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Synthesis and Antimicrobial Activities of Oximes Derived from *O*-Benzylhydroxylamine as FabH Inhibitors

Yin Luo,[a] Li-Rong Zhang,[a] Yang Hu,[a] Shuai Zhang,[a] Jie Fu,[b] Xiao-Ming Wang,*[a] and Hai-Liang Zhu*[a]

Forty-three oxime derivatives were synthesized by allowing *O*-benzylhydroxylamines to react with primary benzaldehydes or salicylaldehydes; these products were gauged as potential inhibitors of β-ketoacyl-(acyl-carrier-protein) synthase III (FabH). Among the 43 compounds, 38 are reported herein for the first time. These compounds were assayed for antimicrobial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Enterococcus faecalis*. Compounds with prominent antibacterial activities were tested for their *E. coli* FabH inhibitory activities. 3-((2,4-Dichlorobenzyloxyimino)methyl)benzaldehyde *O*-2,4-dichlorobenzyl oxime (**44**) showed the best antibacterial activity, with minimum inhibitory concentrations of 3.13–6.25 μg mL^{-1} against the tested bacterial strains, exhibiting the best *E. coli* FabH inhibitory activity, with an IC_{50} value of 1.7 mM. Docking simulations were performed to position compound **44** into the *E. coli* FabH active site in order to determine the most probable binding conformation.

Introduction

The spread of multidrug-resistant bacteria has drastically impaired the efficacy of antibiotics, limiting their clinical use.[1–4] In September 2010, a report of the emergence of Gram-negative *Enterobacteriaceae* with resistance to carbapenem conferred by New Delhi metallo-β-lactamase 1 (NDM-1) drew global attention.[5] To prevent these serious medical threats, the elaboration of new types of antibacterial agents and improvement of drugs in current or prior use are very important tasks.[6]

In recent years, research has been focused on new antibacterial agents that act on novel targets, thus overcoming currently known mechanisms of acquired resistance. One promising target is the fatty acid synthesis (FAS) pathway in bacteria. Fatty acid biosynthesis (FAB) is an essential metabolic process for prokaryotic organisms and is required for cell viability and growth.[7] β-Ketoacyl-(acyl-carrier-protein) (ACP) synthase III, also known as FabH or KAS III, plays an essential and regulatory role in bacterial FAB.[8] The enzyme initiates fatty acid elongation cycles and is involved in the feedback regulation of the biosynthetic pathway via product inhibition.[9,10] FabH proteins from both Gram-positive and Gram-negative bacteria are highly conserved at the sequence and structural levels, while there are no human proteins that are significantly homologous. The residues that compose the FabH active site are essentially invariant across various bacterial sources.[11–13] FabH is a promising target for the design of novel antimicrobial drugs, at it regulates the rate of fatty acid biosynthesis through an initiation pathway, and its substrate specificity is a key factor in membrane fatty acid composition.[14–16] These attributes indicate that small-molecule inhibitors of FabH activity could have therapeutic potential as selective, nontoxic, and broad-spectrum antibacterials.

Several kinds of compounds have been designed and screened in enzyme assays to generate leads that were co-crystallized with various pathogenic FabH proteins and subsequently optimized using structure-guided drug design methods.[17–21] Our group recently summarized the various types of FabH inhibitors, which can be categorized as follows: alkyl-CoA disulfides, thiolactomycin derivatives, 1,2-dithiole-3-(thi)one derivatives, cerulenin derivatives, indole derivatives, benzoylaminobenzoic acid derivatives, alkylsulfonyl derivatives, and thiazolidine-2-one 1,1-dioxide derivatives.[22] Among the published work, Kim and co-workers reported YKAs3003, a Schiff base condensed from 4-hydroxysalicylaldehyde and cyclohexanamine, as a potent inhibitor of *Escherichia coli* FabH with antimicrobial activity.[23] Further optimization of this compound is required to improve its antimicrobial activity. Oxime and oxime ethers have gathered more recent interested owing to their biological activity and their antimicrobial efficiency in particular.[24–28] Figure 1 shows some antimicrobial agents and antifungal drugs equipped with oxime ether groups.

