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Abstract: A series of novel α-(diphenylphosphoryl)- and α-(diphenylphosphorothioyl)cycloalkanone oximes have been synthesized in search for novel bioactive molecules. Their structures were characterized by various spectroscopic methods including IR, NMR (^{1}H, ^{31}P, ^{13}C), mass spectrometry and single crystal X-ray diffraction. The newly synthesized phosphorus-containing oximes were screened for their *in vitro* antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*) and fungal strains (*Candida albicans* and *Candida glabrata*). The biological assays showed that all the studied compounds (**2a-f**) exhibited high antibacterial and antifungal activities at only 0.1-2.1 μg/mL. *In silico* molecular docking studies in FabH enzyme active site were performed in order to predict the possible interaction modes and binding energies of the drug candidates at the molecular level.

Keywords: oximes; phosphine oxides; phosphine sulfides; phosphonyloximes; antibacterial activity; antifungal activity; molecular docking.

Introduction

The emergence of multidrug-resistant strains of bacteria and fungi has brought an urgent need for the discovery of novel antimicrobial agents with other modes of action.[1-3] One possible and useful strategy to attain new classes of drug molecules is to develop synthetic antimicrobials whose chemical structures do not occur in nature, and would thus be evolutionarily foreign to microbes.

For the past few years, oxime derivatives have attracted the increasing interest of researchers due to their wide-range of useful applications as antimicrobial,[4-7] antiparasitic,[8,9]

antiviral,[10] antihistaminic,[11] and anti-HIV[12] agents. Some examples of bioactive oxime derivatives containing commercial drugs are chromanone and nafimidone oximes which have demonstrated good performances as antibacterial agents,[13,14] and oxiconazole, an antifungal medication used to treat skin infections, such as athlete's foot, jock itch and ringworm[15,16] (**Figure 1**).

On the other hand, organophosphorus derivatives are a valuable class of compounds in medicinal chemistry that possess interesting biological effects and different binding affinities to diverse microbiological targets,[17-19] and the introduction of a phosphoryl or thiophosphoryl group to the oxime unit could enhance the antimicrobial activity of such compounds, in accordance with the active substructure combination principle.[20,21] Indeed, some commercialized antibiotic drugs, such as fosfomycin,[22] tedizolid phosphate[23] and phosphopyricin (**Figure 1**),[24] contain in their structures a phosphonic acid, phosphate or diphenylphosphine moiety. Furthermore, a number of antibiotic and antimalarial drugs used in clinical medicine, such as fosmidomycin[25] and FR-900098[26] (**Figure 1**), possess in their core chemical structure a phosphorus moiety as well as an hydroxamic acid functionality which is a structural analog of the oxime pharmacophore.[27]

