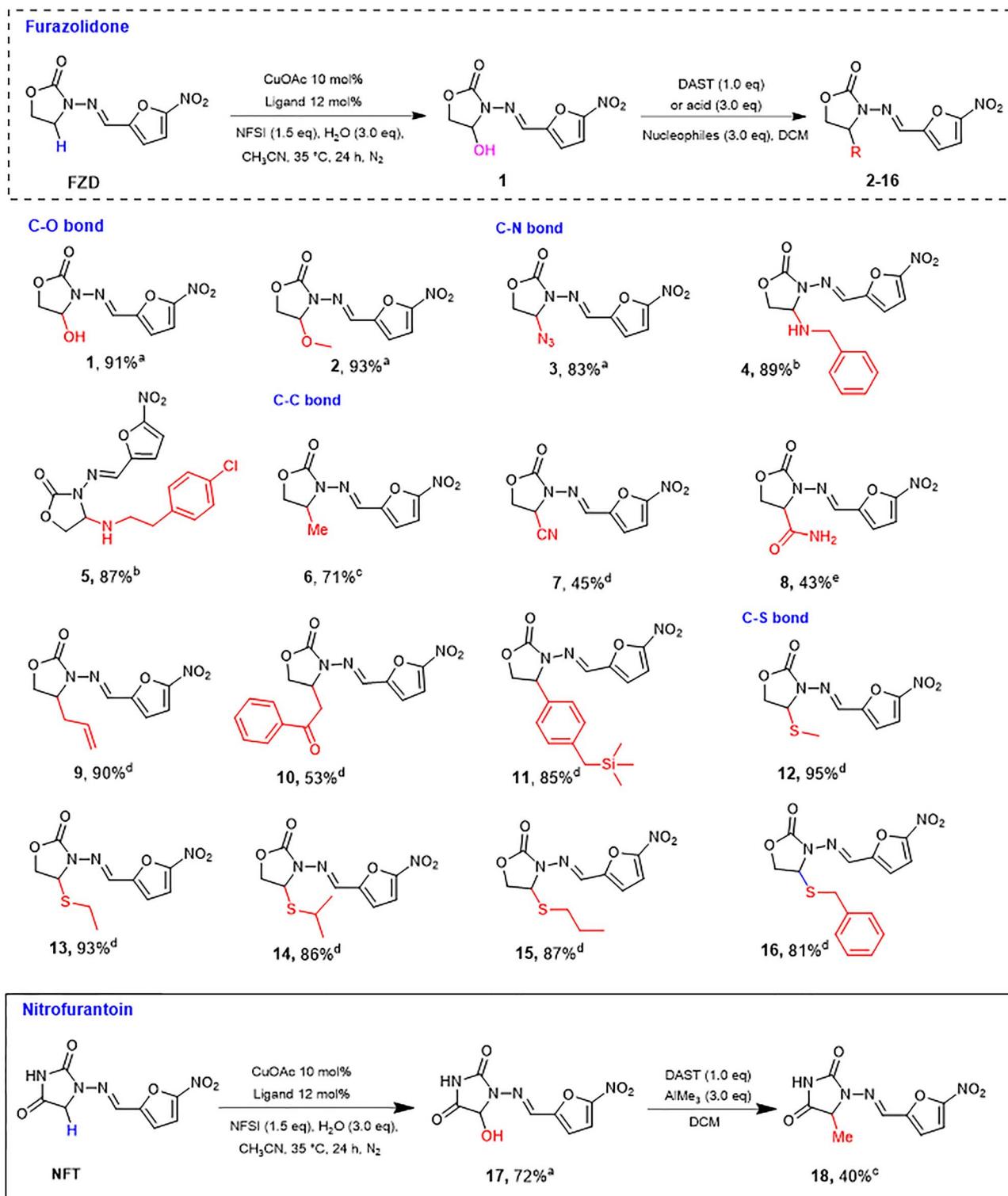


Fig. 2 Late-stage functionalization of FZD and NFT. Reaction conditions: <sup>a</sup>FZD or NFT (0.2 mmol), NFSI (0.3 mmol), ligand (0.024 mmol, 12 mol%) and CuOAc (0.02 mmol, 10 mol%), ROH or TMS-N<sub>3</sub> (0.6 mmol) in CH<sub>3</sub>CN (1.0 ml), under N<sub>2</sub>, 35 °C for 24 h; isolated yields. <sup>b</sup> FZD (0.1 mmol), NFSI (0.15 mmol), ligand (0.012 mmol, 12 mol%) and CuOAc (0.01 mmol, 10 mol%), H<sub>2</sub>O (0.3 mmol) in CH<sub>3</sub>CN (1.0 ml) under N<sub>2</sub>, 35 °C for 24 h; amine (0.3 mmol) added under N<sub>2</sub>, 35 °C for 24 h; overall yield of two steps. <sup>c</sup> 1 or 17 (0.1 mmol), CH<sub>2</sub>Cl<sub>2</sub> (1.0 ml), DAST (0.1 mmol) added under N<sub>2</sub> at -78 °C; rt for 1 h; cooled to -78 °C, AlMe<sub>3</sub> (0.3 mmol) added, stirred for 2 h; rt for 1 h; isolated yields. <sup>d</sup> 1 (0.1 mmol), CH<sub>2</sub>Cl<sub>2</sub> (1.0 ml), acid (0.2 mmol) and nucleophilic reagent (0.3 mmol) added under N<sub>2</sub> at -40 °C, stirred for 1 h, rt for 2 h; isolated yields. <sup>e</sup> 7 (0.1 mmol), LiOH · H<sub>2</sub>O (0.2 mmol), THF (3.0 ml), H<sub>2</sub>O (3.0 ml) under N<sub>2</sub>, H<sub>2</sub>O<sub>2</sub> (36% wt 100 µl) added, rt for 1 h; overall yield of two steps.

and corresponding organic nucleophilic reagents (allyl-trimethylsilane and trimethyl [(1-phenylethyl) oxy] – silane) in yields 90% and 53% respectively. Interestingly, when benzyl-trimethylsilane was used as a nucleophilic reagent, the 4-position of benzene ring was substituted rather than the benzyl position, resulting in product **11** in 85% yield.

In view of the importance of the S atom in pharmaceutical chemistry, we decided to introduce sulfur-containing groups. **1** reacted with sodium methylthiolate and sodium ethanethiolate after adding  $\text{BF}_3\text{-OEt}_2$ , providing products **12** and **13** in excellent yields, (95% and 93%, respectively). Unexpectedly, when propanethiol, isopropylthiol, and benzylthiol were used as nucleophiles,  $\text{BF}_3\text{-OEt}_2$  could not promote the reaction. Other Lewis acids were also used for the same reason but failed. Finally, trifluoroacetic acid (TFA), a type of Brønsted acid, was used to drive the reaction, and products **14–16** were afforded in good yields (81–87%).

NFT derivative heminaminal **17** was obtained in 72% yield under conditions of  $\text{CuOAc/Ligand}$ ,  $\text{H}_2\text{O}$  and NFSI, same conditions as hemiaminal **1**. Methylation of **17** with DAST afforded **18** in moderate yields (41%).

