CJC Chinese Journal of Chemistry
中国化学 - An International Journal

Accepted Article

Title: Design and Synthesis of Sulfanilamide Aminophosphonates as Novel Antibacterial Agents towards *Escherichia Coli*

Authors: Juan Wang, Mohammad Fawad Ansari, Jian-Mei Lin,* and Cheng-He Zhou*

This work may now be cited as: *Chin. J. Chem.* **2021**, *39,* 10.1002/cjoc.202100165.

The final Version of Record (VoR) of it with formal page numbers will soon be published online in Early View: http://dx.doi.org/10.1002/cjoc.202100165.

WILEY-VCH SIOC CCS

ISSN 1001-604X • CN 31-1547/O6
mc.manuscriptcentral.com/cjoc
www.cjc.wiley-vch.de

Cite this paper: *Chin. J. Chem.* **2021**, *39*, XXX—XXX. **DOI: 10.1002/cjoc.202100XXX**

Design and Synthesis of Sulfanilamide Aminophosphonates as Novel Antibacterial Agents towards *Escherichia Coli*

Juan Wang,[a] Mohammad Fawad Ansari,[a] Jian-Mei Lin,*[,b] and Cheng-He Zhou*[,a]

[a] *Institute of Bioorganic & Medicinal Chemistry, Key Laboratory of Luminescence Analysis and Molecular Sensing (Southwest University), Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China*
[b] *School of Medicine, University of Electronic Science and Technology of China, Chengdu 610072, PR China*

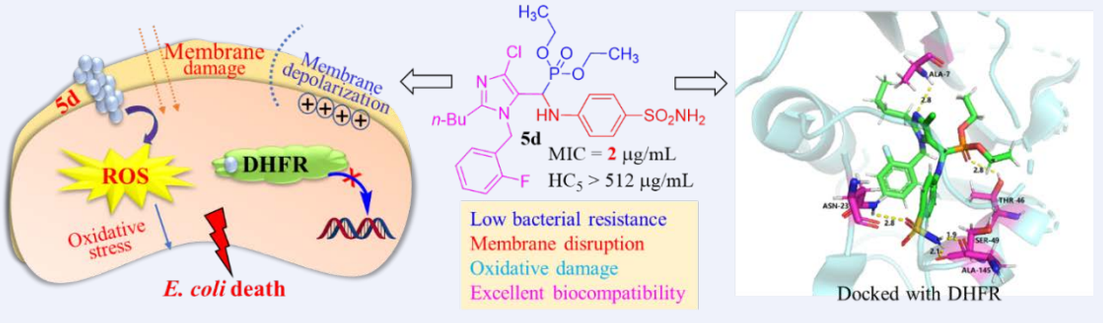
Keywords

Sulfanilamide |Antibacterial |Aminophosphonate |Imidazole |DNA

Main observation and conclusion

The limit ability of traditional antibiotics to treat drug resistant bacteria calls for new therapeutic alternatives. A class of unique sulfanilamide aminophosphonates as new potential agents against microbes was synthesized by one-pot three-component reaction. Noticeably, fluorobenzyl derivative **5d** (MIC = 2 μg/mL) was active against drug resistant *E. coli* infection and exerted no obvious toxicity towards human mammalian cells. Compound **5d** also displayed good anti-biofilm activity and low possibility to induce drug resistance. Mechanism investigation elucidated that molecule **5d** could disrupt *E. coli* membrane through generation of reactive oxygen (ROS) and then intercalate into deoxyribonucleic acid (DNA) to form a steady **5d**-DNA complex, which led to bacterial death. These results indicated that sulfanilamide aminophosphonates would shed light on developing novel potential antibacterial agents.

Comprehensive Graphic Content



*Tel.: +86-23-68254967; Fax: +86-23-68254967;
E-mail: linjianmei@med.uestc.edu.cn (Jian-Mei Lin); zhouch@swu.edu.cn (Cheng-He Zhou)

View HTML Article | Supporting Information

Background and Originality Content

Sulfanilamides are the first synthetic antifolic agents in treatment of a variety of bacterial infections to safeguard human health. [1-3] Sulfanilamide drugs with a structural resemblance to *p*-aminobenzoic acid (PABA) effectively impede the biosynthesis of dihydrofolate and obstruct the generation of bacterial nucleic acid precursor, subsequently triggering the death of microbial pathogens. [4, 5] To date, aromatic heterocycles-derived sulfanilamide drugs, [6] such as sulfadiazine, sulfamethoxypyridazine, sulfathiazole and sulfamethoxazole have been extensively employed in clinical infection therapy, especially for patients intolerant to antibiotics. [7-9] Nevertheless, this kind of antibacterial drugs are also associated with significant risk of adverse reactions. Researches indicated that oxidative metabolism of the anilino fragment in the core skeleton of sulfanilamide generated reactive metabolites that combined with proteins, giving rise to antigen presentation and an immune response, which was the culprit of hypersensitivity reactions [10]. This promoted the development of a series of structurally unique organophosphorus sulfanilamides as novel alternative antibacterial drugs.

Aminophosphonates as significant alternatives for α-amino acids and transition state mimics of peptide hydrolysis, play a vital role in agricultural, medicinal and organic chemistry. [11] A great number of organophosphorus agents have achieved commercial success on account of their multitudinous biological and pharmacological applications, [12] for instance, ACE inhibitor fosinopril for the treatment of hypertension and nitrogen mustards cyclophosphamide and ifosfamide as anticancer agents. [13, 14] Moreover, fosfomycin as a natural antibiotic hindered the synthesis of bacterial cytoderm as well as destroyed the bacterial biofilm, accelerating the death of Gram-positive and Gram-negative bacteria, which encouraged us to develop highly bioactive α-substituted phosphonate derivatives along with low toxicity and residual effects on mammalian cells.

On account of the unlimited biological potentiality and further extension of existed drugs into bioactive compounds, the purpose of this research was to develop a series of new potential antibacterial sulfanilamide agents through hybridization of core skeleton *p*-aminobenzene sulfanilamide, organophosphorus compounds and different aldehydes to overcome the multidrug resistance observed in existing first-line drugs. The design of sulfanilamide aminophosphonates took the following factors into consideration:

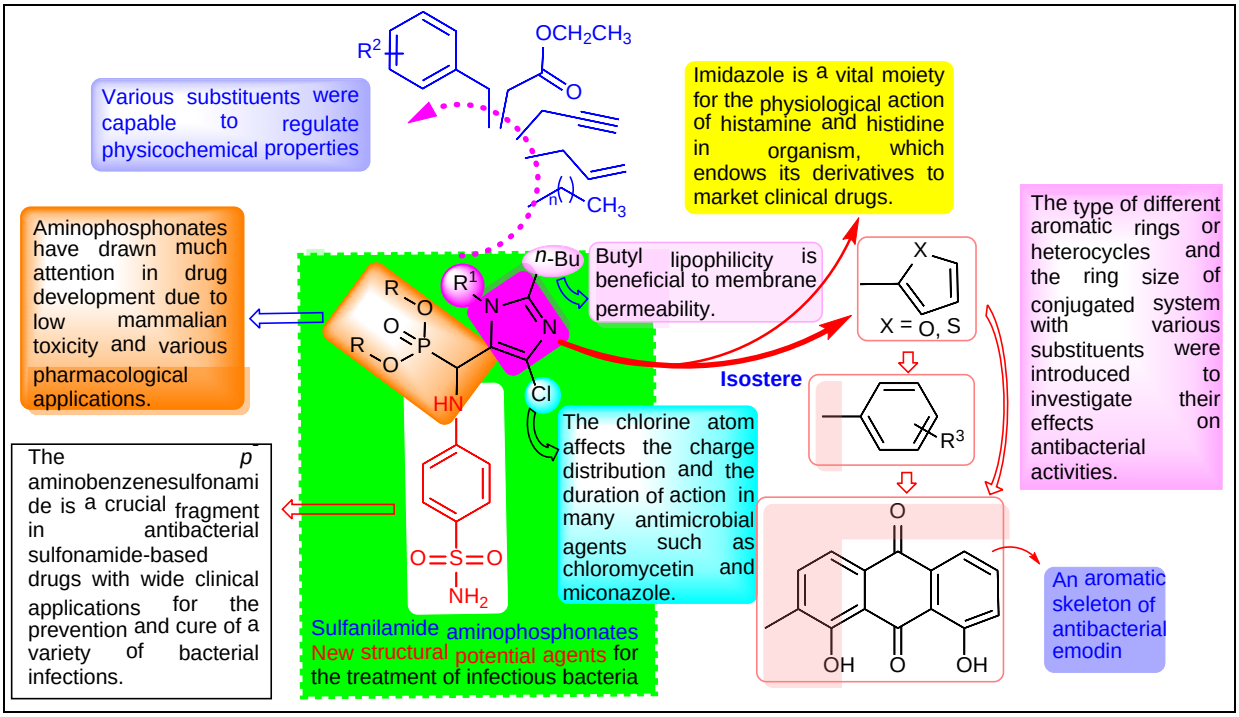


Figure 1 Design of novel sulfanilamide aminophosphonates as potential antibacterial agents

(1) The unique imidazole [15-17] scaffold with electron-rich feature, amphoteric characteristic and high polarity can easily bind to diverse receptors and enzymes in organism through a variety of weak interactions to improve the bioavailability and target selectivity. [18] A lot of imidazole-derived natural products and pharmaceutical molecules with potential therapeutic consequences covering antibacterial, antifungal, anticancer, antiparasitic and other medicinal activities were reported. [19]

(2) Thiophene is an important biological isostere of imidazole in drug design with great development potentiality in the organic and pharmaceutical chemistry. Furan-derived drugs are a class of synthetic antibacterial agents with broad-spectrum antimicrobial efficacy. The biological isosteres thiophene and furan fragments were introduced into α-aminophosphonate sulfanilamides to explore the effect of different five-membered aromatic heterocycles on biological activity.

(3) The type of different aromatic rings or heterocycles and the ring size of conjugated system with various substituents were introduced to investigate their effects on antibacterial activities. Phenyl fragments extensively existed in marketed medicinal [20] agents such as penicillin, amoxicillin, ampicillin and aspirin. The aromatic moieties with different electrical properties could regulate the rigidity and enhance liposolubility and membrane permeability of target compounds to effectively affect drug absorption and metabolism.