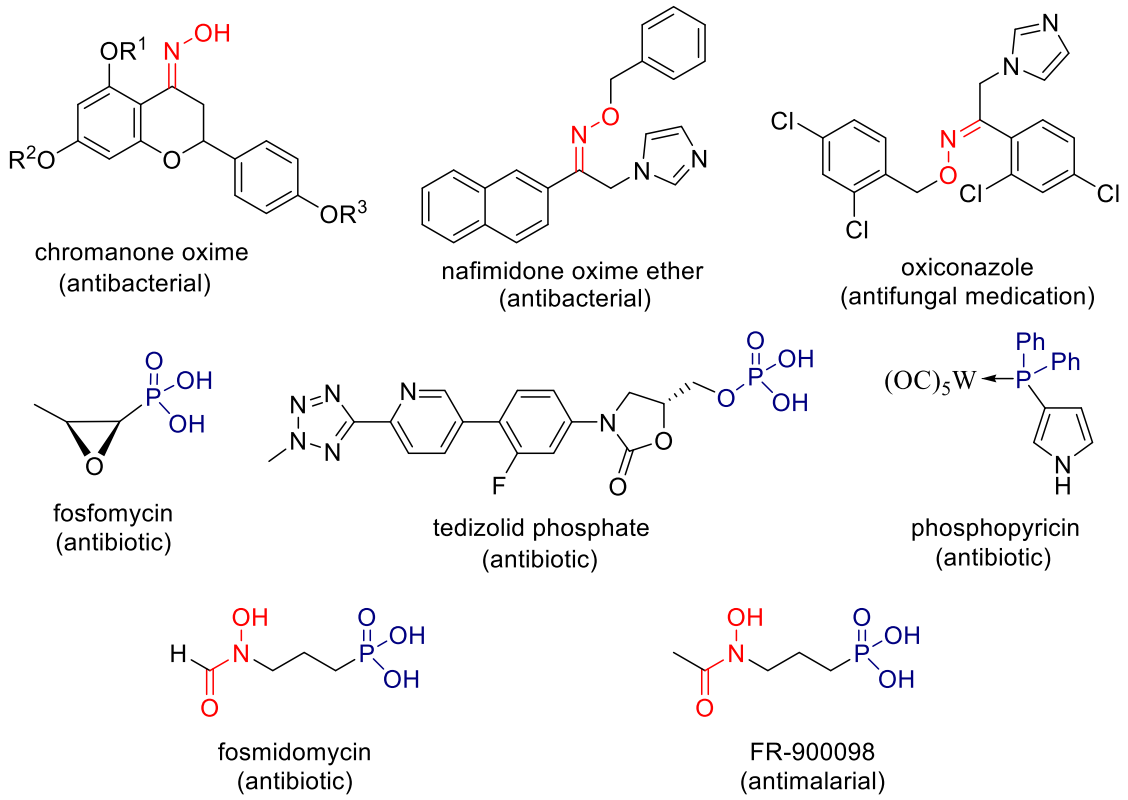


Figure 1. Structures of some antimicrobial agents bearing an oxime or phosphoryl group

In view of the above, and in the continuation of our research program concerning the preparation of novel phosphorus-containing compounds with possible biological properties,[28-31] we now report the synthesis of unprecedented α-(diphenylphosphoryl)- and α-(diphenylphosphorothioyl)cycloalkanone oximes bearing both the phosphoryl / phosphorothioyl and oxime pharmacophores. The antimicrobial and antifungal properties of these compounds were studied *in vitro*, and the results obtained were corroborated by *in silico* molecular docking studies.

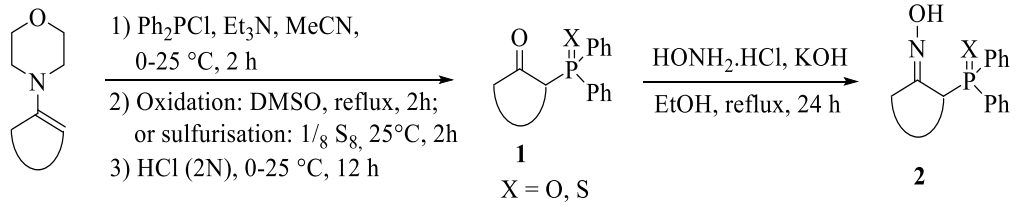
Results and discussion

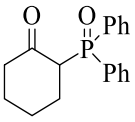
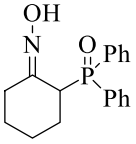
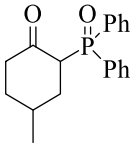
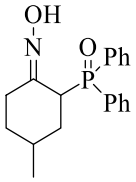
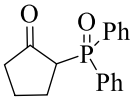
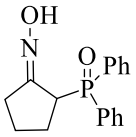
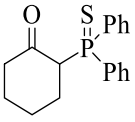
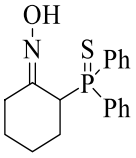
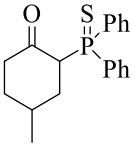
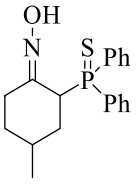
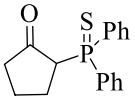
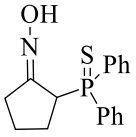
Chemistry

The starting α-(diphenylphosphoryl)- and α-(diphenylphosphorothioyl)cycloalkanones **1** were easily prepared from the reaction of a cyclic enamine with *P*-chlorodiphenylphosphine in the presence of triethylamine, followed by oxidation or sulfurization and hydrolytic work-up, according to the reported procedure.[32-34] Reaction of compounds **1** with hydroxylamine hydrochloride, conducted in refluxing ethanol for 24 h in the presence of an equimolar quantity of potassium hydroxide, led to the desired α-(diphenylphosphoryl)- and α-(diphenylphosphorothioyl)cycloalkanone oximes **2**. The isolated yield of the reaction ranges from 81 to 89% (**Table 1**).

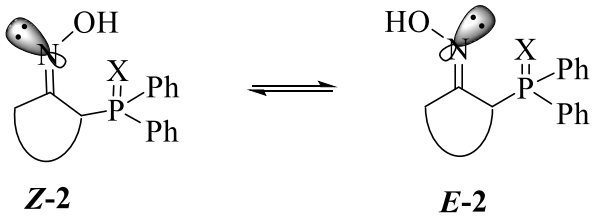
The structures of phosphorus-containing oximes **2** were established from their IR, NMR (^{1}H, ^{31}P, ^{13}C), and mass spectral data, which indicate that they are obtained as a mixture of two inseparable *Z* and *E* isomers (**Scheme 1**). Their relative proportions were estimated from the ^{31}P NMR spectra where a singlet for each isomer is present (**Table 1**). The *Z* and *E* configurations were assigned on the basis of the ^{13}C chemical shifts of carbons in *α* position with respect to the C=N double bond. Indeed, according to some literature data regarding the stereochemistry of oximes,[35-37] the carbon adjacent to the C=N double bond resonates at higher field when it is in the *syn* position relative to the oxime OH group. It should be mentioned that our attempts to separate the *Z* and *E* isomers by column chromatography or by fractional crystallization, have failed.