For this study, we designed and synthesized a new series of oxime derivatives with a methyleneaminoxy group (C=N–O) derived from *O*-benzylhydroxylamines and primary benzaldehydes or salicylaldehydes, and then studied their antimicrobial activities and inhibitory activities toward *E. coli* FabH. Docking

[a] *Dr. Y. Luo,[+] Dr. L.-R. Zhang,[+] Dr. Y. Hu, Dr. S. Zhang, Prof. X.-M. Wang, Prof. H.-L. Zhu*
State Key Laboratory of Pharmaceutical Biotechnology
Nanjing University, Nanjing 210093 (P.R. China)
E-mail: zhuhl@nju.edu.cn

[b] *Dr. J. Fu*
State Key Laboratory of Pollution Control & Resource Reuse
Nanjing University, Nanjing 210046 (P.R. China)

[+] *These authors contributed equally to this work.*

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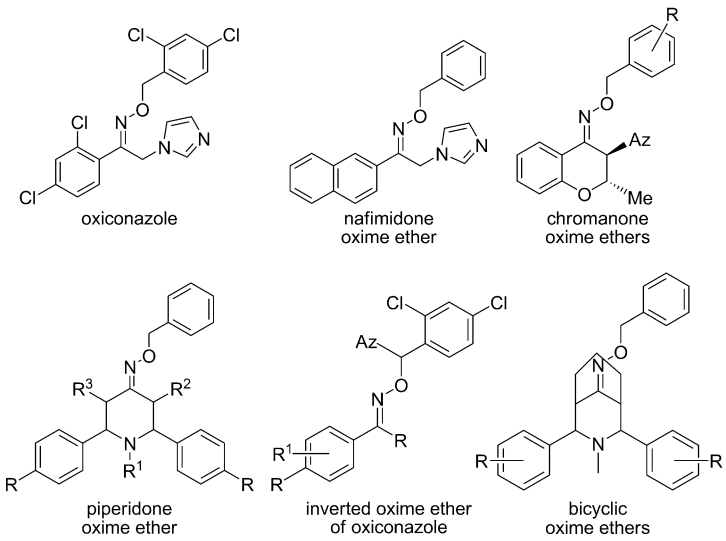


Figure 1. Structures of some analogous antimicrobial agents bearing an oxime group; Az = 1-methyl-1*H*-imidazole.

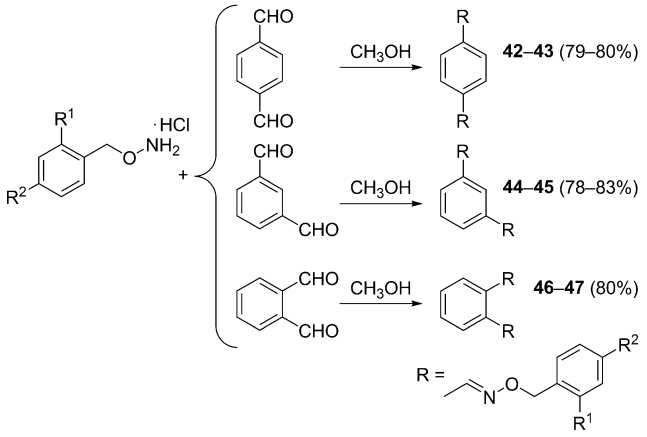
simulations were performed using the X-ray crystallographic structure of *E. coli* FabH in complex with the most active inhibitor, compound **44**, to explore the binding mode of the compound at the active site. This series of compounds was found to be more efficient than most of the previously reported FabH inhibitors, and their structures are also different from most reported inhibitors.

Results and Discussion

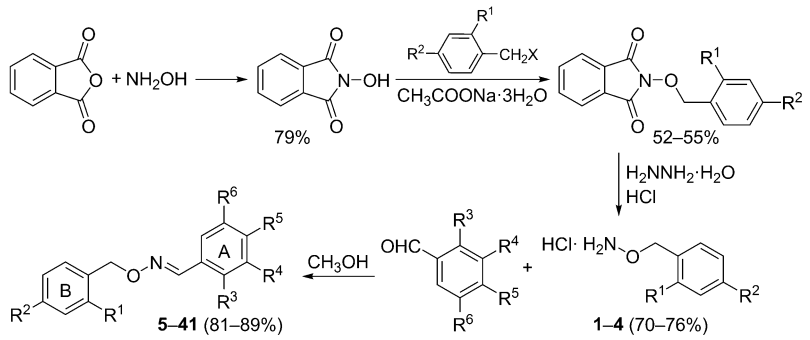
Chemistry

O-Benzylhydroxylamines **1**–**4** were synthesized by the routes illustrated in Scheme 1 according to the method of Karakurta et al.[29] *O*-Benzylhydroxylamine was prepared by reaction of benzyl halide and *N*-hydroxyphthalimide in dimethyl sulfoxide (DMSO) in the presence of sodium acetate trihydrate followed by hydrolysis of the imidic group by treatment with hydrazine hydrate.