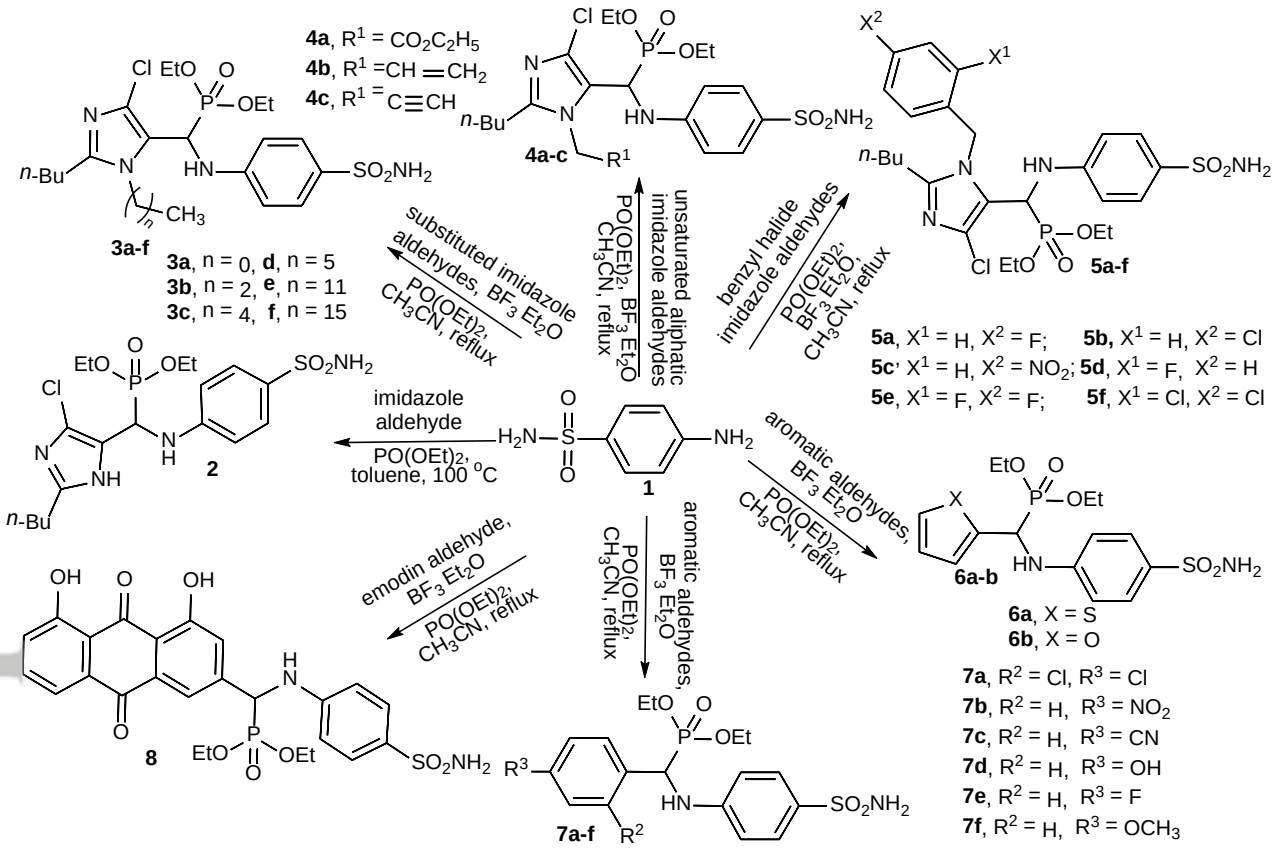
(4) Aloe-emodin (AE) with a history of over 2000 years is a naturally anthraquinone derivative and an active ingredient of various Chinese herbs, such as *Aloe vera* and *Rheum officinale*. [21] However, the pharmaceutical applications of AE were still in a fledging period, because most researches focused on illustrating the mechanism of action of existing aloe-emodin drugs rather than the preparation of novel AE medicinal compounds. [22] Herein, the hybridization of AE and sulfanilamide produced a target molecule to investigate its biological potency.

Considering of these circumstances, a series of novel sulfanilamide aminophosphonates was developed (**Figure 1**). Their antimicrobial activities, bactericidal kinetics and bacterial resistance were evaluated. Cytotoxicity assay was performed to ensure that this kind of compounds was safe for mammalian cells. To further explore the antibacterial mechanism of the promising molecule, biofilm elimination, membrane disruption, oxidative damage, interaction with DNA as well as molecular docking with dihydrofolate reductase were carried out.

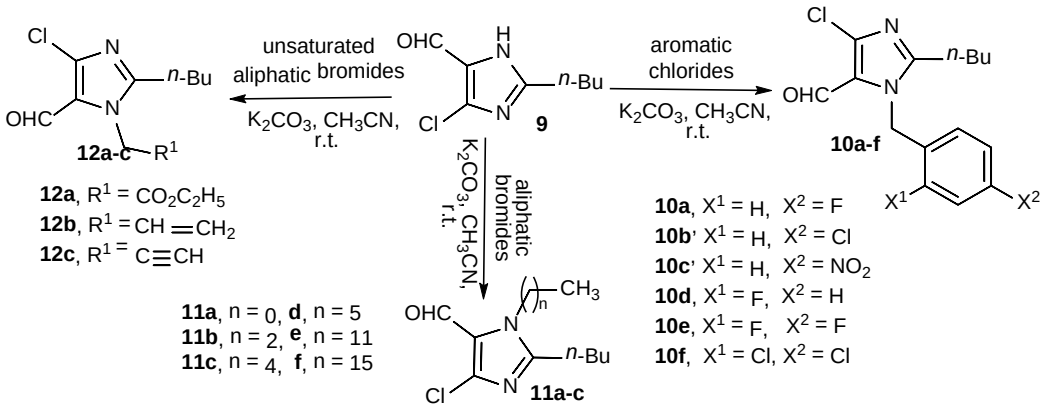
Results and Discussion

Chemistry. A practical synthetic process for sulfanilamide aminophosphonates was described in **Scheme 1**. The target benzenesulfonamide derivatives were developed from sulfanilamide, diethyl phosphite and various aldehydes by Kabachnik–Fields reaction. The imidazole derivative **2** in a yield of 47.1% was prepared by the reaction of sulfanilamide, diethyl phosphite and 2-butyl-4-chloro-5-formylimidazole in toluene at 110 °C. However, further structural modifications of compound **2** were not successful by aromatic chlorides and aliphatic bromides. Therefore, the reaction conditions for the synthesis of α-aminophosphonate derivatives were further optimized. A number of imidazole aldehydes were afforded in acceptable yields based on the reported syntheses (**Scheme 2**). A mixture of intermediates **10**–**12**, sulfanilamide and diethyl phosphite was stirred in acetonitrile using boron trifluoride etherate as the catalyst to afford sulfanilamide aminophosphonates **3**–**5** in yields of 42.0–85.5%. Subsequently, the target compounds **6**–**8** were readily prepared by the reaction of sulfanilamide, diethyl phosphite and various aromatic aldehydes under the same condition with yields ranging from 14.0% to 88.1%.

Scheme 1 Preparative process of sulfanilamide aminophosphonates



Scheme 2 Synthesis route of imidazole aldehydes



Antibacterial activity. The antibacterial efficacy [23] of sulfanilamide aminophosphonates **2**–**8** was evaluated and represented in **Table 1**. The ClogP values of compounds 2–8 were calculated and listed in **Table S1**. Alkyl-modified sulfanilamide derivatives **3a**–**f** and

ester-substituted analogue **4a** gave similar antibacterial activities against the tested germs in comparison to corresponding unsubstituted compound **2**. Especially, molecules **3a** (MIC = 16 μg/mL) and **3c** (MIC = 16 μg/mL) displayed moderate inhibitory potencies against *P. aeruginosa* ATCC 27853 and MRSA, respectively. However, the antibacterial efficiency of dodecyl and cetyl derivatives **3e** (ClogP = 6.576) and **3f** (ClogP = 9.756) decreased possibly because high lipid solubility. This fact declared that alkyl chains of different lengths almost had no positive effect on the bioactivity. The introduction of unsaturated alkyl chain into imidazole yielded compounds **4b–c**, in particular, alkynyl modified molecule **4c** (ClogP = 1.921) was active against the listed bacterium (MIC = 8–32 μg/mL). The above results suggested that incorporation of unsaturated fragments could be beneficial to inhibit the growth of the tested microbes.

Table 1 Biological data (MIC, μg/mL) and hemolytic activity (HC_5, μg/mL) of compounds **2–8** [a,b]

Compounds	Gram-positive bacteria					Gram-negative bacteria						HC_5[c]
	MRSA	*E. f.*	*S. a.* 6538	*S. a.* 25923	*S. a.* 29213	*K. p.*	*E. c.*	*E. c.* 25922	*P. a.*	*P. a.* 27853	*A. b.*	RBCs[d]
2	128	16	32	128	64	64	128	64	64	32	64	> 512
3a	64	64	32	64	128	64	64	64	32	16	32	> 512
3b	64	64	64	128	64	64	64	128	128	64	64	> 512
3c	16	64	64	128	64	32	64	128	64	64	32	> 512
3d	128	128	128	128	128	32	128	128	128	128	128	38
3e	128	128	128	128	128	128	128	128	128	128	128	93
3f	128	128	128	256	128	256	128	256	256	256	256	155
4a	128	32	64	64	128	64	64	64	64	32	64	> 512
4b	64	128	128	128	32	32	128	64	64	32	64	> 512
4c	32	16	16	8	8	16	16	32	32	16	8	> 512
5a	128	32	32	64	16	16	16	64	64	16	8	> 512
5b	16	32	128	32	128	32	32	64	128	16	64	> 512
5c	32	128	32	64	16	64	64	64	128	32	32	> 512
5d	16	32	8	16	4	16	2	32	16	32	16	> 512
5e	64	64	64	128	16	64	64	64	128	32	32	> 512
5f	128	64	64	64	128	32	128	128	64	64	64	> 512
6a	128	128	8	64	128	32	64	64	128	32	64	> 512
6b	64	32	4	32	64	64	32	64	128	64	16	> 512
7a	32	64	16	32	16	64	128	64	128	64	32	> 512
7b	128	128	8	32	32	64	128	128	128	64	64	> 512
7c	128	128	128	8	64	16	64	64	128	64	64	> 512
7d	128	128	128	256	128	128	256	128	128	125	128	> 512
7e	128	128	128	256	256	256	256	128	256	256	128	> 512
7f	128	64	256	256	256	256	256	256	256	256	256	> 512
8	128	64	64	64	32	64	128	32	128	64	64	> 512
A[e]	16	8	8	8	4	8	16	32	32	8	16	ND[g]
B[f]	8	4	8	32	2	16	8	16	8	32	8	ND[g]

MRSA, Methicillin-resistant *Staphylococcus aureus* (N315); *E. f.*, *Enterococcus faecalis* (V583); *S. a.* 6538, *Staphylococcus aureus* ATCC 6538; *S. a.* 25923, *Staphylococcus aureus* ATCC 25923; *S. a.* 29213, *Staphylococcus aureus* ATCC 29213; K. p., *Klebsiella pneumonia* KPN325; *E. c.*, *Escherichia coli* (JM109); *E. c.* 25922, *Escherichia coli* ATCC 25922; *P. a.*, *Pseudomonas aeruginosa* ST235; *P. a.* 27853, *Pseudomonas aeruginosa* ATCC 27853; A. b, *Acinetobacter baumannii* R674. The experiments were performed with three duplicates. [c] HC_5, Concentration causing 5% haemolysis RBCs, [d] Human red blood cells. [e] A = Chloramphenicol, [f] B = Norfloxacin [g] ND = Not determined.