Table 1. Synthesis of α-(diphenylphosphoryl)- and α-(diphenylphosphorothioyl)-cycloalkanone oximes **2**



Entry	Cycloalkanones **1**	Oximes **2**	*Z*-**2** (%)[b]	*E*-**2** (%)[b]
1	 **1a**, 96%[a]	 **2a**, 89%[a]	73	27
2	 **1b**, 92%	 **2b**, 86%	55; 35[c]	10
3	 **1c**, 88%	 **2c**, 81%	91	9
4	 **1d**, 91%	 **2d**, 87%	42	58
5	 **1e**, 89%	 **2e**, 85%	21	68; 11[d]
6	 **1f**, 84%	 **2f**, 82%	86	14

[a] Isolated yield. [b] Determined from the ^{31}P NMR spectra. [c] Two *Z*-diastereoisomers are present due to asymmetric carbons. [d] Two *E*-diastereoisomers are present due to asymmetric carbons.



***Z*-2** ***E*-2**

Scheme 1. *Z* and *E* isomers for oximes **2a-f**

X-ray crystallography

Unambiguous structure elucidation of the α-(diphenylphosphoryl)cyclohexanone oxime **2a** was achieved through single-crystal X-ray diffraction analysis. The crystallographic data and structural refinement parameters are summarized in **Table 2**. Single crystals of **2a** were obtained by slow evaporation at room temperature of a methanol/hexane/ethyl acetate solution. Oxime **2a** crystallized in the monoclinic space group P21/n, with one molecule in the asymmetric unit (**Figure 2a**).

The molecule exhibits a regular spatial configuration with habitual distances and angles (**Table S1**). The dihedral angle between the two phenyl groups on the phosphorus atom is 104.29°, showing a quasi-orthogonal arrangement of the two phenyl rings due to steric reasons.

The cyclohexanone oxime ring is in the characteristic chair conformation and its geometrical parameters are reported in **Table S1** indicating that the C-C and C-N bond lengths as well as the C-C-C and C-C-N angles are in conformity with those observed in similar molecules.[38] The conformation of the cyclohexanone oxime ring can be described in terms of Cremer and Pople puckering coordinates,[39] by evaluating the parameters Q (total puckering amplitude), q2, q3, θ and φ. Their calculated values for the C1-C2-C3-C4-C5-C6 ring are: Q = 0.5370 Å, q2 = 0.0811Å, q3 = 0.5308Å, θ = 176.34° and φ = 8.69°, indicating that the cyclohexanone oxime ring has been distorted from the standard chair conformation by the oxime moiety.

The oxime function is predominantly observed in the *Z* configuration. However, a slight disorder was observed for this functionality, adopting an *E* configuration. The disorder was properly refined in two parts with final occupancy factors of 0.73 and 0.27, for the *Z* and *E* configurations, respectively (**Figure 2b**). This was in perfect agreement with the NMR results, which showed the same ratio (73/27) for the *Z* and *E* isomers, respectively (**Table 1**).

The crystal packing indicates that the molecules are interconnected *via* C=N-O-H…O=P intermolecular hydrogen bonds to form chains along the a-axis (**Figure S1**).

Table 2. Crystallographic data and structure refinement parameters for compound **2a**.

Empirical formula	$C_{18}H_{20}NO_2P$
Formula weight [g mol^{-1}]	313.32
Temperature (K)	100
Wavelength	1.54184
Crystal system	Monoclinic
Space group	P21/n
a [Å]	7.4395(3)
b [Å]	22.9744(9)
c [Å]	9.4000(4)
α (°)	90
β (°)	96.447(4)
γ (°)	90
V (Å^3)	1596.47(11)
Z	4
ρcalc [g cm^{-3}]	1.304
Absorption coefficient μ [mm^{-1}]	1.577
F(000)	664.0
Crystal size (mm^3)	0.218 × 0.111 × 0.091
θ range [°]	7.696 to 147.254
h, k, l ranges	9, 28,11
Reflections collected	15096
Independent reflections [R_{int}]	3181 (0.0494)
Completeness to θ	98.7%
Refinement method	Full-matrix least squares on F^2
Data/restraints/parameters	3181/10/208
Goodness-of-fit on F^2	1.045
Final R indices [I > 2σ(I)]	R_1 = 0.0611, wR_2 = 0.1700
R indices (all data)	R_1 = 0.0738, wR_2 = 0.1853
Largest diff. peak/hole [e Å^{-3}]	0.52/-0.40