O-Benzylhydroxylamines were then allowed to react with substituted benzaldehydes and salicylaldehydes to prepare the corresponding oxime derivatives **5**–**47** (Schemes 1 and 2). The structural substituents of these oxime derivatives are listed in Table 1. These compounds gave satisfactory elementary analyses (±0.4%); moreover, ^{1}H NMR, ^{13}C NMR, and HRMS data are consistent with the assigned structures. Among these compounds, **5**–**18**, **22**–**26**, **28**–**36**, and **38**–**47** are reported herein for the first time. Compounds **5**–**21** are oxime derivatives that were prepared by combining substituted *O*-benzylhydroxylamines with benzaldehydes. Compounds **22**–**41** were prepared by the combination of substituted *O*-benzylhydroxylamines and salicylaldehydes. Furthermore, to demonstrate the importance of the oxime group for activity, compounds **42**–**47**, with two methyleneaminoxy groups (C=N–O) were synthesized by treating substituted *O*-benzylhydroxylamines with benzenedicarboxaldehydes.



Scheme 2. Synthesis of oxime derivatives **42**–**47**.



Scheme 1. Synthesis of oxime derivatives **5**–**41**.

Antimicrobial activity and cytotoxicity

All compounds synthesized were screened for antibacterial activities by the serial dilution method against three Gram-negative strains: *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Pseudomonas fluorescens* P13; compounds were also screened against three Gram-positive strains: *Bacillus subtilis* ATCC 530, *Staphylococcus aureus* ATCC 6538, and *Enterococcus faecalis* ATCC29212. The MICs of the compounds against these microorganisms are listed in Table 2, along with the activity of the reference compound fleroxacin. The results show that most of the synthesized compounds are endowed with significant antimicrobial activity. Out of the 43 synthetic oxime deriva-

Table 1. Structures of compounds **5**–**47**.

Compd	R^1	R^2	R^3	R^4	R^5	R^6
5	Cl	Cl	H	H	$PhCH_2O$	H
6	Cl	Cl	H	H	NO_2	H
7	Cl	Cl	H	H	Br	H
8	Cl	Cl	H	H	CH_3	H
9	Cl	Cl	H	H	Cl	H
10	Cl	Cl	H	H	F	H
11	Cl	Cl	F	H	H	H
12	Cl	Cl	H	H	$N(CH_3)_2$	H
13	Cl	H	H	H	NO_2	H
14	Cl	H	H	H	$N(CH3)_2$	H
15	Cl	H	H	H	Br	H
16	F	H	H	H	NO_2	H
17	F	H	H	H	$N(CH3)_2$	H
18	F	H	H	H	Br	H
19	H	H	H	H	NO_2	H
20	H	H	H	H	H	H
21	H	H	H	H	$N(CH_3)_2$	H
22	Cl	Cl	OH	H	H	H
23	Cl	Cl	OH	Br	H	Br
24	Cl	Cl	OH	H	H	Br
25	Cl	Cl	OH	Cl	H	Cl
26	Cl	Cl	OH	H	H	Cl
27	Cl	H	OH	H	H	H
28	Cl	H	OH	Br	H	Br
29	Cl	H	OH	H	H	Br
30	Cl	H	OH	Cl	H	Cl
31	Cl	H	OH	H	H	Cl
32	F	H	OH	H	H	H
33	F	H	OH	Br	H	Br
34	F	H	OH	H	H	Br
35	F	H	OH	Cl	H	Cl
36	F	H	OH	H	H	Cl
37	H	H	OH	H	H	H
38	H	H	OH	Br	H	Br
39	H	H	OH	H	H	Br
40	H	H	OH	Cl	H	Cl
41	H	H	OH	H	H	Cl
42	Cl	Cl	–	–	–	–
43	Cl	H	–	–	–	–
44	Cl	Cl	–	–	–	–
45	Cl	H	–	–	–	–
46	Cl	Cl	–	–	–	–
47	Cl	H	–	–	–	–