Among benzyl modified imidazole ones, 2-fluorobenzyl derivative **5d** (ClogP = 3.482) with an appropriate lipid solubility displayed the best bacteriostatic activity towards the evaluated bacteria with MIC values of 2–32 μg/mL while 4-fluorobenzyl derivative **5a** showed a weaker activity against the listed germ, which pointed out that the substituted position of fluorine in phenyl ring showed remarkable influence in the antibacterial potency. Especially, compound **5d** exhibited excellent inhibition potency against *E. coli* with a low MIC value of 2 μg/mL, which was 4-fold greater than norfloxacin (MIC = 8 μg/mL) and 8-fold better than chloramphenicol (MIC = 16 μg/mL). Replacement of 4-fluorobenzyl group by 4-chlorobenzyl moiety produced molecule **5b** with similar bioactivities against Gram-negative microbes to compound **5a**. Furthermore, the introduction of nitro group with strong electron-attracting property showed little effect on the improvement of biological activity. Additionally, *N*-substitution in the imidazole with 2, 4-difluorobenzyl or dichlorobenzyl group produced the corresponding molecules **5e** and **5f** with a slight loss of potency than mono-substituted analogue.

Five aromatic heterocycle derivatives **6a** and **6b** were not sensitive to most of the microbes except that compound **6b** had a quite better selectively inhibitory activity to *S. aureus* ATCC 6538 (MIC = 4 μg/mL). Furthermore, the antibacterial effects of phenyl-modified compounds **7a–f** were evaluated. Nitro-bearing compound **7b**

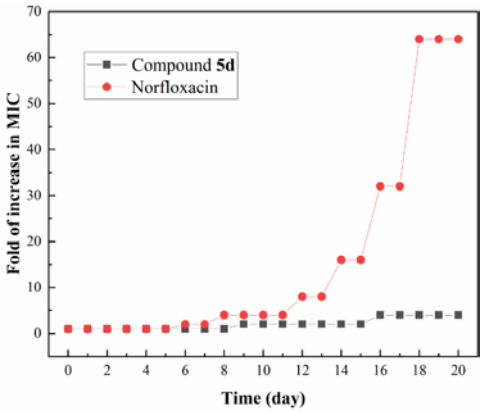


Figure 2 Resistance study for fluorobenzyl derivative **5d** against drug resistant *E. coli*. Norfloxacin was employed as a positive control.

was favorable for anti-*S. aureus* ATCC 6538 efficiency (MIC = 8 μg/mL). Simultaneously, cyano-containing derivative **7c** (MIC = 8 μg/mL) gave a 4-fold improved inhibition toward *S. aureus* ATCC 25923 in contrast with norfloxacin. However, compound **7d–f** was not sensitive to the listed strains. This result implied that introduction of electron-attracting groups on the benzene ring was beneficial to biological activity. A hybrid **8** of aloe-emodin and sulfanilamide exerted inferior inhibitory effects.

Tendency to induce bacterial resistance. Bacteria resistance has become a threat for human health due to the decreasing effectiveness of existed drugs, [24] which means that human infections are becoming too difficult to be eliminated and create a challenging problem on a global scale. The experimental result in **Figure 2** displayed that the susceptibility of *E. coli* against fluorobenzyl derivative **5d** almost unaffected, while the MIC value of norfloxacin toward *E. coli* got dramatically increased after several passages, which showed that *E. coli* was more difficult to develop resistance against compound **5d** than norfloxacin.

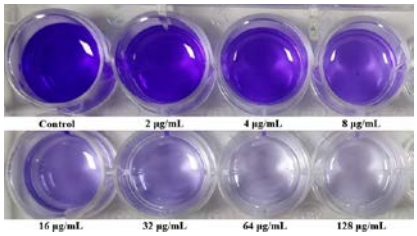


Figure 3 Influence of fluorobenzyl derivative **5d** on the viability of *E. coli* biofilm. DMSO was used as a negative control. The experiments were performed with three duplicates.

Biofilm disruption. The sinister nature of bacterial resistance is amplified when the microbes are kept in isolation in biofilms that shield them from effective antimicrobials and/or the innate immune system, [25] simultaneously resulting that microbes are invulnerable to most marketed antibiotics. [26] In consequence, bacterial adhesion and biofilm formation are vital targets for the development of new drugs. The fluorobenzyl derivative **5d** was selected to evaluate the anti-biofilm activity against *E. coli* by crystal violet staining assay. The negative control of water showed no effect on *E. coli* biofilm damage because the biofilm layer remained intact, while active molecule **5d** completely eradicated performed *E. coli* biofilm at the concentration of 4 × MIC (**Figure 3**). The result indicated that fluorobenzyl derivative **5d** was able to detach and disperse *E. coli* biofilm into planktonic culture, which might reduce bacterial resistance.

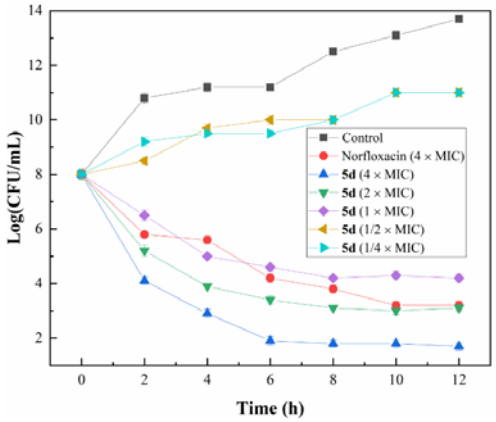


Figure 4 Bactericidal kinetic of fluorobenzyl derivative **5d** at (1/4 × MIC, 1/2 × MIC, 1 × MIC, 2 × MIC, 4 × MIC) against *E. coli*. Norfloxacin at 4 × MIC was used as a positive control and *E. coli* cells untreated was employed as growth control. The experiments were performed with three duplicates.

Time-dependent killing assay. The time-kill kinetic assay was executed to explore the ability of fluorobenzyl derivative **5d** on eliminating *E. coli* over a short period of time. [27] As shown in **Figure 4**, compound **5d** (4 × MIC) could kill viable *E. coli* and decrease the bacterial amount of 2.5 Log (CFU/mL) within 2 hours. The rapid bactericidal capacity might be related to the disruption of cell membrane integrity.

Depolarization of cytoplasmic membrane. The depolarization of cytoplasmic membrane can disturb membrane potential of bacterial cells, which subsequently resulting in the physical damage of the lipid bilayer or membrane disruption, thus inducing the death of microbes. [28, 29] The fluorobenzyl derivative **5d** was screened to investigate the ability of causing bacterial membrane depolarization using fluorescence dye diSC35. The results clearly indicated that compound **5d** could interact with the *E. coli* membrane and rapidly dissipate the membrane potential (**Figure 5**), which provided a new method for enhancing the antibacterial potency.

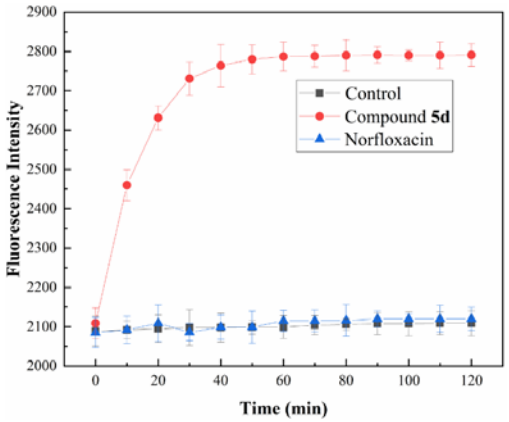


Figure 5 Transformation of membrane potential of *E. coli* in the existence of fluorobenzyl derivative **5d** at 4 × MIC. DMSO was used as a negative control. Norfloxacin was used as a positive control. The experiments were performed with three duplicates.

Outer membrane permeability. The outer membrane composing of lipopolysaccharide, phospholipid bilayer and lipoprotein shields bacteria from effective antimicrobials and toxic external factors, which creates difficulties to kill Gram-negative organisms. [30, 31] The hydrophobic dye *N*-phenyl naphthylamine (NPN) was used to research outer membrane permeabilization, which generally passed through the impaired outer membrane of bacteria, increasing the intensity of fluorescence. The enhancement of fluorescence intensity in *E. coli* after treatment with compound **5d** at the concentration of 4 × MIC (**Figure 6**) indicated that fluorobenzyl derivative **5d** damaged the outer membrane effectively.

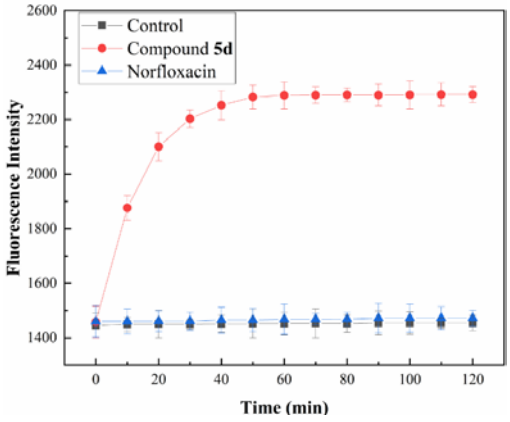


Figure 6 Variation of membrane permeability in *E. coli* after treatment with fluorobenzyl derivative **5d** at 4 × MIC. DMSO was used as a negative control. Norfloxacin was used as a positive control. The experiments were performed with three duplicates.

Inner membrane damage. Bacterial inner membrane disruption of fluorobenzyl derivative **5d** was explored using a common dye propidium iodide (PI), which entered merely damaged membrane of *E. coli* and fluoresced strongly after interaction with cellular DNA. [32, 33] The maximum fluorescence intensity was observed after treatment with compound **5d** (at 4 × MIC) within 80 minutes as shown in **Figure 7A**, indicating fluorobenzyl derivative **5d** (4 × MIC) caused certain damage to *E. coli* membrane on account of the existence of PI-DNA complex possibly. Meanwhile, compound **5d** could permeabilize the membrane of the Gram-negative bacteria in a dose-dependent behavior, leading to cell death **(Figure S1A** and **Figure 7B)**. The morphology of bacterial membrane were analyzed by scanning electron microscopy (SEM), the internal substance leaked to the surrounding districts of *E. coli* cell (**Figure S1B**) because fluorobenzyl derivative **5d** could influence the membrane function and eventually lyse the cells. The above results showed that molecule **5d** enhanced the antibacterial effect against *E. coli* by destroying the bacterial membrane.