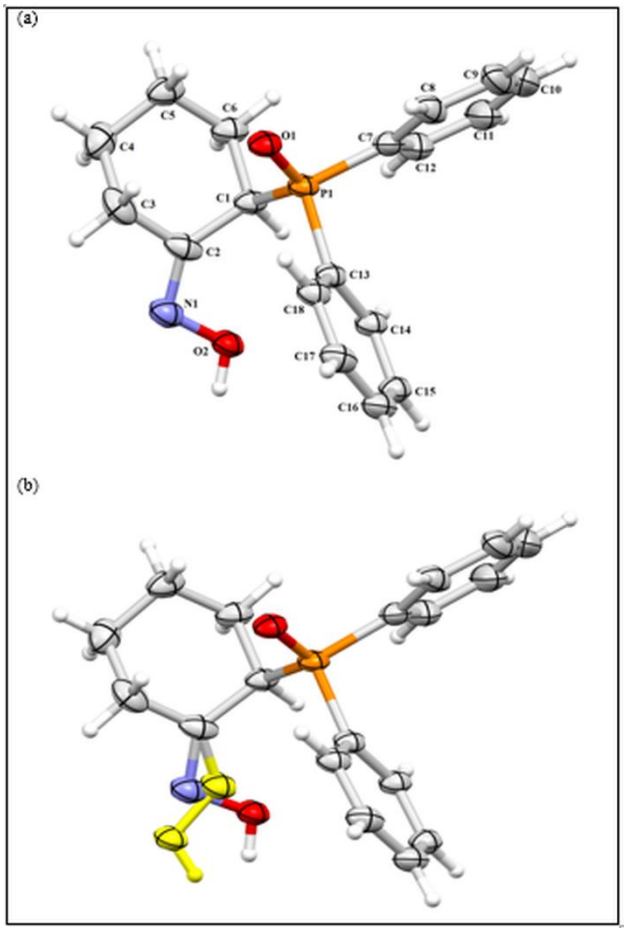


Figure 2. (a) X-ray molecular structure of compound **2a**. (b) X-ray molecular structure of **2a** showing both *Z* and *E* (in yellow) isomers. Thermal displacements ellipsoids are drawn at the 30% probability level.

Antimicrobial activity

The antimicrobial activity of the synthesized compounds (**2a-f**) was assessed against Gram-negative bacteria (*Escherichia coli* JW 1772, *Salmonella typhimurium* ATCC 14028), Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633), and fungal strains (*Candida albicans* ATCC 28367, *Candida glabrata* ATCC 15126) using the broth microdilution method according to the guidelines provided by the National Committee for Clinical Laboratory Standards.[40,41] Stock solutions of tested compounds (**2a-f**) (0.1 to 60 μg/mL final concentration) were incubated with an aliquot of each microorganism (0.5 McFarland turbidity standard scale) overnight at 37 °C and 29 °C for bacteria and fungi, respectively. Ciprofloxacin and fluconazole were used as standard antibacterial and antifungal drugs, respectively.

To establish the antimicrobial activity of the synthesized compounds on the bacterial and fungal growth, their respective minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) [*i.e.*, the minimum fungicidal concentration (MFC) for *C albicans* and *C. glabatra*] were determined by optical density (OD_{600}) reading and the agar plating method, respectively (see Supplementary Information for detailed protocols).[42]

The critical MIC, MBC (and MFC) values of the tested compounds against each pathogenic strain are summarized in **Table 3** together with the activity of the reference molecules ciprofloxacin and fluconazole.

The inhibition growth kinetics against *E. coli*, *S. typhimurium*, *S. aureus*, and *B. subtilis* containing different concentrations of the synthesized compounds **2a-f** (**Figure S2**) point out a significant inhibition of all bacterial strains. Indeed, the results show that all the synthesized compounds (**2a-f**) displayed good antibacterial activity, better than the standard drug ciprofloxacin, against all bacterial strains, with MIC values ranging from 0.7 to 1.8 μg/mL. The five-membered ring compounds **2c** and **2f** exhibited the best antibacterial activity with MIC values 3 to 8-fold more potent than those of the standard drug ciprofloxacin. Interestingly, the tested compounds (**2a-f**) were found to be more effective against Gram-positive bacteria (MIC = 0.7-1.2 μg/mL) than Gram-negative ones (MIC = 1.2-1.8 μg/mL), which could be due to the difference in bacterial membrane composition.[43]

Furthermore, a significant bactericidal activity (99.9%) was noticed for all the studied compounds (**2a-f**) with MBC values ranging from 2.0 to 2.1 μg/mL. Five-membered ring compounds **2c** and **2f** were confirmed to be the most potent agents, with **2c** showing the

strongest MBC activity against *S. typhimurium* and *S. aureus*, and **2f** giving the highest MBC activity against *E. coli* and *B. subtilis*.

Importantly, the ratio MBC/MIC was found in the range of 1.1-2.9 (**Table 4**), indicating that all the synthesized compounds (**2a-f**) were bactericidal in nature and not bacteriostatic.[44-47]

Table 3. MIC, MBC and MFC (µg/mL) of compounds **2a-f** and standard drugs against selected human pathogens.