tives, compound **44** exhibited the best antibacterial activity, with MICs of 6.25, 3.13, 3.13, 3.13, 3.13, and 3.13 $\mu g\,mL^{-1}$ against *E. coli*, *P. aeruginosa*, *P. fluorescens*, *B. subtilis*, *S. aureus* and *E. faecalis*, respectively; these values are close to those of the broad-spectrum antibiotic fleroxacin on Gram-positive strains, with corresponding MICs of 0.39, 0.78, 3.13, 1.56, 1.56, and 1.56 $\mu g\,mL^{-1}$. Moreover, compound **10** exhibited similar activity to that of compound **44**. Overall, most of the compounds with two oxime groups (compounds **42**–**47**) showed good antibacterial activities. In general, most synthesized compounds either showed better antimicrobial activities toward Gram-negative (*E. coli*, *P. aeruginosa*, and *P. fluorescens*) than toward Gram-positive bacteria (*B. subtilis*, *S. aureus*, and *E. faecalis*), or their activities were similar against both Gram-negative and Gram-positive bacteria. In addition, the reported FabH inhibitor DDCP (figure 3, compound **5** in reference [30]) was tested for its antibacterial activities, with MICs of 1.56, 3.13, 6.25, 12.5, 25, 6.25 $\mu g\,mL^{-1}$ against *E. coli*, *P. aeruginosa*, *P. fluorescens*, *B. subtilis*, *S. aureus*, and *E. faecalis*, respectively. Its activity was better than most synthesized oximes on Gram-negative strains; however, it was not more active than certain oximes (such as compound **44**) on Gram-negative strains.

To demonstrate the importance of the oxime group, compounds **48** and **49** (Figure 2) were synthesized; their structures are similar to that of compound **10**. Their antimicrobial activities were tested, and the results are given in Table 2. The sole difference between compounds **10** and **48** is the presence of a methylene unit in the latter in place of the oxygen atom of compound **10**. Compound **10** showed much higher antibacterial activity than compounds **48** and **49**. The MICs of compound **10** against *E. coli*, *P. aeruginosa*, *P. fluorescens*, *B. subtilis*, *S. aureus*, and *E. faecalis* are 6.25, 3.13, 6.25, 6.25, 6.25, and 12.5 $\mu g\,mL^{-1}$, respectively, while the respective MICs for compound **49** are 25, 12.5, 25, 50, 50, and 100 $\mu g\,mL^{-1}$. These results suggest that the oxime group influences antimicrobial activity.

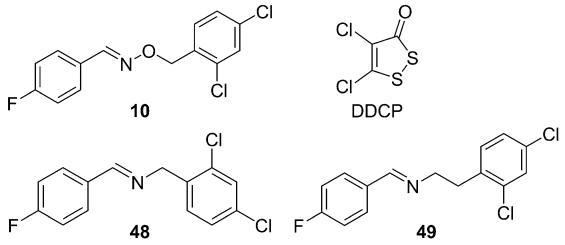


Figure 2. Structures of compounds **10**, DDCP, **48**, and **49**.

It is well known that some fluorinated compounds such as norfloxacin, ciprofloxacin, levofloxacin, and linezolid exhibit favorable antibacterial activities, as the fluorine atom may play an important role in improving the pharmacokinetic properties.[31–34] Among compounds **5**–**12**, which have the same substituents on the B ring (R^1 and R^2 groups), those with electron-withdrawing groups at the R^5 position (**6**, **7**, **9**, **10**, and **11**) exhibited higher antibacterial activities than those with electron-donating groups (**5**, **8**, and **12**). Moreover, compound **10**, with a *para*-fluoro group, showed greater antibacterial activity than the *ortho*-fluorinated compound **11**, suggesting that the position of this substituent is important for antibacterial activity. In assessing compounds with the same substituents on the A ring (R^3, R^4, R^5 and R^6 groups), such as compounds **12**, **14**, **17**, and **21**, the activity is ranked as: **12** ≈ **14** > **17** > **21**. Among these, compound **12**, with two chloro groups, showed activity similar to that of the mono-chlorinated compound **14** and greater than the mono-fluorinated compound **17**. Compound **21**, lacking any halogen substitution, showed the weakest activity.

As mentioned above, compounds **22**–**41** were synthesized by combining substituted *O*-benzylhydroxylamines and salicylaldehydes. Various salicylaldehyde substituents such as 3,5-dichloro, 5-chloro, 3,5-dibromo, and 5-bromo led to varied antibacterial activities observed for these oxime derivatives. Com-

Table 2. Antibacterial activities of synthesized compounds.