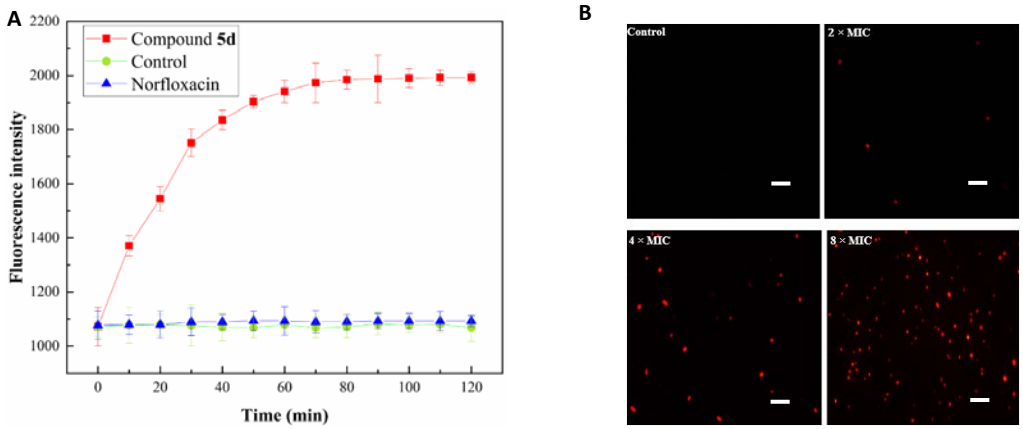


Figure 7 Inner membrane disruption, DMSO was used as a negative control. Norfloxacin was used as a positive control. (**A**) Inner membrane disruption of drug-resistant *E. coli* after treatment with fluorobenzyl derivative **5d** at the concentration of 4 × MIC. (**B**) Propidium iodide staining in *Escherichia coli* treated with fluorobenzyl derivative **5d** at concentrations of 2 × MIC, 4 × MIC and 8 × MIC for 24 h. The images were captured with fluorescence microscope at magnification of 400X magnification. Scale bar for all images is 100 μm. The experiments were performed with three duplicates.

Evaluation of intracellular oxidative stress. In order to investigate the reason of membrane disruption, 2,7-dichlorofluorescein diacetate (DCFH-DA) was used to detect the existence of reactive oxygen species (ROS). The oxidative damage interferes the basic components of cells. [34] The fluorescence intensity is positively associated with the concentration of peroxide produced. It was observed that treatment of *E. coli* cells with fluorobenzyl derivative **5d** led to the increased ROS accumulation in a dose-dependent manner (**Figure 8**). This was consistent with dose-dependent data observed for membrane damage, which indicated generation of ROS could disrupt cell membrane, thereby killing microbes.

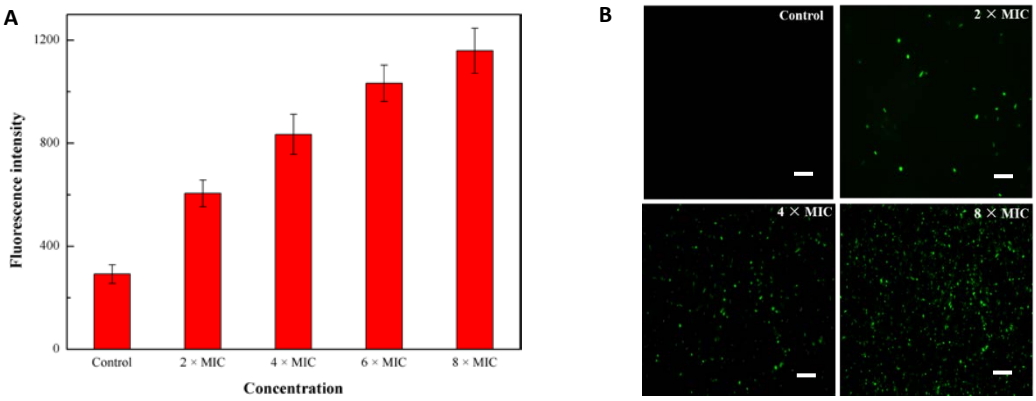


Figure 8 Intracellular ROS production in *E. coli* treated with increasing concentrations of fluorobenzyl derivative **5d** (at 2 × MIC, 4 × MIC, 6 × MIC and 8 × MIC), DMSO was employed as a negative control. (**A**) Intracellular oxidative stress; (**B**) Intracellular ROS was measured by DCFH-DA staining after treatment with fluorobenzyl derivative **5d** for 24 h. The intensity of the green fluorescence indicates ROS concentration in *Escherichia coli*. The images were captured with fluorescence microscope at magnification of 400X magnification. Scale bar for all images is 100 μm. The experiments were performed with three duplicates.

Interaction between fluorobenzyl derivative 5d and DNA. DNA is a significant drug target in many therapeutic agents and has been widely exploited for rational design of novel efficient DNA-targeting antibacterial drugs with low drug resistance. [35] Calf thymus DNA is often regarded as DNA model. Herein, *in vitro* interaction between active fluorobenzyl derivative **5d** with calf thymus DNA was investigated regarding AO (Acridine orange) as a spectral probe to investigate the possible mechanism of action at a molecular level.

Variation of DNA in the existence of fluorobenzyl derivative 5d. Hyperchromism and hypochromism are extremely crucial spectral features examined by absorption spectroscopy to certify the variation of double-helical DNA. [35] The enhancement in maximum absorption of DNA was proportional to the increased concentration of fluorobenzyl derivative **5d** along with a slight red-shift under a fixed content of DNA (**Figure 9A**). Meanwhile, the **5d**-DNA complex showed lower absorption value than free DNA and free fluorobenzyl derivative **5d**. This observed hypochromism was occasioned by intercalation of fluorobenzyl derivative **5d** into DNA double-helical structure and good aromatic π-π overlap with DNA bases, which disclosed the formation of compound **5d**-DNA complex.

Competition between fluorobenzyl derivative 5d and AO with DNA. The organic dye acridine orange (AO) has been proven to be

a very sensitive fluorescent probe and emits green fluorescence when binds to DNA. [36] The fluorescence intensity of DNA-AO complex decreased significantly at 537 nm with the increased addition of fluorobenzyl derivative **5d** (**Figure 9B**), indicating that it could embed in the double-helical DNA by competing with the AO. The purpose was to understand the mechanism of drug treatment and provided theoretical basis for the design of new and effective drugs.

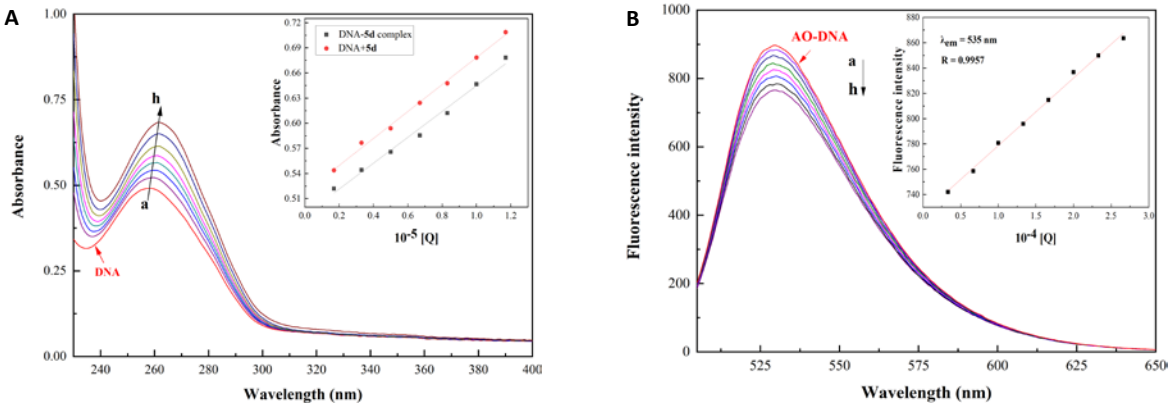


Figure 9 Interaction between fluorobenzyl derivative 5d and DNA. (**A**) UV absorption spectra of calf thymus DNA with different contents of fluorobenzyl derivative **5d** (pH = 7.4, T = 290 K). ***c***(DNA) = 7.41 × 10^{-5} mol L^{-1} and ***c***(fluorobenzyl derivative **5d**) = 0–1.17 × 10^{-5} mol L^{-1}. Inset: Comparison of the absorption at 260 nm between the value of fluorobenzyl derivative **5d**–DNA complex and the sum values of free DNA and free fluorobenzyl derivative **5d**. (**B**) Competition between fluorobenzyl derivative **5d** and AO with DNA. ***c***(DNA) = 7.41 × 10^{-5} mol L^{-1} and ***c***(fluorobenzyl derivative **5d**) = 0–1.17 × 10^{-5} mol L^{-1}. The experiments were performed with three duplicates.

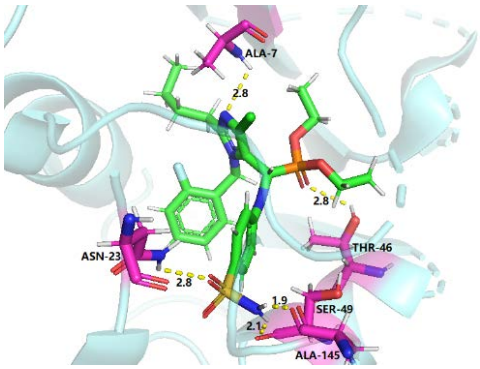


Figure 10 3D conformation of fluorobenzyl derivative **5d** docked in dihydrofolate reductase (PDB code: 3DRC)

Interaction with dihydrofolate reductase. Dihydrofolate reductase is essential for the biosynthesis of nucleic acids in bacteria. The lack of dihydrofolate reductase will hinder the formation of tetrahydrofolate and ultimately affect the biosynthesis of bacterial nucleic acid precursor to restrain the survival of germ. The fluorobenzyl derivative **5d** was explored to interact with the dihydrofolate reductase (**Figure 10**). The oxygen atom of phosphorus oxygen double bond was adjacent to THR-46 residue *via* hydrogen bond with a space distance of 2.8 Å. The imidazole nitrogen atom of compound **5d** also took part in the noncovalent interaction with ALA-7 residue by hydrogen bond reciprocity with a space distance of 2.8 Å. Moreover, the oxygen atom of sulfonamido fragment could participate in the hydrogen bonding interaction with ASN-23 residue with a space distance of 2.8Å, hydrogen atoms of benzenesulfonamido fragment could form hydrogen bonds with the SER-49 and ALA-145 residues with the corresponding distances of 1.9Å and 2.1Å, respectively, which indicated that sulfonamido group was an indispensable and important existence in pharmaceutical molecules. All of the hydrogen bonds might help to stabilize the supramolecular complex of fluorobenzyl derivative **5d** with dihydrofolate reductase, which possibly provided a theoretical basis for good antibacterial potency of fluorobenzyl derivative **5d**.