Compound	Gram-negative bacteria				Gram-positive bacteria				Fungi	
	E.C. JW 1772		S.T. ATCC 14028		S.A. ATCC 25923		B.S. ATCC 6633		C.A. ATCC 28367	C.G. ATCC 15126
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MFC	MFC
2a	1.8	2.116	1.6	2.030	1.0	2.086	1.0	2.132	0.1	0.1
2b	1.8	2.094	1.8	2.135	0.9	2.070	0.9	2.083	0.1	0.1
2c	1.6	2.115	1.2	2.006	0.7	2.056	0.8	2.090	0.1	0.1
2d	1.8	2.106	1.8	2.032	1.2	2.089	0.9	2.080	0.1	0.1
2e	1.8	2.103	1.5	2.016	1.0	2.114	1.0	2.083	0.1	0.1
2f	1.6	2.059	1.3	2.022	0.8	2.082	0.7	2.065	0.1	0.1
Ciprofloxacin	10.9	62.5	5.8	31.2	5.8	31.2	1.9	15.6	-	-
Fluconazole	-	-	-	-	-	-	-	-	64.0	64.0

E.C.: *Escherichia coli*; **S.T.**: *Salmonella typhimurium* **S.A.**: *Staphylococcus aureus*; **B.S.**: *Bacillus subtilis*; **C.A.**: *Candida albicans*; **C.G.**: *Candida glabrata*.

Table 4. Ratio MBC/MIC of compounds **2a-f**.

Strains \ Compounds	2a	2b	2c	2d	2e	2f
Escherichia coli	1.17	1.16	1.32	1.17	1.16	1.28
Salmonella typhimurium	1.26	1.18	1.67	1.12	1.34	1.55
Staphylococcus aureus	2.08	2.30	2.93	1.74	2.11	2.60
Bacillus subtilis	2.13	2.31	2.61	2.31	2.08	2.95

The antifungal activity of the synthesized compounds was assessed through the inhibition of *Candida albicans* and *Candida glabrata* as fungal strains. As shown in **Table 3**, all the tested compounds (**2a-f**) also displayed good fungicidal activity, better than the standard drug fluconazole, with an excellent MFC value of 0.1 μg/mL.

Considering their overall efficacy, the five-membered ring oximes **2c** and **2f** were found to be the most potent of all the tested compounds. However, the increase in the size of the ring and its substitution resulted only in a very slight decrease of the antibacterial activity. Thus, compounds **2c** and **2f** can be regarded as a new valuable source to produce novel oxime derivatives against bacterial and fungal strains. This indicates that the introduction of significant phosphorus substituants to cyclopentanone oximes was as effective in obtaining of potent antimicrobial agents and might be a potential source for synthetic products for the pharmaceutical industry against bacterial and fungal strains.

Molecular docking analysis

Among the molecular targets in pathogenic strains, the β-ketoacyl-acyl carrier protein synthase III (FabH), which initiates the fatty acid elongation cycles by catalyzing the first condensation step between acetyl-CoA and malonyl-ACP,[48] has been validated as an attractive drug target.[49] Moreover, FabH is not only highly conserved at the sequence and structural levels among key pathogens; but above all, the amino acid residues (*i.e.*, Cys-His-Asn) forming its catalytic triad are essentially invariant across Gram-positive and Gram-negative organisms.[50,51]

Given the fact that oxime derivatives have been previously described to inhibit *E. coli* FabH,[49] and the promising antibacterial activity of compounds **2c** and **2f** against Gram-positive and Gram-negative, in an attempt to correlate such activity with the putative binding mode of these latter inhibitors in the active site of this enzyme, *in silico* molecular docking experiments were conducted as described previously,[52-54] using the reported crystal structure of *E. coli* FabH (*Ec*FabH) in complex with Malonyl-CoA in its active site (PDB entry code: 1HNJ).[55]

The best scoring position obtained (*i.e.*, lowest energy complex) showed that compounds **2c** and **2f** may be located at the entrance of the *Ec*FabH active site with predicted binding energy values $\Delta E_{\mathbf{2c}} = -7.0$ kcal/mol and $\Delta E_{\mathbf{2f}} = -6.6$ kcal/mol, respectively. In both cases, **2c** and **2f** would be located at the right entrance of the catalytic cleft, at a distance of around 9 Å from the catalytic Cys^{112}, thus excluding any covalent interaction (**Figure 3A**).

Each of the docked **2c**-*Ec*FabH and **2f**-*Ec*FabH complexes was further subjected to interactions analysis using Ligplot+ v.1.4.[56] The Ligplot+ diagram schematically depicts the hydrogen bonds and hydrophobic interactions between the ligand (*i.e.*, oximes **2c** or **2f**), and the active site residues of the protein (*i.e.*, *Ec*FabH) during the binding process. In the obtained binding model, the two bulky phenyl groups of **2c** may be involved in π-stacking interactions with Trp^{32}, Thr^{37}, GLy^{152}, Ile^{156}, Met^{207}, Gly^{209}, Phe^{213}, and Ala^{246}; while the cyclopentanone oxime would be stabilized by 2 H-bonding with Arg^{36} and Asn^{247} residues and interact with Asn^{210} and Trp^{32} residues (**Figure 3B**). Although similar hydrophobic interactions were observed with compound **2f**, only one H-bonding involving Asn^{247} residue would be detected (**Figure 3C**).