## In vitro biological activity

### Antibacterial activity

All synthesized compounds (**1–18**) were screened *in vitro* for antibacterial activities (Table 1) against *S. aureus* strain ATCC 29213, *E. coli* strain ATCC 25922 and *C. albicans* strain ATCC14053, which are representative of the Gram-positive bacteria, Gram-negative bacteria and fungi, respectively. Compound **1**, **6**, and **FZD** were evaluated the ability against *H. pylori* strain SS1 additionally. All compounds were assessed by

a standard two-fold microdilution assay against these four strains.

Hydroxyl is an important active group in drugs, able to both change physicochemical properties and enhance hydrogen bonding interactions with the target proteins.<sup>10</sup> Compared with the parent compound **FZD**, compound **1** has an additional –OH group at the *N*- $\alpha$  position, which gives it stronger activity against *S. aureus* with a MIC value of  $1.5625 \mu\text{g ml}^{-1}$ , 2-fold superior to that of **FZD**. However, compound **2**, with an additional –OCH<sub>3</sub>, exhibited low activity against these three bacterial strains. We speculated that the enhanced activity of compound **1** is due to the active hydrogen on the hydroxyl group. Compounds **3–5** containing C–N bonds also showed strong inhibition against *S. aureus* and *E. coli*. Neither compound **6** nor **7** which containing small groups such as –CN, –CH<sub>3</sub> showed better activity. It is worth mentioning that we synthesized compound **8**, an amide with two active hydrogens based on compound **7**. Intramolecular hydrogen bonds might form in compound **8**, but the activity was poor. Antibacterial result showed **8** even had lower activity than compound **7**. Coupling with allyl group afforded compound **9**, which led to a substantial decrease in activity. Introducing an aromatic moiety into *N*- $\alpha$  position resulted in analogues **10** and **11**, which were found to have a complete loss of activity in comparison to **FZD**. This may have been because the aromatic moieties of **10** and **11** influenced their binding ability to the target. Furthermore, the introduction of sulfur did not afford better activity for compounds **12–16**. In addition, representative compounds **1** (**OH-FZD**) and **6** (**Me-FZD**) were chosen and their ability against *H. pylori* was tested. MICs of **FZD**, **1**, and **6** were all less than 0.0976. It was revealed that our modification maintained their anti-*H. pylori* efficacy.