Hemolytic assay. The capacity of compounds to lyse red blood cells (RBCs) is an assessment of their toxicity to mammalian cells. The hemolytic activity of the sulfanilamide aminophosphonates were expressed as their HC_5 values. [37] Cytotoxicity of sulfanilamide derivatives **3c–f** with different aliphatic chain was found to enhance on account of the increase of alkyl chain length except molecules **3a** and **3b** with HC_5 values of > 512 μg/mL, respectively. The cetyl-modified compound **3f** with the longest chain in this series had a HC_5 value of 154 μg/mL. Except for compounds substituted with long alkyl chains were toxic to red blood cells, other types of target molecules were found to HC_5 values greater than 512 μg/mL. Fluorobenzyl derivative **5d**, one of the most potent antibacterial compounds gave good selectivity (S = HC_5/MIC) against *E. coli* (> 256). The erythrocyte morphology did not change after treatment with fluorobenzyl derivative **5d** at the concentration of 2 μg/mL in comparison to control (**Figure S3A**). Simultaneously, there was no obvious hemolysis when human red cells were treated with the increased concentration of the fluorobenzyl derivative **5d** within 3 hours (**Figure S3B**), indicating the compound **5d** was a low-toxic molecule.

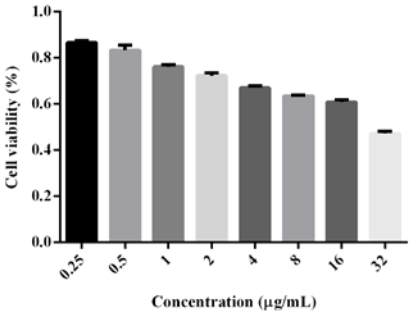


Figure 11 Cytotoxic assay of compound **5d** on 293T cell line evaluated by MTT method. The experiments were performed with three duplicates.

Cytotoxicity. Viability of 293T human renal epithelial cells treated with different contents of fluorobenzyl derivative **5d** was determined using MTT assay to evaluate the toxicity against mammalian cell. [37] No obvious toxicity was observed at the concentration of 2 μg/mL (**Figure 11**). The result clearly indicated that highly selective nature of compound **5d** to cause toxicity toward *E. coli* cells specifically, which further ascertained the therapeutic potential to bacterial infection.

Brief chemical biological summary of synthesized target sulfanilamide aminophosphonates. From the above observation, the hybrids of sulfanilamide with imidazole and phosphonates pro-

duced the desired sulfanilamide aminophosphonates with large inhibitory potentiality against the screened microbes, especially drug-resistance *Escherichia coli* by biofilm disruption, DNA intercalation and oxidative damage *etc*. The antimicrobial behavior of synthesized sulfanilamide aminophosphonates could be described as following in **Figure 12**.

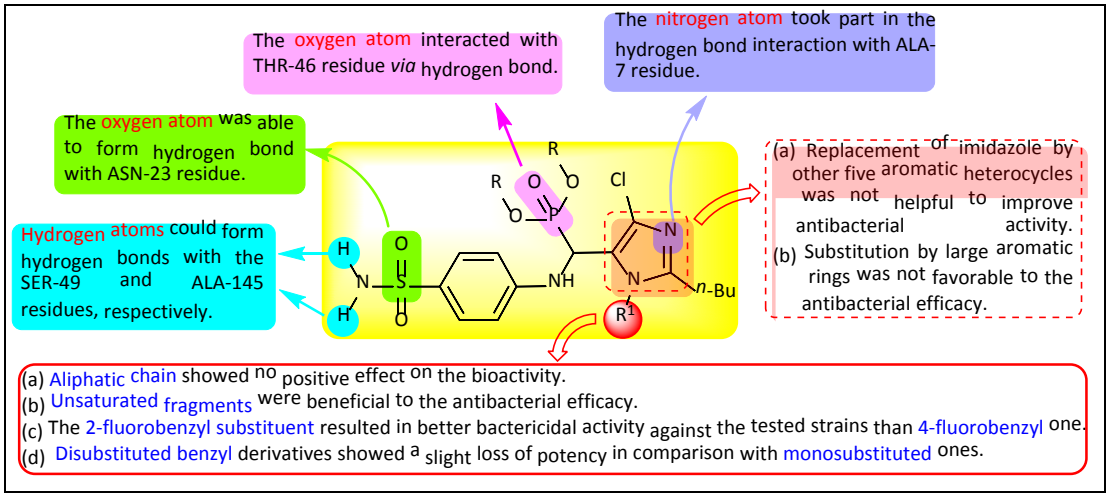


Figure 12 Brief chemical biological summary of synthesized target sulfanilamide aminophosphonates

Conclusions

In this work, a class of novel sulfanilamide aminophosphonates as antibacterial agents was developed through a practical protocol. The structure-activity relationship revealed that the imidazole-derived compounds exerted a significant effect on bioactivity. Noticeably, fluorobenzyl derivative **5d** was active against *E. coli* (MIC = 2 μg/mL) and exerted no obvious toxicity against mammalian cells. Mechanism investigation elucidated that molecule **5d** could disrupt the *E. coli* membrane through generation of reactive oxygen (ROS) and then intercalate into deoxyribonucleic acid (DNA) to form a steady **5d**-DNA complex, which resulted in bacterial death. This research provided a new platform to develop highly active compounds with multiple antibacterial mechanisms of action.

Experimental

General methods

All the reactions were monitored by thin layer chromatography (TLC). Melting points were measured with X-6 melting point instrument. ^{1}H and ^{13}C NMR spectra were recorded on the Bruker AVANCE III 600MHz spectrometer regarding tetramethylsilane (TMS) as an internal standard. The high resolution mass spectra (HRMS) were performed on an IonSpec FTICR mass spectrometer. Microbalance with 0.1mg resolution was applied to weigh sample masses. All chemicals and solvents were commercially available and were used without further purification.

Imidazole aldehydes (10a–f, **11a–f** and **12a–c):** Compounds **10a–f**, **11a–f** and **12a–c** were obtained on the basis of the synthetic processes reported in literature. [38]

Compound (2): A mixture of sulfanilamide (200 mg, 1.16 mmol), 2-butyl-4-chloro-1H-imidazole-5-carbaldehyde (217 mg, 1.16 mmol) and diethyl phosphite (161 mg, 1.16 mmol) in toluene (20 mL) was refluxed under N_2 protection for 3 h. After cooling to the room temperature, the compound **2** (500 mg) was obtained by filtration as yellow solid. Yield: 47.1%; mp: 232–233 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.96 (s, 1H, Im-3-N*H*), 7.53 (d, *J* = 8.7 Hz, Ph-3,5-2*H*), 6.98 (s, 2H, SO_2NH_2), 6.78 (d, *J* = 8.8 Hz, 2H, Ph-2,6-2*H*), 6.35 (s, 1H, Ph-N*H*), 4.90 (dd, *J* = 23.6, 9.1 Hz, 1H, C*H*), 4.09 (dd, *J* = 11.3, 4.4 Hz, 2H, OCH_2CH$_3$), 3.96 (dd, *J* = 7.3, 2.8 Hz, 1H, OCH_2CH$_3$), 3.83 (dd, *J* = 12.2, 4.9 Hz, 1H, OCH_2CH$_3$), 2.54–2.50 (m, 2H, Im-CH$_2$CH_2CH$_2$CH$_3$), 1.54 (m, *J* = 15.3, 7.6 Hz, 2H, Im-CH$_2$CH$_2$CH_2CH$_3$), 1.27–1.19 (m, 5H, Im-CH_2CH$_2$CH$_2$CH$_3$, OCH$_2$CH_3), 1.11 (t, *J* = 7.0 Hz, 3H, OCH$_2$CH_3), 0.85 (t, *J* = 7.4 Hz, 3H, Im-CH$_2$CH$_2$CH$_2$CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) δ 149.8, 147.9, 133.2, 127.6, 113.0, 63.6, 63.2, 30.2, 28.0, 21.9, 16.7, 16.5, 14.0; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.60; HRMS (ESI) calcd. for $C_{18}H_{28}ClN_4O_5PS$ $[M + H]^+$, 479.1285; found, 479.1275.

Compound (3a)*:* A mixture of sulfanilamide (201 mg, 1.17 mmol), compound **11a** (280 mg, 1.40 mmol), diethyl phosphite (256 mg, 1.91 mmol) and a catalytic amount of boron trifluoride etherate in acetonitrile (10 mL) was refluxed under N_2 protection for 6 h. Upon completion of the reaction, the solvent was evaporated under reduced pressure. The mixture was further purified by silica gel column chromatography (eluent, petroleum ether/ethyl acetate (4/1, V/V)) to afford the desired product **3a** (360 mg) as yellow solid. Yield: 62.9%; mp: 156–157 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.6 Hz, 2H, Ph-3,5-2*H*), 6.67 (d, *J* = 8.7 Hz, 2H, Ph-2,6-2*H*), 5.44 (t, *J* = 8.0 Hz, 1H, N*H*), 5.35 (s, 2H, SO_2NH_2), 5.01 (dd, *J* = 26.2, 6.9 Hz, 1H, C*H*), 4.24–4.16 (m, 2H, OCH_2CH$_3$), 4.00 (m, *J* = 21.6, 7.2 Hz, 1H, OCH_2CH$_3$), 3.88 (dd, *J* = 15.7, 8.2 Hz, 1H, OCH_2CH$_3$), 3.67 (s, 3H, NCH_3), 2.58 (t, *J* = 7.7 Hz, 2H, Im-CH_2CH$_2$CH$_2$CH$_3$), 1.70–1.59 (m, 2H, Im-CH$_2$CH_2CH$_2$CH$_3$), 1.35 (dd, *J* = 13.7, 6.8 Hz, 5H, Im-CH$_2$CH$_2$CH_2CH$_3$, OCH$_2$CH_3), 1.19 (t, *J* = 7.0 Hz, 3H, OCH$_2$CH_3), 0.90 (t, *J* = 7.3 Hz, 3H, Im-CH$_2$CH$_2$CH$_2$CH_3); ^{13}C NMR (151 MHz, $CDCl_3$) δ 149.3, 131.6, 128.4, 118.0, 112.8, 64.1, 63.9, 47.6, 46.6, 31.9, 29.5, 26.7, 22.3, 16.4, 16.2, 13.7; ^{31}P NMR (243 MHz, $CDCl_3$) δ 19.24; HRMS (ESI) calcd. for $C_{19}H_{30}ClN_4O_5PS$ $[M + H]^+$, 493.1441; found, 493.1438.