Compd	MIC [μg mL^{-1}]					
	Gram-positive			Gram-negative		
	B. subtilis ATCC 530	*S. aureus* ATCC 25923	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*P. fluorescens* P13
5	50	25	50	25	25	25
6	12.5	25	50	12.5	12.5	25
7	12.5	25	25	12.5	12.5	25
8	25	50	25	25	25	12.5
9	12.5	25	12.5	12.5	12.5	25
10	6.25	6.25	12.5	6.25	3.13	6.25
11	6.25	12.5	12.5	12.5	12.5	25
12	50	50	25	12.5	12.5	12.5
13	12.5	25	25	12.5	12.5	6.25
14	50	25	50	12.5	25	12.5
15	25	12.5	50	25	25	50
16	50	50	>100	25	12.5	25
17	50	50	>100	25	25	25
18	50	50	>100	25	25	25
19	>100	>100	>100	50	25	25
20	25	12.5	12.5	12.5	12.5	25
21	>100	>100	>100	50	50	50
22	25	25	50	25	50	12.5
23	50	25	50	25	100	50
24	50	>100	>100	25	50	25
25	25	50	12.5	25	25	12.5
26	50	50	25	25	25	25
27	50	100	25	50	50	12.5
28	50	>100	50	50	25	50
29	>100	>100	50	>100	>100	50
30	25	25	25	25	12.5	25
31	100	100	50	50	50	25
32	50	50	50	50	100	50
33	>100	>100	>100	50	50	>100
34	100	50	>100	50	50	>100
35	50	25	25	25	25	50
36	50	25	25	25	50	50
37	50	50	50	25	50	25
38	50	100	100	25	50	50
39	50	100	100	25	100	25
40	12.5	25	25	12.5	12.5	25
41	50	50	50	12.5	25	12.5
42	12.5	12.5	25	6.25	12.5	6.25
43	3.13	12.5	12.5	12.5	3.13	6.25
44	3.13	3.13	3.13	6.25	3.13	3.13
45	12.5	6.25	6.25	6.25	12.5	12.5
46	12.5	12.5	12.5	6.25	12.5	12.5
47	12.5	12.5	25	6.25	12.5	6.25
48	50	100	100	50	25	25
49	50	50	100	25	12.5	25
Fleroxacin	1.56	1.56	1.56	0.39	0.78	3.13
DDCP	12.5	25	6.25	1.56	3.13	6.25

pared with compounds **5**–**21**, these compounds showed lower antibacterial activity. In particular, compounds **20** and **37** were synthesized by combining unsubstituted benzaldehyde and salicylaldehyde with unsubstituted *O*-benzylhydroxylamine. Compound **20** showed better antibacterial activity than compound **37** across most of the strains tested, suggesting that the presence of a hydroxy group could decrease potency.

To gauge the tested compounds for potential cytotoxicity, and therefore a lack of selective antibacterial activity, compound **44** was also monitored for its cytotoxicity toward Vero, BHK-21, and Raw 264.7 cells; the results are listed in Table 3. The IC_{50} values were consistently >40 mg mL^{-1}, demonstrating that compound **44** elicits no clear cytotoxicity toward eukaryotic cells.

Table 3. Cytotoxicity results of compound **44**.

Cell line	IC_{50} [mg mL^{-1}]
Vero	44.27
BHK-21	54.39
Raw264.7	49.46

E. coli FabH inhibitory activity

The potencies of the synthesized oximes in inhibiting *E. coli* FabH activity were examined using compounds found to have the highest (**42**–**47**, **10**, **18**, **9**, and **11**) and lowest (**21** and **29**) antibacterial activities; the results are summarized in Table 4. Most of the tested compounds displayed significant *E. coli* FabH inhibitory activity. Among them, compound **44** was found to be the most potent, with an IC_{50} value of 1.7 μM; compound **21** was weakest, with an IC_{50} value of 59.3 μM. This result supports the prominent antibacterial activity observed for **44**.

Compound **10**, with a *para*-fluoro group, showed higher FabH inhibition than its *ortho*-fluorinated counterpart, compound **11**. The results of *E. coli* FabH inhibition assays correspond to the structure–activity relationships of the antibacterial activities of these compounds, demonstrating that their antibacterial action is likely correlated to FabH inhibition.

Binding model of compound 44 and *E. coli* FabH

Molecular docking of compound **44** into *E. coli* FabH was performed on a binding model based on the crystal structure of the FabH–CoA complex (PDB code: 1HNJ).[35] All docking runs were performed with the LigandFit Dock protocol of Discovery Studio 3.1. The model of compound **44** docked with *E. coli* FabH is depicted in Figure 3a. In the binding model, com-

Table 4. *E. coli* FabH inhibitory activities of top compounds.