Table 1 Minimum inhibitory concentration of the compounds against microbial strains ( $\mu\text{g ml}^{-1}$ )

Compounds	Mol. weight (g mol <sup>-1</sup> )	MIC ( $\mu\text{g ml}^{-1}$ )			
		<i>S. Aureus</i> ATCC 29213	<i>E. coli</i> ATCC 25922	<i>C. Albicans</i> ATCC14053	<i>H. pylori</i> SS1
<b>FZD</b>	225	3.125	0.39065	>50	<0.0976
<b>1</b>	241	1.5625	1.5625	>50	<0.0976
<b>2</b>	255	>50	>50	>50	
<b>3</b>	266	3.125	1.5625	>50	
<b>4</b>	330	3.125	3.125	>50	
<b>5</b>	378	3.125	3.125	>50	
<b>6</b>	239	25	6.25	>50	<0.0976
<b>7</b>	250	12.5	12.5	>50	
<b>8</b>	268	25	25	>50	
<b>9</b>	265	>50	>50	>50	
<b>10</b>	343	>50	>50	>50	
<b>11</b>	387	>50	>50	>50	
<b>12</b>	271	25	25	>50	
<b>13</b>	285	12.5	50	>50	
<b>14</b>	299	12.5	>50	>50	
<b>15</b>	299	12.5	>50	>50	
<b>16</b>	347	3.125	>50	>50	
<b>NFT</b>	238	25	12.5	>50	
<b>17</b>	254	50	50	>50	
<b>18</b>	252	25	6.25	>50	



LSF of **NFT** was also performed, and compounds **17** and **18** containing  $-\text{OH}$  and  $-\text{CH}_3$  were synthesized, respectively. Unexpectedly, compound **18** with a methyl group showed stronger anti-*E. coli* activity than its parent drug, with a 2-fold superior efficacy to the MIC of **NFT**, demonstrating the “magic methyl” effect, which differed from the results from the methylation of **FZD**.

The results indicated that parent drugs **FZD** and **NFT** had very low inhibition on fungal strain. The MIC values were more than  $50 \mu\text{g ml}^{-1}$  against *C. Albicans*. Analogue **1–18** after modification didn't afford better activity.

### Cytotoxicity analysis

We chose HepaRG cells, which are liver bipotent progenitors,<sup>33–35</sup> to assess the hepatotoxicity of the analogues. 5 potent derivatives (**1, 3, 4, 5 & 18**) and the parent drug **FZD** were selected for cell viability study with MTT assay method. Cells were incubated for 24 h at different concentrations of **FZD** and derivatives. As shown in Table 2, all compounds have low toxicity, and the values of  $\text{IC}_{50}$  are more than  $100 \mu\text{M}$ .

As shown in Fig. 3, results from MTT assay showed that after 24 h exposure, **FZD** exhibited almost 75% cell viability at concentration of  $0.01\text{--}1 \mu\text{M}$  ( $p < 0.05$ ), while all other derivatives exhibited nearly 100% cell viability in the same concentration. Furthermore, all compounds still exhibited more than 50% cell viability even though treated in a high concentration of  $100 \mu\text{M}$ .

Table 2 Cytotoxicity of FZD and potent compounds **1, 3, 4, 5, and 18**

Compounds	Cytotoxicity $\text{IC}_{50} (\mu\text{M})$
<b>FZD</b>	>100
<b>1</b>	>100
<b>3</b>	>100
<b>4</b>	>100
<b>5</b>	>100
<b>18</b>	>100

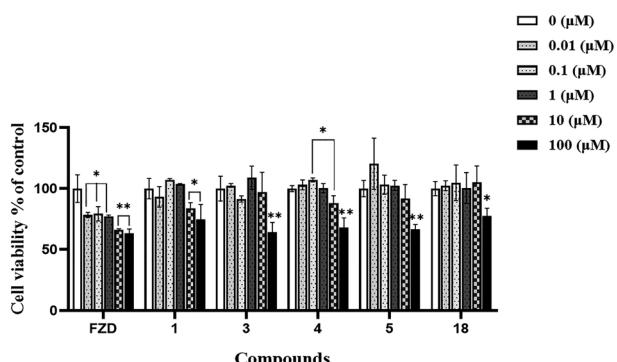


Fig. 3 Cytotoxicity of FZD and potent compounds against HepaRG cells. MTT assay for cell viability. Cells were treated with compounds **FZD**, **1, 3, 4, 5, and 18** at concentrations of  $0.01$  to  $100 \mu\text{M}$  for 24 h (mean  $\pm$  SD,  $n = 3$ ). \* $p < 0.05$ , \*\* $p < 0.01$  vs. the control.