Compound (3b): Compound **3b** was prepared in the same way as compound **3a**, starting from sulfanilamide (198 mg, 1.15 mmol), compound **11b** (270 mg, 1.18 mmol) and diethyl phosphite (181 mg, 1.31 mmol), the desired product **3b** (356 mg) was obtained as white solid. Yield: 58.8%; mp: 189–190 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.52 (d, *J* = 8.8 Hz, 2H, Ph-3,5-2*H*), 7.22 (s, 1H, *N*H), 6.96 (SO_2NH_2, 2H), 6.77 (d, *J* = 8.6 Hz, 2H, Ph-2,6-2*H*), 4.90 (dd, *J* = 27.3, 6.7 Hz, 1H, C*H*), 4.29 (s, 1H, OCH_2CH$_3$), 4.18–4.07 (m, 2H, OCH_2CH$_3$), 3.94–3.88 (m, 1H, OCH_2CH$_3$), 3.86–3.80 (m, 1H, NCH_2CH$_2$CH$_3$), 3.74 (dd, *J* = 16.4, 7.9 Hz, 1H, NCH_2CH$_2$CH$_3$), 2.58 (m, *J* = 15.4, 7.6 Hz, 1H, Im-CH_2CH$_2$CH$_2$CH$_3$), 1.59 (dd, *J* = 14.8, 7.6 Hz, 2H, Im-CH$_2$CH_2CH$_2$CH$_3$), 1.41 (s, 1H, Im-CH_2CH$_2$CH$_2$CH$_3$), 1.31 (dd, *J* = 14.9, 7.4 Hz, 2H, Im-CH$_2$CH$_2$CH_2CH$_3$), 1.24 (t, *J* = 7.0 Hz, 3H, OCH$_2$CH_3), 1.06 (t, *J* = 7.0 Hz, 3H, OCH$_2$CH_3), 0.90–0.83 (m, 6H, Im-CH$_2$CH$_2$CH$_2$CH_3, NCH$_2$CH$_2$CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.1, 148.7, 132.7, 127.7, 118.2, 112.0, 63.4, 47.1, 46.2, 46.0, 45.9, 29.6, 26.0, 24.1, 22.1, 16.7, 16.4, 14.2, 11.2; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.19; HRMS (ESI) calcd.

for $C_{21}H_{34}ClN_4O_5PS$ [M + H]$^+$, 521.1754; found, 521.1751.

Compound (3c): Compound **3c** was prepared in the same way as compound **3a**, starting from sulfanilamide (200 mg, 1.16 mmol), compound **11c** (293 mg, 1.14 mmol) and diethyl phosphite (157 mg, 1.14 mmol), the desired product **3c** (420 mg) was obtained as white solid. Yield: 66.0%; mp: 217–218 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.53 (d, *J* = 8.5 Hz, 2H, Ph-3,5-2*H*), 7.18 (s, 1H, N*H*), 6.97 (s, 2H, SO_2NH_2), 6.78 (d, *J* = 8.2 Hz, 2H, Ph-2,6-2*H*), 4.92 (dd, *J* = 27.0, 5.3 Hz, 1H, C*H*), 4.32 (d, 1H, $NCH_2CH_2CH_2CH_2CH_3$), 4.19–4.10 (m, 2H, OCH_2CH_3), 3.97–3.84 (m, 2H, OCH_2CH_3), 3.75 (d, *J* = 7.4 Hz, 1H, $NCH_2CH_2CH_2CH_2CH_3$), 2.59 (m, *J* = 15.2, 7.5 Hz, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.60 (dd, *J* = 14.5, 7.2 Hz, 2H, $NCH_2CH_2CH_2CH_2CH_3$), 1.42–1.28 (m, 5H, Im-$CH_2CH_2CH_2CH_3$, OCH_2CH_3), 1.26 (t, *J* = 7.0 Hz, 4H, $NCH_2CH_2CH_2CH_2CH_3$), 1.20 (d, *J* = 6.5 Hz, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.07 (t, *J* = 6.9 Hz, 3H, OCH_2CH_3), 0.88 (t, *J* = 7.3 Hz, 3H, Im-$CH_2CH_2CH_2CH_3$), 0.81 (t, *J* = 7.1 Hz, 3H, $NCH_2CH_2CH_2CH_2CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.0, 148.6, 132.8, 127.6, 112.0, 63.4, 47.1, 46.1, 44.9, 30.5, 29.6, 28.6, 26.0, 22.3, 22.1, 16.7, 16.4, 14.3, 14.1; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.19; HRMS (ESI) calcd. for $C_{33}H_{38}ClN_4O_5PS$ [M + H]$^+$, 549.2067; found, 549.2061.

Compound (3d): Compound **3d** was prepared in the same way as compound **3a**, starting from sulfanilamide (100 mg, 0.58 mmol), compound **11d** (57 mg, 0.58 mmol) and diethyl phosphite (81 mg, 0.58 mmol), the desired product **3d** (156 mg) was obtained as white solid. Yield: 47.7%; mp: 234–235 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.52 (d, *J* = 7.9 Hz, 2H, Ph-3,5-2*H*), 6.92 (s, 1H, N*H*), 6.77 (d, *J* = 7.3 Hz, 2H, Ph-2,6-2*H*), 6.44 (s, 2H, SO_2NH_2), 4.91 (d, *J* = 27.5 Hz, 1H, C*H*), 4.29 (s, 2H, OCH_2CH_3), 4.14 (d, *J* = 6.6 Hz, 2H, OCH_2CH_3), 3.91 (d, *J* = 7.8 Hz, 2H, $NCH_2(CH_2)_4CH_3$), 3.76 (s, 2H, Im-$CH_2CH_2CH_2CH_3$), 2.57 (d, *J* = 7.1 Hz, 2H, $NCH_2CH_2(CH_2)_3CH_3$), 1.64–1.55 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.33–1.30 (m, 2H, $NCH_2CH_2CH_2(CH_2)_2CH_3$), 1.25 (d, *J* = 6.7 Hz, 6H, Im-$CH_2CH_2CH_2CH_3$, $NCH_2CH_2CH_2CH_2CH_2CH_3$), 1.21 (d, *J* = 6.9 Hz, 3H, OCH_2CH_3), 1.07 (d, *J* = 6.5 Hz, 3H, OCH_2CH_3), 0.90–0.80 (m, 6H, Im-$CH_2CH_2CH_2CH_3$, $NCH_2(CH_2)_4CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.0, 148.6, 132.8, 127.6, 118.3, 112.0, 63.5, 47.1, 46.1, 44.9, 31.3, 31.1, 30.8, 29.6, 26.0, 22.4, 22.1, 16.7, 16.4, 14.3, 14.1; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.17; HRMS (ESI) calcd. for $C_{34}H_{41}ClN_4O_5PS$ [M + H]$^+$, 563.2224; found, 563.2222.

Compound (3e): Compound **3e** was prepared in the same way as compound **3a**, starting from sulfanilamide (100 mg, 0.58 mmol), compound **11e** (316 mg, 0.64 mmol) and diethyl phosphite (90 mg, 0.65 mmol), the desired product **3e** (181 mg) was obtained as white solid. Yield: 48.3%; mp: 242–243 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.51 (d, *J* = 8.1 Hz, 2H, Ph-3,5-2*H*), 6.95 (s, 1H, N*H*), 6.76 (d, *J* = 8.0 Hz, 2H, Ph-2,6-2*H*), 6.50 (s, 2H, SO_2NH_2), 4.91 (d, *J* = 27.3 Hz, 1H, C*H*), 4.29 (s, 1H, OCH_2CH_3), 4.19–4.11 (m, 2H, OCH_2CH_3), 3.90 (dd, *J* = 15.9, 7.7 Hz, 2H, $NCH_2(CH_2)_9CH_3$), 3.75 (d, *J* = 7.1 Hz, 1H, OCH_2CH_3), 2.60–2.51 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.58 (m, *J* = 13.5, 6.8 Hz, 2H, $NCH_2CH_2(CH_2)_8CH_3$), 1.31 (dd, *J* = 14.9, 7.5 Hz, 3H, OCH_2CH_3), 1.23 (t, *J* = 20.0 Hz, 20H, Im-$CH_2CH_2CH_2CH_3$, $NCH_2CH_2(CH_2)_8CH_3$), 1.06 (t, *J* = 6.9 Hz, 3H, OCH_2CH_3), 0.86 (dd, *J* = 13.6, 7.0 Hz, 6H, Im-$CH_2CH_2CH_2CH_3$, $N(CH_2)_{10}CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 148.6, 127.6, 118.3, 112.0, 63.5, 47.1, 44.9, 40.5, 40.4, 40.2, 39.9, 39.7, 39.6, 31.8, 31.1, 30.8, 29.6, 29.5, 29.3, 29.1, 29.0, 26.3, 26.0, 22.5, 22.1, 16.7, 16.4, 14.4, 14.1; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.16; HRMS (ESI) calcd. for $C_{33}H_{53}ClN_4O_5PS$ [M + H]$^+$, 647.3163; found, 647.3156.

Compound (3f): Compound **3f** was prepared in the same way as compound **3a**, starting from sulfanilamide (100 mg, 0.58 mmol), compound **11f** (329 mg, 0.60 mmol) and diethyl phosphite (92 mg, 0.65 mmol), the desired product **3f** (198 mg) was obtained as white solid. Yield: 46.3%; mp: 213–214 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.52 (d, *J* = 8.7 Hz, 2H, Ph-3,5-2*H*), 6.95 (s, 2H, SO_2NH_2), 6.87 (s, 1H, N*H*), 6.78 (d, *J* = 8.5 Hz, 2H, Ph-2,6-2*H*), 4.92 (dd, *J* = 26.8, 6.3 Hz, 1H, C*H*), 4.30 (s, 1H, OCH_2CH_3), 4.19–4.11 (m, 2H, OCH_2CH_3), 3.97–3.84 (m, 2H, $NCH_2(CH_2)_{14}CH_3$), 3.76 (d, *J* = 7.2 Hz, 1H, OCH_2CH_3), 2.61–2.52 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.60 (m, *J* = 13.2, 6.4 Hz, 2H, $NCH_2CH_2(CH_2)_{13}CH_3$), 1.35 (d, *J* = 7.3 Hz, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.26 (d, *J* = 8.3 Hz, 28H, $NCH_2CH_2(CH_2)_{13}CH_3$, Im-$CH_2CH_2CH_2CH_3$), 1.20 (s, 3H, OCH_2CH_3), 1.07 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 0.89 (d, *J* = 7.4 Hz, 3H, $NCH_2(CH_2)_{14}CH_3$), 0.87–0.85 (m, 3H, Im-$CH_2CH_2CH_2CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 148.6, 132.8, 127.9, 127.6, 112.9, 63.4, 47.1, 46.1, 44.9, 40.5, 40.3, 40.0, 39.9, 39.7, 31.8, 30.8, 29.5, 29.3, 29.2, 29.0, 26.3, 26.0, 22.5, 22.1, 16.7, 16.4, 14.4, 14.1; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.17; HRMS (ESI) calcd. for $C_{34}H_{61}ClN_4O_5PS$ [M + H]$^+$, 703.3789; found, 703.3784.