Compd	IC_{50} [μM]
9	25.2 ± 1.8
10	4.2 ± 0.3
11	23.9 ± 2.5
18	5.9 ± 0.4
20	59.3 ± 4.1
28	47.2 ± 4.5
41	22.6 ± 1.8
42	31.4 ± 2.5
43	1.7 ± 0.2
44	8.7 ± 0.7
45	15.4 ± 1.4
46	17.9 ± 0.9
DDCP	2.1 ± 0.1

pound **44** is nicely bound to the FabH kinase through four interactions with a total binding energy of $-28.39\ kJ\,mol^{-1}$. The ammonium groups of residues Arg36 and Arg151 respectively form π–cation interactions with the same dichlorophenyl ring of compound **44**; the same ring of **44** also forms a π–π conjugate bond with the terminal amine of Trp32, which might enhance the binding affinity between FabH and ligand **44**. Additionally, the terminal amine of Ala246 forms a σ–π bond with the other dichlorophenyl ring of compound **44**. Figure 3b shows the surface model between compound **44** and FabH, indicating that the hydrophobic channel of the binding site is suitably occupied by the compound. The results show that the three key interactions are all π interactions with aromatic rings, implying that aromatic groups might play an important role in FabH inhibitory activity. This docking model in combination with the biological assay data suggest that compound **44** is an inhibitor of FabH.

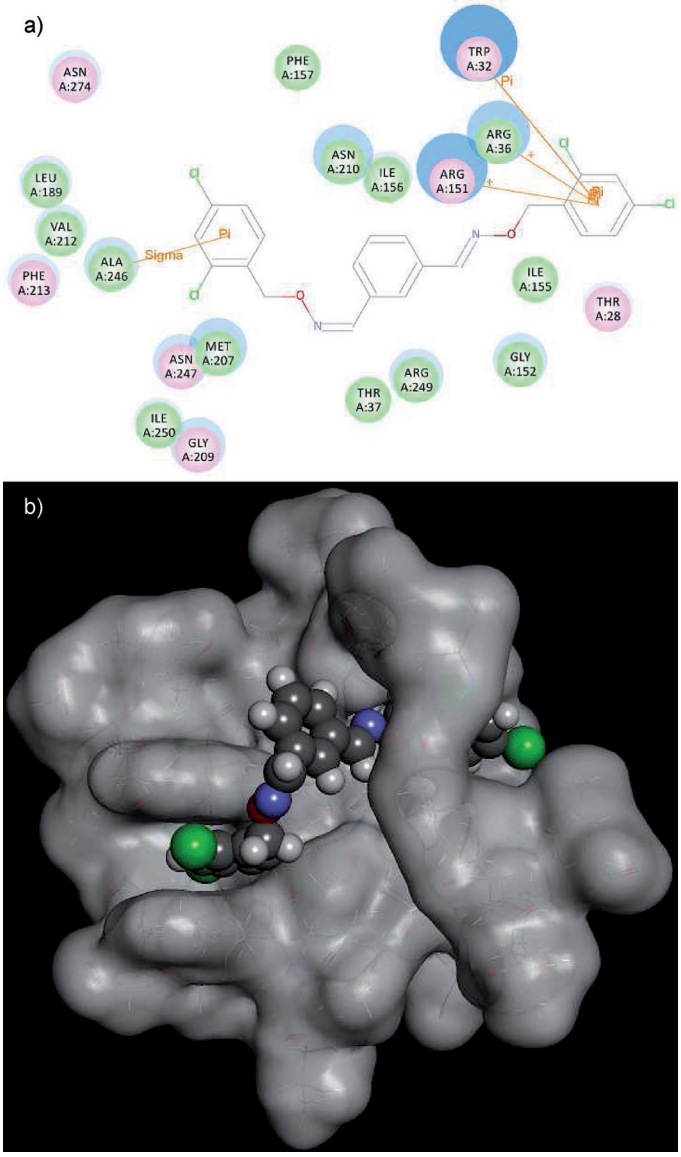


Figure 3. a) 2D ligand interaction diagram of compound **44** with FabH with the essential amino acid residues at the binding site highlighted in circles (image constructed using the Discovery Studio software package). Purple circles indicate residues that participate in hydrogen bonding, electrostatic, or polar interactions; green circles show residues involved in van der Waals interactions. b) Surface structure model of compound **44** in binding complex with FabH.

Conclusions

A series of novel oxime derivatives generated by combining substituted benzaldehydes and salicylaldehydes with substituted *O*-benzylhydroxylamines were assayed for their antibacterial activities against *E. coli*, *P. aeruginosa*, *P. fluorescens*, *B. subtilis*, *S. aureus*, and *E. faecalis*. Compound **44**, 3-((2,4-dichlorobenzyloxyimino)methyl)benzaldehyde *O*-2,4-dichlorobenzyl oxime, showed the best antibacterial activity with MICs of 3.13–6.25 $\mu g\,mL^{-1}$ against the strains tested, and also exhibited the highest *E. coli* FabH inhibitory activity, with IC_{50} 1.7 μM. The introduction of hydrophobic and electron-withdrawing groups was conducive to the antibacterial and FabH inhibitory activities. Docking simulations were performed to position compound **44** into the *E. coli* FabH active site to determine the probable binding conformation.