Such binding modes for both **2c** and **2f**, would suggest that these two compounds might be able to block (or at least partially) the access of the substrate into the *Ec*FabH protein's active site, therefore resulting in the potential enzyme activity inhibition.

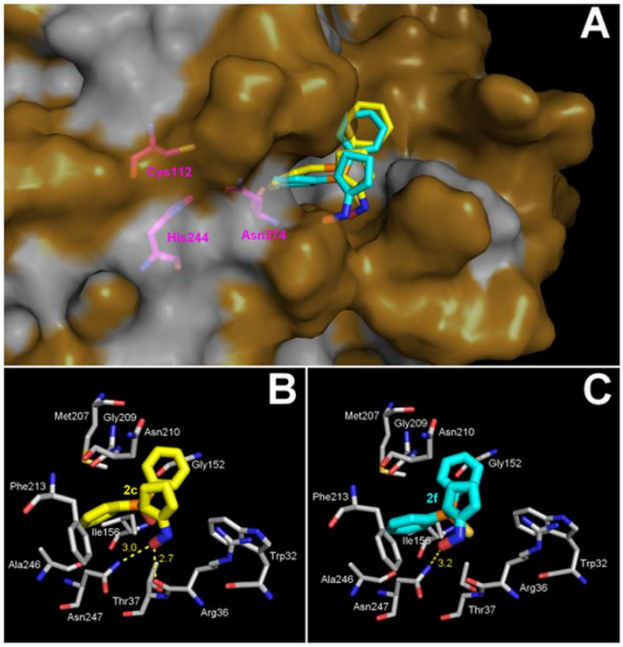


Figure 3. (**A**) *In silico* molecular docking of cycloalkanone oximes **2c** (*yellow* color) and **2f** (*cyan* color) into the crystallographic structure of *E. coli* FabH in a van der Waals surface representation. The Cys^{112}-His^{244}-Asn^{274} catalytic residues of *Ec*FabH active site are in *magenta stick* representation. Hydrophobic residues (alanine, leucine, isoleucine, valine, tryptophan, tyrosine, phenylalanine, proline, and methionine) are highlighted in *white*. (**B**-**C**) Ligplot+ analyses results: 3D representation of the schematic binding mode of compounds **2c** (**B**; *yellow* color) and **2f** (**C**; *cyan* color) in complex with *Ec*FabH showing both hydrogen bonds (*yellow dashed lines*) and hydrophobic interactions. Selected residues of the *Ec*FabH binding pocket are shown as *gray sticks*. The stick representation uses the following atom color-code: nitrogen, blue; oxygen, red; sulfur, yellow; and phosphorus, orange. Structures were drawn with PyMOL Molecular Graphics System (version 1.4, Schrödinger, LLC) using the PDB file 1HNJ.[55]

Conclusion

We have described a two-step approach to unprecedented α-(diphenylphosphoryl)- and α-(diphenylphosphorothioyl)cycloalkanone oximes (**2a-f**). Their structures were characterized by various spectroscopic methods including IR, NMR (^{1}H, ^{31}P, ^{13}C), mass spectrometry and single crystal X-ray diffraction. The synthesized compounds were screened for their *in vitro* antibacterial and antifungal activities against several Gram-negative, Gram-positive and fungal strains. The biological assays showed that all the studied compounds (**2a-f**) exhibited high antibacterial and antifungal activities, better than the standard drugs ciprofloxacin and fluconazole, with MIC, MBC and MFC values in the range of 0.7-1.8, 2.0 and 0.1 μg/mL, respectively. Five-membered ring compounds **2c** and **2f** were found to be the most potent antibacterial agents. We tried to correlate these results with those obtained in the *in silico* molecular docking study with the *E. coli* β-ketoacyl-acyl carrier protein synthase III (*Ec*FabH). These experiments have brought useful and informative data regarding the potential binding mode of oximes **2c** and **2f** inside the *Ec*FabH enzyme's active site, therefore providing some clues for developing new lead compounds as potent antibacterial agents. Moreover, from the molecular docking results, one cannot exclude that the antibacterial activity of compounds **2c** and **2f** towards Gram-negative and Gram-positive bacteria might result from the inhibition of FabH protein.