( $p < 0.05$ ), indicating a non-cytotoxic against HepaRG cells. Hence, these compounds in future could be used as good candidates for drugs in the field of medicinal chemistry.

## Conclusions

In summary, we developed an effective method for the synthesis of substituted **FZD** and **NFT** by late-stage  $\text{C}(\text{sp}^3)\text{-H}$  functionalization, and the modification of the  $\alpha$ -position of the N-heterocycles. This method was firstly applied to the coupling of C–N and C–S bonds. A series of functional groups were added including  $-\text{OH}$ ,  $-\text{OCH}_3$ ,  $-\text{N}_3$ ,  $-\text{CN}$ , etc. We successfully synthesized 16 **FZD** derivatives and 2 **NFT** derivatives in moderate to good yields. Three bacteria strains and one fungi strain were measured, the modified compounds had broad-spectrum antibacterial activity, especially derivatives **1** and **18**. Compounds **1, 3, 4, and 5** exhibited identical or better activity than that of the parent compound **FZD** against *S. aureus*. Cell cytotoxicity assays of potent compounds **1, 3, 4, 5, and 18** indicated non-cytotoxic against HepaRG cells. This study is an attempt to explore the application of copper(i) catalyzed C–H LSF, which provides good results and proves the practicability of this method. We believe our study could provide a reference for the application of LSF in pharmaceutical chemistry.

## Author contributions

Y. X. Y. and D. P. Z. designed the study. G. S. Y. C. performed experiments, acquired and analyzed data and wrote this manuscript. Z. C., P. Y. and S. W. assisted with experiments and analyzed data. D. P. Z. and X. L. L. contributed to completing the revision. Y. X. Y. contributed to funding support and submission of the article. All authors have given approval to the final version of the manuscript.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

This work was supported by the National Natural Science Foundation of China (No.81960278), the Outstanding Youth Funds of Science and Technology Department of Gansu Province (No.20JR5RA371).

## Notes and references

- P. Das, M. D. Delost, M. H. Qureshi, D. T. Smith and J. T. Njardarson, *J. Med. Chem.*, 2019, **62**, 4265–4311.
- B. Das, A. T. K. Baidya, A. T. Mathew, A. K. Yadav and R. Kumar, *Bioorg. Med. Chem.*, 2022, **56**, 116614.
- O. Bouteureira and G. J. Bernardes, *Chem. Rev.*, 2015, **115**, 2174–2195.
- Q. Zhao, L. Xin, Y. Liu, C. Liang, J. Li, Y. Jian, H. Li, Z. Shi, H. Liu and W. Cao, *J. Med. Chem.*, 2021, **64**, 10557–10580.



5 R. Z. Wu, H. Y. Zhou, J. F. Song, Q. H. Xia, W. Hu, X. D. Mou and X. Li, *J. Med. Chem.*, 2021, **64**, 17627–17655.

6 S. Alam Khan and M. Jawaid Akhtar, *Bioorg. Chem.*, 2022, **120**, 105599.

7 M. D. Cummings and S. Sekharan, *J. Med. Chem.*, 2019, **62**, 6843–6853.

8 K. Korvorapun, R. Kuniyil and L. Ackermann, *ACS Catal.*, 2020, **10**, 435–440.

9 O. Abdulla, A. D. Clayton, R. A. Faulkner, D. M. Gill, C. R. Rice, S. M. Walton and J. B. Sweeney, *Chem. Eur. J.*, 2017, **23**, 1494–1497.

10 B. Hong, T. Luo and X. Lei, *ACS Cent. Sci.*, 2020, **6**, 622–635.

11 C. A. Kuttruff, M. Haile, J. Kraml and C. S. Tautermann, *ChemMedChem*, 2018, **13**, 983–987.

12 M. Moir, J. J. Danon, T. A. Reekie and M. Kassiou, *Expert Opin. Drug Discovery*, 2019, **14**, 1137–1149.

13 H. Robinson, S. A. Oatley, J. E. Rowedder, P. Slade, S. J. F. Macdonald, S. P. Argent, J. D. Hirst, T. McInally and C. J. Moody, *Chem. Eur. J.*, 2020, **26**, 7678–7684.