Experimental Section

Chemistry

All chemicals used (reagent grade) were commercially available. Melting points (uncorrected) were determined on an XT4 MP apparatus (Taike Corp., Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer. ^1H and ^{13}C NMR spectra were recorded on Bruker PX500 or DPX300 spectrometers at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts (δ) are reported in ppm. Elemental analyses were performed on a CHN-O-Rapid instrument, and are within ± 0.4 % of the theoretical values.

General method for the synthesis of oxime derivatives: The substituted benzyloxy amines **1**–**4** were prepared according to procedures reported by Karakurta et al.[29] Equimolar quantities (0.5 mmol) of substituted benzaldehydes and salicylaldehydes and the substituted benzyloxy amines **1**–**4** with one amino group were dissolved in methanol (10 mL) and stirred at room temperature for

4–6 h. The precipitates were separated by filtration, recrystallized from methanol, washed with methanol three times, and dried in a vacuum desiccator containing anhydrous $CaCl_2$ to give oxime derivatives **5**–**41** in yields of 81–89%.

Terephthalaldehydes (0.25 mmol) with two aldehyde groups and the substituted *O*-benzylhydroxylamines **1**–**4** hydrochloride with one amino group were dissolved in methanol (10 mL) and stirred at room temperature for 4–6 h. The precipitates were separated by filtration, recrystallized from methanol, washed with methanol three times, and dried in a vacuum desiccator containing anhydrous $CaCl_2$ to give oxime derivatives **42**–**47** in yields of 78–83%.

General method for the synthesis of compounds 48 and 49: Compounds **48** and **49** were prepared according to the procedure used for oxime derivatives. The *O*-benzylhydroxylamine was replaced by the corresponding benzylamine and 2-phenylethylamine, and yields were 80–82%.

Biology

Antibacterial activity: The antibacterial activities of the synthesized compounds were tested against three Gram-negative bacterial strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *P. fluorescens* P13, along with three Gram-positive strains: *B. subtilis* ATCC 530, *S. aureus* ATCC 25923, and *E. faecalis* ATCC 29212 using methods recommended by the Clinical Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS).[36]

In vitro activities of the compounds were tested in nutrient broth (NB) for bacteria by the twofold serial dilution method. Seeded broth (broth containing microbial spores) was prepared in NB from 24-hour-old bacterial cultures on nutrient agar (Hi-media) at 37 ± 1 °C. The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^4–10^5 CFU mL^{-1}. The tested compounds and reference drugs were prepared by twofold serial dilution to obtain the required concentrations of 100, 50, 25, 12.5, 6.25, 3.13 μg mL^{-1}, and even lower concentrations. The tubes were incubated in BOD incubators at 37 ± 1 °C for bacteria. The MICs were recorded by visual observations after 24 h incubation (for bacteria). Fleroxacin and DDCP were used as standards.

Cytotoxicity assays: The cytotoxicity of compound **44** was evaluated against normal Vero, BHK-21, and Raw 264.7 cell lines. Target tumor cell lines were grown to log phase in RPMI 1640 medium supplemented with 10% fetal bovine serum. After diluting to 2×10^4 cells mL^{-1} with the complete medium, 100 μL of the obtained cell suspension was added to each well of 96-well culture plates. The subsequent incubation was carried out at 37 °C under an atmosphere containing 5% CO_2 for 24 h before cytotoxicity assessments. Tested samples at pre-set concentrations were added to six wells. After an exposure period of 48 h, PBS (40 μL) containing 2.5 mg mL^{-1} 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) was added to each well. Four hours later, 100 μL extraction solution (10% SDS, 5% isobutyl alcohol, 0.01 M HCl) was added. After an overnight incubation at 37 °C, the optical density was measured at λ 570 nm on an ELISA microplate reader. In all experiments three replicate wells were used for each drug concentration. Each assay was carried out at least three times. The results are summarized in Table 3.