Experimental Section

Chemistry

Commercially available reagents and solvents were used without further purification. Melting points were determined in open glass capillaries and are uncorrected. The infrared spectra were recorded in the 400-4000 cm^{-1} range with a Nicolet IR200 FT-IR spectrometer using a neat sample at ambient temperature. The number of scans was 32 and the resolution 4 cm^{-1}. NMR spectra were recorded at 400 and 500 MHz (^{1}H), 161 and 202 MHz (^{31}P), 100 and 126 MHz (^{13}C APT) in $CDCl_3$ at a concentration of 50 mg/mL and at 25 °C. Chemical shifts (δ) are reported in part per million (ppm) relative to the residual solvent peak. The coupling constants are reported in Hz. The multiplicities of signals are indicated by the following abbreviations: s: singlet; d: doublet; t: triplet; and m: multiplet. High-resolution-MS spectra were performed on a JOEL JMSGCmateII mass spectrometer. Column chromatography was performed on silica gel (70-230 mesh ASTM) using the reported eluent. Thin layer

chromatography (TLC) was carried out on 5 x 20 cm plates with a layer thickness of 0.25 mm (Silica gel 60 F254).

Synthesis of cyclic enamines

The starting cyclic enamines were prepared according to the procedure reported by Stork[57] with slight modification:

A mixture of cyclic ketone (1 mol) and morpholine (1.7 mol) in dry toluene (30 mL) was heated at reflux, with Dean-Stark separation of water, for 4 h. The solvent was then removed under vacuum and the residue obtained was distilled under reduced pressure to give pure enamine in more than 90% yield.

General procedure for the synthesis of α-(diphenylphosphoryl)- and α-(diphenylphosphorothioyl)cycloalkanones 1a-1f

The α-(diphenylphosphoryl)- and α-(diphenylphosphorothioyl)cycloalkanones **1** were prepared according to the procedure reported by Barkallah et al: [32]

To a well stirred solution of enamine (1 mmol) and triethylamine (1.1 mmol) in dry acetonitrile (15 mL), maintained under an inert atmosphere (N_2) and cooled at 0 °C, *P*-chlorodiphenylphosphine (1 mmol) in dry acetonitrile (3 mL) was added dropwise within 15 min. The resulting solution was warmed up to room temperature and stirred for 2 h. The reaction mixture was then treated with DMSO or sulphur as follows:

- Oxidation: DMSO (1 mmol) was added and the mixture was heated under reflux for 2 h. After cooling, 2N aqueous HCl solution (30 mL) was added dropwise at 0 °C and stirring was continued at room temperature for 12 h. The mixture was then extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was dried over $MgSO_4$ and concentrated under vacuum. The residue obtained was chromatographed on a silica gel column using *n*-hexane/ethyl acetate (50:50 % *v*/*v*) as eluent.

- Sulfurization: Ground sulfur (1 mmol) was added and the reaction mixture was stirred at room temperature until complete dissolution of the sulfur in 2 h. 2N aqueous HCl solution (30 mL) was then added dropwise at 0 °C and stirring was continued at room temperature for 12 h. The mixture was then extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was dried over $MgSO_4$ and concentrated under vacuum. The residue obtained was chromatographed on a silica gel column using *n*-hexane/ethyl acetate (50:50 % *v*/*v*) as eluent.

General procedure for the synthesis of α-(diphenylphosphoryl)- and α-(diphenylphosphorothioyl)cycloalkanone oximes (2a-2f)

To a mixture of hydroxylamine hydrochloride (0.01mol), potassium hydroxide (0.01 mol) and dry ethanol (20 mL), a solution of α-(diphenylphosphoryl)- or α-(diphenylphosphorothioyl)cycloalkanones **1** (0.01 mol) was added dropwise with stirring, at 25°C, in dry ethanol (10 mL). The reaction mixture was then heated under reflux for 24 h. After cooling, the solvent was removed under reduced pressure. The residue obtained was diluted with $CHCl_3$ (30 mL) and washed with water (2×20 mL). The organic phase was dried over Na_2SO_4 and concentrated under vacuum. After workup, Column chromatography was performed when necessary using *n*-hexane/ethyl acetate (60:40 % *v*/*v*) as eluent.

X-ray diffraction data

For the structure of compound $C_{18}H_{20}NO_2P$ ***(2a)***, X-ray intensity data was collected at 100 K, on a Rigaku Oxford Diffraction Supernova Dual Source (Cu at zero) diffractometer equipped with an Atlas CCD detector using ω scans and CuKα (λ = 1.577 Å) radiation. The images were interpreted and integrated with the program CrysAlisPro (Rigaku Oxford Diffraction).[58] Using Olex2,[59] the structure was solved by direct methods using the ShelXS[60] structure solution program and refined by full-matrix least-squares on F^2 using the ShelXL[61] program package. Supplementary data related to the X-ray diffraction can be found at https://www.ccdc.cam.ac.uk. CCDC 1989019 contains the supplementary crystallographic data for this paper and can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336033; or deposit@ccdc.cam.ac.uk).