Compound (4a): Compound **4a** was prepared in the same way as compound **3a**, starting from sulfanilamide (200 mg, 1.16 mmol), compound **12a** (319 mg, 1.17 mmol) and diethyl phosphite (174 mg, 1.26 mmol), the desired product **4a** (444 mg) was obtained as white solid. Yield: 67.7%; mp: 191–192 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.48 (d, *J* = 8.8 Hz, 2H, Ph-3,5-2*H*), 7.17 (s, 1H, N*H*), 6.95 (s, 2H, SO_2NH_2), 6.71 (d, *J* = 8.7 Hz, 2H, Ph-2,6-2*H*), 6.52 (s, 2H, Im-CH_2), 5.22 (d, *J* = 18.5 Hz, 1H, OCH_2CH_3), 5.05 (d, *J* = 18.5 Hz, 1H, OCH_2CH_3), 4.86 (dd, *J* = 26.8, 5.7 Hz, 1H, C*H*), 4.18 – 4.10 (m, 2H, OCH_2CH_3), 3.94 (dt, *J* = 14.4, 7.2 Hz, 2H, OCH_2CH_3), 3.78 (d, *J* = 7.4 Hz, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.54 (dd, *J* = 15.0, 7.2 Hz, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.29 (dd, *J* = 14.8, 7.4 Hz, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.23 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.10–1.03 (m, 6H, OCH_2CH_3, $CO_2CH_2CH_3$), 0.85 (t, *J* = 7.4 Hz, 3H, Im-$CH_2CH_2CH_2CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.8 (C=O), 150.2, 150.0, 132.9, 127.4, 119.1, 112.2, 63.6, 61.5, 47.1, 46.3, 46.0, 28.9, 25.8, 22.1, 16.7, 16.4, 14.4, 14.3, 14.2; ^{31}P NMR (243 MHz, DMSO-d_6) δ 18.96; HRMS (ESI) calcd. for $C_{22}H_{34}ClN_4O_7PS$ [M + H]$^+$, 565.1653; found, 565.1651.

Compound (4b): Compound **4b** was prepared in the same way as compound **3a**, starting from sulfanilamide (200 mg, 1.16 mmol), compound **12b** (260 mg, 1.15 mmol) and diethyl phosphite (170 mg, 1.23 mmol), the desired product **4b** (235 mg) was obtained as white solid. Yield: 42.0%; mp: 171–172 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.50 (d, J = 8.8 Hz, 2H, Ph-3,5-2*H*), 7.09 (s, 1H, N*H*), 6.95 (s, 2H, SO_2NH_2), 6.73 (d, J = 8.6 Hz, 2H, Ph-2,6-2*H*), 5.70 (s, 1H, C*H*=CH_2), 5.08 (d, J = 15.6 Hz, 1H, NHC*H*), 4.92 (dd, J = 26.8, 7.5 Hz, 2H, CH=CH_2), 4.65 (d, J = 17.4 Hz, 2H, Im-CH_2), 4.20–4.10 (m, 2H, OCH_2CH_3), 3.95 (dd, J = 12.3, 4.9 Hz, 1H, OCH_2CH_3), 3.81 (dd, J = 16.1, 8.1 Hz, 1H, OCH_2CH_3), 2.56–2.51 (m, 1H, Im-$CH_2CH_2CH_2$), 2.48–2.40 (m, 1H, Im-$CH_2CH_2CH_2$), 1.57 (dd, J = 14.9, 7.5 Hz, 2H, Im-$CH_2CH_2CH_2$), 1.28 (dd, J = 14.8, 7.4 Hz, 2H, Im-$CH_2CH_2CH_2$), 1.24 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 1.08 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 0.85 (t, J = 7.4 Hz, 3H, Im-$CH_2CH_2CH_2CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.1, 149.2 (C=CH_2), 133.9, 132.7, 127.5 (C=CH_2), 118.8, 116.6, 116.0, 112.2, 63.5, 47.1, 46.9, 45.9, 29.3, 26.1, 22.1, 16.7, 16.5, 14.1; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.18; HRMS (ESI) calcd. for $C_{21}H_{32}ClN_4O_5PS$ [M + H]$^+$, 519.1598; found, 519.1594.

Compound (4c): Compound **4c** was prepared in the same way as compound **3a**, starting from sulfanilamide (100 mg, 0.581 mmol), compound **12c** (131 mg, 0.581 mmol) and diethyl phosphite (81 mg, 0.581 mmol), the desired product **4c** (131 mg) was obtained as white solid. Yield: 43.3%; mp: 142–143 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.50 (d, *J* = 8.8 Hz, 2H, Ph-3,5-2*H*), 7.05 (d, *J* = 51.8 Hz, 1H, N*H*), 6.94 (s, 2H, SO_2NH_2), 6.81 (d, *J* = 8.0 Hz, 2H, Ph-2,6-2*H*), 5.22 (d, *J* = 18.5 Hz, 1H, NHC*H*), 4.99 (d, *J* = 1.9 Hz, 2H, NCH_2CCH), 4.18–4.12 (m, 2H, OCH_2CH_3), 3.99 (dd, *J* = 15.9, 8.3 Hz, 2H, OCH_2CH_3) , 2.71–2.58 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.66–1.58 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.32 (dd, *J* = 14.9, 7.4 Hz, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.24 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.10 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 0.87 (t, *J* = 7.4 Hz, 3H, Im-$CH_2CH_2CH_2CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 149.91, 148.9, 132.8, 127.4, 118.9, 112.5, 78.7 (C≡CH), 76.3 (C≡CH), 63.8, 63.5, 46.9, 45.8, 34.4, 31.1, 29.1, 26.2, 22.2, 16.7, 16.5, 14.1; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.00; HRMS (ESI) calcd. for $C_{21}H_{30}ClN_4O_5PS$ [M + H]$^+$, 517.1441; found, 517.1435.

Compound (5a): Compound **5a** was prepared in the same way as compound **3a**, starting from sulfanilamide (100 mg, 0.58 mmol), compound **10a** (190 mg, 0.65 mmol) and diethyl phosphite (90 mg,

0.65 mmol), the desired product **5a** (189 mg) was obtained as white solid. Yield: 55.4%; mp: 177–178 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.41 (d, *J* = 7.3 Hz, 2H, Ph-3,5-2*H*), 6.97 (s, 5H, 4-F-Ph-2,3,5,6-4*H*, N*H*), 6.76 (s, 2H, Ph-2,6-2*H*), 6.48 (s, 2H, SO_2NH_2), 5.65 (d, *J* = 16.3 Hz, 1H, NCH_2), 5.28 (d, *J* = 17.1 Hz, 1H, NCH_2), 4.94 (dd, *J* = 27.1, 7.6 Hz, 1H, C*H*), 4.19–4.08 (m, 2H, OCH_2CH_3), 4.04–3.81 (m, 2H, OCH_2CH_3), 2.33 (d, *J* = 72.5 Hz, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.46–1.32 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.23 (t, *J* = 6.8 Hz, 3H, OCH_2CH_3), 1.20–1.14 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.12 (t, *J* = 6.7 Hz, 3H, OCH_2CH_3), 0.74 (d, *J* = 6.8 Hz, 3H, Im-$CH_2CH_2CH_2CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.9, 149.8, 133.4, 132.7, 128.5, 127.4, 119.3, 116.0, 115.7, 115.5, 112.1, 63.6, 47.5, 47.1, 46.0, 29.2, 26.3, 22.0, 16.7, 16.5, 14.0; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.04; HRMS (ESI) calcd. for $C_{25}H_{33}ClFN_4O_5PS$ $[M + H]^+$, 587.1660; found, 587.1655.

Compound (5b): Compound **5b** was prepared in the same way as compound **3a**, starting from sulfanilamide (100 mg, 0.58 mmol), compound **10b** (198 mg, 0.64 mmol) and diethyl phosphite (89 mg, 0.64 mmol), the desired product **5b** (164 mg) was obtained as white solid. Yield: 46.9%; mp: 204–205 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.42 (d, *J* = 8.3 Hz, 2H, Ph-3,5-2*H*), 7.17 (s, 2H, 4-Cl-Ph-3,5-2*H*), 6.97 (s, 3H, 4-Cl-Ph-2,6-2*H*, N*H*), 6.72 (s, 2H, SO_2NH_2), 6.48 (d, *J* = 8.2 Hz, 2H, Ph-2,6-2*H*), 5.66 (d, *J* = 16.5 Hz, 1H, NCH_2), 5.30 (d, *J* = 17.4 Hz, 1H, NCH_2), 4.95 (dd, *J* = 27.2, 7.9 Hz, 1H, C*H*), 4.20–4.09 (m, 2H, OCH_2CH_3), 4.04–3.81 (m, 2H, OCH_2CH_3), 2.42–2.20 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.48–1.33 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.23 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.15 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.11 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 0.73 (t, *J* = 7.3 Hz, 3H, Im-$CH_2CH_2CH_2CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 149.8, 149.4, 136.3, 132.7, 132.2, 128.7, 128.4, 127.4, 119.4, 112.0, 63.6, 47.5, 47.0, 46.0, 29.1, 26.3, 22.0, 16.7, 16.5, 14.0; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.02; HRMS (ESI) calcd. for $C_{25}H_{33}Cl_2N_4O_5PS$ $[M + H]^+$, 603.1365; found, 603.1356.