***E. coli* FabH purification and activity assay:** Full-length *E. coli* acyl-carrier protein (ACP), acyl-carrier-protein synthase (ACPS), and β-ketoacyl-ACP synthase III (FabH) were individually cloned into pET expression vectors with an N-terminal His tag (ACP, ACPS in pET19; FabH in pET28). All proteins were expressed in *E. coli* strain BL21 (DE3). Transformed cells were grown on Luria–Bertani (LB) agar plates supplemented with kanamycin (30 μg mL^{-1}). SDS-PAGE analysis was used to screen colonies for overexpression of proteins. One such positive colony was used to inoculate 10 mL LB medium with 30 μg mL^{-1} kanamycin; this was grown overnight at 37 °C, 1 mL of which was used to inoculate 100 mL LB medium supplemented with 30 mg mL^{-1} kanamycin. The culture was shaken for 4 h at 37 °C, and then induced with 0.5 mM isopropyl β-D-thiogalactopyranoside (IPTG). The culture was grown for 4 h, and harvested by centrifugation (30 min at 21 000 *g*).

Harvested cells containing His-tagged ACP, ACPS, and FabH were lysed by sonication in 20 mM Tris (pH 7.6), 5 mM imidazole, and 0.5 M NaCl and centrifuged at 37 100 *g* for 30 min. The supernatant was applied to a Ni–NTA agarose column, washed, and eluted using a gradient of 5–500 mM imidazole over 20 column volumes. Eluted protein was dialyzed against 20 mM Tris (pH 7.6), 1 mM DTT, and 100 mM NaCl. Purified FabH was concentrated to 2 mg mL^{-1} and stored at −80 °C in 20 mM Tris (pH 7.6), 100 mM NaCl, 1 mM DTT, and 20% glycerol for enzyme assays.

Purified ACP contains the apo form that needs to be converted into the holo form. The conversion reaction is catalyzed by ACP synthase (ACPS). In a final volume of 50 mL, 50 mg ACP, 50 mM Tris, 2 mM DTT, 10 mM $MgCl_2$, 600 μM CoA, and 0.2 μM ACPS were incubated for 1 h at 37 °C. The reaction was then adjusted to pH ~ 7.0 using 1 M potassium phosphate. Holo-ACP was purified by fractionation of the reaction mixture by Source Q-15 ion-exchange chromatography using a 0–500 mM NaCl gradient over 25 column volumes.

In a final 20 μL reaction, 20 mM Na_2HPO_4/NaH_2PO_4 (pH 7.0), 0.5 mM DTT, 0.25 mM $MgCl_2$, and 2.5 μM holo-ACP were mixed with 1 nM FabH, and H_2O was added to 15 μL. After 1 min incubation, a 2 μL mixture of 25 μM acetyl-CoA and 0.75 μCi [^{3}H]acetyl-CoA was added for FabH reaction for 25 min. The reaction was stopped by adding 20 mL ice-cold 50% TCA, incubating for 5 min on ice, and centrifuging (16 000 *g*, 10 min) to pellet the protein. The pellet was washed with 10% ice-cold TCA and resuspended in 5 μL 0.5 M NaOH. Incorporation of the ^{3}H signal in the final product was determined by liquid scintillation. For IC_{50} determination, inhibitors were added from a concentrated DMSO stock such that the final concentration of DMSO did not exceed 2%.

Docking simulations

Molecular docking of compound **44** into the 3D X-ray structure of *E. coli* FabH (PDB code: 1HNJ) was carried out with Discovery Studio software (version 3.1) as implemented through the graphical user interface DiscoveryStudio CDOCKER protocol.

The 3D structures of the aforementioned compounds were constructed using ChemBio 3D Ultra 11.0 software [Chemical Structure Drawing Standard, Cambridge Soft Corp., USA (2008)]; they were then energy-minimized by using MMFF94 with 5000 iterations and a minimum RMS gradient of 0.10. The crystal structures of *E. coli* FabH were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). All bound water and ligands were removed from the protein, and polar hydrogen atoms were added. The entire 1HNJ structure was defined as a receptor and the site sphere was selected based on the active center of 1HNJ according to a previous report,[28] then compound **44** was placed in during the molecular docking procedure. The types of interac-

tions between the docked ligand and protein were analyzed after the end of the molecular docking procedure.

Declaration

The authors declare no conflict of interest. The material herein is original and has not been submitted for publication elsewhere. The main work in the synthesis of oxime compounds, evaluation of their biological activities, data analysis, and manuscript preparation were performed by Yin Luo, Li-Rong Zhang, and Jie Fu. Docking simulations were done by Yang Hu. Shuai Zhang contributed to the synthesis, biological assays, and data analysis. Xiao-Ming Wang and Hai-Liang Zhu are the corresponding authors.

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