Antimicrobial activity

Microorganisms and growth conditions

Six different strains of pathogenic microorganisms, including Gram-negative bacteria: *Escherichia coli* and *Salmonella typhimurium,* Gram-positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis*, and fungi: *Candida albicans* and *Candida glabrata*, were employed to test the antimicrobial activity of all synthesized compounds. All bacteria were cultured in Nutrient Broth (BK003HA; Biokar) at 37 °C for 24 h; while the fungi were grown on Sabouraud Dextrose Broth (AM5088; Microxpress; Tulip Diagnostics) for 48 h at 29 °C.

Bioassays

The broth microdilution method was used to determine the antimicrobial activity according to CLSI guidelines.[40,41] Overnight microorganism's cultures were diluted with the appropriate sterile broth to obtain a suspension of about 1.37×10^6 CFU/mL. Stock solutions of tested compounds (**2a-f**) were prepared in DMSO by serial two-fold dilution in the previous appropriate sterile broth to obtain a concentration range from 0.1 to 60 µg/mL. An aliquot of working suspension (100 µL) was added to a sterile 96-well plate containing (100 µL) of tested stock solutions. Wells containing DMSO-microorganism inoculum, and those only microorganism suspensions were served as negative control. The *in vitro* activities of ciprofloxacin against bacteria and fluconazole against fungi were evaluated as positive control with the same serial two-fold dilution as the tested compounds. The microplates were incubated for 24 h at 37 °C and 29 °C for bacteria and fungi, respectively. Then, the minimum inhibitory concentration (MIC) was defined as the lowest concentration of the tested compounds that completely inhibited bacterial growth *via* OD_{600} reading. The minimum bactericidal and fungicidal concentrations (MBC and MFC) were recorded as the lowest concentration giving no visible colony on agar plate. These parameters were measured according to Lehtinen et al.,[42] with some adjustments. We determined the optical density OD_{600} using a 96-well microplate reader (VersaMax with SOFTmax). Each broth medium is used as the background, and the MIC was defined by an optical density equal to 0.2. The agar plate was used to define the MBC and MFC by counting the colonies (CFU/mL) using the following equation:

$$CFU/mL = \frac{\text{N colonies on plate}}{\text{dilution} \times \text{volume plate}}$$

Briefly, at the end of the 24 h-incubation periods, 100 µL of the suspension was plated onto Nutrient Broth Agar and Sabouraud Dextrose Broth Agar medium. Agar plates were incubated overnight at the desire temperature described above. Compounds with MIC values close to the MBC values, were further evaluated through microdilutions, in the concentration range from 2.000 to 2.300 µg/mL, to precisely determine individual MBC value. Each analysis was performed in triplicate.

Molecular docking experiments

A computational docking and scoring procedure using the Autodock Vina program[62] was used to generate the putative binding modes of cycloalkanone oximes **2c** and **2f** into the active

site of the *E. coli* β-ketoacyl-acyl carrier protein synthase III (*Ec*FabH) as previously described.[63,64] The PyMOL Molecular Graphics System (version 1.4, Schrödinger, LLC) was used as working environment with an in-house version of the AutoDock/Vina PyMOL plugin.[65] The X-ray crystallographic structure of *Ec*FabI in complex with Malonyl-CoA (PDB entry code: 1HNJ; 1.46 Å resolution)[55] available at the Protein Data Bank was used as receptor. Docking runs were performed after removing the ligand (*i.e.*, Malonyl-CoA) from the enzyme active site. The box size used for the receptor was chosen to fit the whole active site cleft and to allow non-constructive binding positions. The three-dimensional structures of the aforementioned compounds were constructed using Chem 3D ultra 11.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2007)], and their geometry was refined using the Avogadro open-source molecular builder and visualization tool (version 1.2.0. http://avogadro.cc/)

To check the accuracy of our docking experiment, the *N*-((3'-(hydroxymethyl)biphenyl-4-yl)methyl)benzenesulfonamide inhibitor (*i.e.*, **Cpd6**) for which a 3D structure in complex with *Ec*FabH is available (PDB entry code: 5BNM)[66] was submitted to *in silico* molecular docking into *Ec*FabH active site. Comparison of the generated **Cpd6** docking pose with the available binding geometry observed in the 5BNM crystal structure revealed root mean square deviations of 0.484 Å for the top ranked pose (predicted binding energy value, $\Delta E_{\mathbf{Cpd6}}$ = -8.2 kcal/mol). This re-/cross-docking experiment thus confirms the validity and reliability of our computational approach.

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Author Contribution Statement

N. Jebli synthesized the compounds and analyzed the data. S. Hamimed conducted the antimicrobial assays. K.V. Hecke performed the X-ray diffraction analysis. J-F. Cavalier carried out the *in silico* molecular docking studies. S. Touil designed and supervised the work and analyzed the data.

Supplementary data

Supplementary data (which contain NMR and mass spectra and other informations regarding the crystal structure and bioactivity of the title compounds) associated with this article can be found, in the online version, at http://dx.doi.org.

Conflict of interest

There are no conflicts to declare.

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Graphical Abstract