14 D. Aynedtinova, M. C. Callens, H. B. Hicks, C. Y. X. Poh, B. D. A. Shennan, A. M. Boyd, Z. H. Lim, J. A. Leitch and D. J. Dixon, *Chem. Soc. Rev.*, 2021, **50**, 5517–5563.

15 E. J. Barreiro, A. E. Kümmerle and C. A. Fraga, *Chem. Rev.*, 2011, **111**, 5215–5246.

16 C. S. Leung, S. S. Leung, J. Tirado-Rives and W. L. Jorgensen, *J. Med. Chem.*, 2012, **55**, 4489–4500.

17 H. Schönherr and T. Cernak, *Angew. Chem., Int. Ed.*, 2013, **52**, 12256–12267.

18 A. Vasilopoulos, S. W. Krska and S. S. Stahl, *Science*, 2021, **372**, 398–403.

19 Y. Wang, Y. Du and N. Huang, *Future Med. Chem.*, 2018, **10**, 2713–2728.

20 H. Fang, Y. Dou, J. Ge, M. Chhabra, H. Sun, P. Zhang, Y. Zheng and Q. Zhu, *J. Org. Chem.*, 2017, **82**, 11212–11217.

21 E. L. Ellsworth, T. P. Tran, H. D. Showalter, J. P. Sanchez, B. M. Watson, M. A. Stier, J. M. Domagala, S. J. Gracheck, E. T. Joannides, M. A. Shapiro, S. A. Dunham, D. L. Hanna, M. D. Huband, J. W. Gage, J. C. Bronstein, J. Y. Liu, D. Q. Nguyen and R. Singh, *J. Med. Chem.*, 2006, **49**, 6435–6438.

22 C. Marc, B. Vrignaud, K. Levieux, A. Robine, C. G. Guen and E. Launay, *J. Child Health Care*, 2016, **20**, 530–536.

23 E. Vitaku, D. T. Smith and J. T. Njardarson, *J. Med. Chem.*, 2014, **57**, 10257–10274.

24 X. H. Su, B. X. Wang, W. J. Le, Y. R. Liu, C. Wan, S. Li, R. A. Alm, J. P. Mueller and P. A. Rice, *Antimicrob. Agents Chemother.*, 2016, **60**, 621–623.

25 C. Shi, Y. Zhang, T. Wang, W. Lu, S. Zhang, B. Guo, Q. Chen, C. Luo, X. Zhou and Y. Yang, *J. Med. Chem.*, 2019, **62**, 2950–2973.

26 P. Govender, R. Müller, K. Singh, V. Reddy, C. J. Eyermann, S. Fienberg, S. R. Ghorpade, L. Koekemoer, A. Myrick, D. Schnappinger, C. Engelhart, J. Meshanni, J. A. W. Byl, N. Osheroff, V. Singh, K. Chibale and G. S. Basarab, *J. Med. Chem.*, 2022, **65**, 6903–6925.

27 N. H. Zuma, F. J. Smit, R. Seldon, J. Aucamp, A. Jordaan, D. F. Warner and D. D. N'Da, *Bioorg. Chem.*, 2020, **96**, 103587.

28 J. C. Spain, *Annu. Rev. Microbiol.*, 1995, **49**, 523–555.

29 M. Mohammadi, B. Attaran, R. Malekzadeh and D. Y. Graham, *Dig. Dis. Sci.*, 2017, **62**, 1890–1896.

30 N. H. Zuma, J. Aucamp and D. D. N'Da, *Eur. J. Pharm. Sci.*, 2019, **140**, 105092.

31 Z. T. Zheng and Y. B. Wang, *J. Gastroenterol. Hepatol.*, 1992, **7**, 533–537.

32 Z. Chang, J. Huang, S. Wang, G. Chen, H. Zhao, R. Wang and D. Zhao, *Nat. Commun.*, 2021, **12**, 4342.

33 A. M. Timperio, H. A. Kuiper and L. Zolla, *Xenobiotica*, 2003, **33**, 153–167.

34 P. Gripon, S. Rumin, S. Urban, J. Le Seyec, D. Glaise, I. Cannie, C. Guyomard, J. Lucas, C. Trepo and C. Guguen-Guillouzo, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 15655–15660.

35 R. Parent, M. J. Marion, L. Furio, C. Trépo and M. A. Petit, *Gastroenterology*, 2004, **126**, 1147–1156.