Compound (5c): Compound **5c** was prepared in the same way as compound **3a**, starting from sulfanilamide (71 mg, 0.10 mmol), compound **10c** (132 mg, 0.41 mmol) and diethyl phosphite (59 mg, 0.43 mmol), the desired product **5c** (109 mg) was obtained as yellow solid. Yield: 43.7%; mp: 209–210 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.93 (s, 2H, SO_2NH_2), 7.36 (d, *J* = 8.4 Hz, 2H, 4-NO_2-Ph-3,5-2*H*), 7.07 (s, 1H, N*H*), 6.88 (d, *J* = 34.2 Hz, 4H, 4-NO_2-Ph-2,6-2*H*, Ph-3,5-2*H*), 6.43 (d, *J* = 8.3 Hz, 2H, Ph-2,6-2*H*), 5.83 (d, *J* = 18.0 Hz, 1H, NCH_2), 5.51 (d, *J* = 18.2 Hz, 1H, NCH_2), 4.97 (dd, *J* = 27.1, 7.0 Hz, 1H, C*H*), 4.18–4.08 (m, 2H, OCH_2CH_3), 3.98 (dd, *J* = 15.7, 8.3 Hz, 1H, OCH_2CH_3), 3.85 (d, *J* = 7.6 Hz, 1H, OCH_2CH_3), 2.42–2.33 (m, 1H, Im-$CH_2CH_2CH_2CH_3$), 2.25 (d, *J* = 7.0 Hz, 1H, Im-$CH_2CH_2CH_2CH_3$), 1.51–1.36 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.23 (t, *J* = 6.9 Hz, 3H, OCH_2CH_3), 1.17 (d, *J* = 4.8 Hz, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.11 (t, *J* = 6.9 Hz, 3H, OCH_2CH_3), 0.74 (t, *J* = 7.2 Hz, 3H, Im-$CH_2CH_2CH_2CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 149.6, 147.0, 145.0, 132.7, 127.3, 123.7, 119.4, 111.8, 63.6, 47.6, 46.9, 45.9, 29.1, 26.1, 22.0, 16.7, 16.6, 14.0; ^{31}P NMR (243 MHz, DMSO-d_6) δ 18.97; HRMS (ESI) calcd. for $C_{25}H_{33}ClN_5O_7PS$ $[M + H]^+$, 614.1605; found, 614.1599.

Compound (5d): Compound **5d** was prepared in the same way as compound **3a**, starting from sulfanilamide (100 mg, 0.58 mmol), compound **10d** (189 mg, 0.64 mmol) and diethyl phosphite (87 mg, 0.63 mmol), the desired product **5d** (251 mg) was obtained as white solid. Yield: 73.6%; mp: 169–170 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.33 (d, *J* = 7.6 Hz, 2H, Ph-3,5-2*H*), 7.21 (s, 2H, 2F-Ph-3,4-2*H*), 6.95 (s, 4H, 2F-Ph-5,6-2*H*, SO_2NH_2), 6.69 (s, 1H, N*H*), 6.39 (d, *J* = 6.5 Hz, 2H, Ph-2,6-2*H*), 5.70 (d, *J* = 15.8 Hz, 1H, NCH_2), 5.35 (d, *J* = 17.6 Hz, 1H, NCH_2), 4.93 (dd, *J* = 26.9, 7.2 Hz, 1H, C*H*), 4.17–4.09 (m, 2H, OCH_2CH_3), 4.00 (d, *J* = 7.2 Hz, 1H, OCH_2CH_3), 3.87 (s, 1H, OCH_2CH_3), 2.47–2.42 (m, 1H, Im-$CH_2CH_2CH_2CH_3$), 2.31 (s, 1H, Im-$CH_2CH_2CH_2CH_3$), 1.51–1.40 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.23 (t, *J* = 6.4 Hz, 3H, OCH_2CH_3), 1.18 (d, *J* = 6.5 Hz, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.12 (t, *J* = 6.2 Hz, 3H, OCH_2CH_3), 0.75 (d, *J* = 6.5 Hz, 3H, Im-$CH_2CH_2CH_2CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 149.8, 149.8, 149.4, 132.6, 127.3, 124.8, 124.1, 119.5, 112.0, 63.7, 63.6, 63.6, 42.4, 29.1, 26.0, 22.0, 16.7, 16.7, 16.5, 16.5, 14.0; ^{31}P NMR (243 MHz, DMSO-d_6) δ 18.99; HRMS (ESI) calcd. for $C_{25}H_{33}ClFN_4O_5PS$ $[M + H]^+$, 587.1660; found, 587.1654.

Compound (5e): Compound **5e** was prepared in the same way as compound **3a**, starting from sulfanilamide (100 mg, 0.58mmol), compound **10e** (198 mg, 0.63 mmol) and diethyl phosphite (85 mg, 0.62 mmol), the desired product **5e** (300 mg) was obtained as yellow solid. Yield: 85.5%; mp: 135–136 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.49 (d, *J* = 8.4 Hz, 2H, Ph-3,5-2*H*), 7.31 (t, *J* = 9.7 Hz, 1H, 2,4-2F-Ph-6-*H*), 7.03 (t, *J* = 7.8 Hz, 1H, 2,4-2F-Ph-5-*H*), 6.97 (s, 2H, SO_2NH_2), 6.84 (d, *J* = 8.4 Hz, 2H, Ph-2,6-2*H*), 6.70 (dd, *J* = 14.9, 8.0 Hz, 1H, 2,4-2F-Ph-3-*H*), 6.60 (dd, *J* = 8.3, 5.2 Hz, 1H, N*H*), 5.19 (s, 2H, NCH_2), 4.86 (dd, *J* = 22.8, 9.2 Hz, 1H, C*H*), 4.07 (dd, *J* = 18.3, 11.3 Hz, 2H, OCH_2CH_3), 3.98 (m, *J* = 14.1, 7.2 Hz, 1H, OCH_2CH_3), 3.88 (dd, *J* = 16.1, 8.3 Hz, 1H, OCH_2CH_3), 2.63–2.55 (m, 2H, Im$CH_2CH_2CH_2CH_3$), 1.48 (dd, *J* = 14.6, 7.2 Hz, 2H, Im$CH_2CH_2CH_2CH_3$), 1.27–1.24 (m, 2H, Im$CH_2CH_2CH_2CH_3$), 1.21 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.10 (t, *J* = 6.9 Hz, 3H, OCH_2CH_3), 0.80 (t, *J* = 7.3 Hz, 3H, Im$CH_2CH_2CH_2CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.4, 148.4, 132.3, 130.2, 129.6, 127.4, 120.2, 114.7, 112.7, 112.3, 104.9, 104.7, 104.6, 62.9, 49.0, 48.0, 29.5, 26.8, 22.0, 16.8, 16.6, 14.0; ^{31}P NMR (243 MHz, DMSO-d_6) δ 20.48; HRMS (ESI) calcd. for $C_{25}H_{32}ClF_2N_4O_5PS$ $[M + H]^+$, 605.1566; found, 605.1566.

Compound (5f): Compound **5f** was prepared in the same way as compound **3a**, starting from sulfanilamide (100 mg, 0.58 mmol), compound **10f** (205 mg, 0.59 mmol) and diethyl phosphite (90 mg, 0.65 mmol), the desired product **5f** (170 mg) was obtained as white solid. Yield: 45.9%; mp: 139–140 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.63 (s, 1H, 2,4-2Cl-Ph-3-*H*), 7.39 (d, *J* = 8.6 Hz, 2H, Ph-3,5-2*H*), 6.95 (s, 3H, 2,4-2Cl-Ph-5-*H*, SO_2NH_2), 6.82 (s, 1H, 2,4-2Cl-Ph-6-*H*), 6.45 (d, *J* = 8.6 Hz, 2H, Ph-2,6-2*H*), 5.69 (d, *J* = 16.7 Hz, 2H, NCH_2), 5.30 (d, *J* = 18.3 Hz, 1H, N*H*), 4.95 (dd, *J* = 27.0, 7.4 Hz, 1H, C*H*), 4.14 (dd, *J* = 14.7, 7.3 Hz, 2H, OCH_2CH_3), 4.03–3.96 (m, 1H, OCH_2CH_3), 3.90–3.83 (m, 1H, OCH_2CH_3), 2.39 (dd, *J* = 15.0, 8.2 Hz, 1H, Im-$CH_2CH_2CH_2CH_3$), 2.24 (s, 1H, Im-$CH_2CH_2CH_2CH_3$), 1.50–1.41 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.23 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.20–1.16 (m, 2H, Im-$CH_2CH_2CH_2CH_3$), 1.13 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 0.75 (t, *J* = 7.3 Hz, 3H, Im-$CH_2CH_2CH_2CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 149.8, 149.6, 133.7, 133.0, 132.8, 132.2, 129.0, 128.1, 127.4, 119.5, 112.0, 63.6, 46.9, 45.9, 45.7, 29.0, 25.9, 22.0, 16.7, 16.5, 14.0; ^{31}P NMR (243 MHz, DMSO-d_6) δ 18.87; HRMS (ESI) calcd. for $C_{25}H_{32}Cl_3N_4O_5PS$ $[M + H]^+$, 637.0975; found, 637.0972.

Compound (6a): Compound **6a** was prepared in the same way as compound **3a**, starting from sulfanilamide (250 mg, 1.45 mmol), 2-thenaldehyde (253 mg, 2.26 mmol) and diethyl phosphite (613 mg, 4.44 mmol), the desired product **6a** (391 mg) was obtained as white solid. Yield: 66.6%; mp: 239–240 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.48 (d, *J* = 8.6 Hz, 2H, Ph-3,5-2*H*), 7.43 (d, *J* = 4.7 Hz, 1H, thiophene-4-*H*), 7.22 (s, 1H, N*H*), 7.03–6.97 (m, 2H, Ph-2,6-2*H*), 6.97–6.91 (m, 4H, thiophene-2,3-2*H*, SO_2NH_2), 5.50 (dd, *J* = 24.1, 9.5 Hz, 1H, C*H*), 4.14–4.03 (m, 2H, OCH_2CH_3), 4.03–3.96 (m, 1H, OCH_2CH_3), 3.88 (m, *J* = 17.3, 8.5 Hz, 1H, OCH_2CH_3), 1.18 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.13 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.4, 140.0, 132.4, 127.6, 127.3, 127.2, 126.3, 112.9, 63.3, 63.1, 50.6, 49.6, 16.8, 16.5; ^{31}P NMR (243 MHz, DMSO-d_6) δ 20.52; HRMS (ESI) calcd. for $C_{15}H_{21}N_2O_5PS_2$ $[M + H]^+$, 405.0708; found, 405.0701.

Compound (6b): Compound **6b** was prepared in the same way as compound **3a**, starting from sulfanilamide (248 mg, 1.44 mmol), furfural (213 mg, 2.22 mmol) and diethyl phosphite (593 mg, 4.29 mmol), the desired product **6b** (385 mg) was obtained as white solid. Yield: 68.3%; mp: 219–220 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.62 (s, 1H, N*H*), 7.49 (d, *J* = 8.6 Hz, 2H, Ph-3,5-2*H*), 6.94 (d, *J* = 3.7 Hz, 4H, Ph-2,6-2*H,* SO_2NH_2), 6.81 (s, 1H, furan-5-*H*), 6.45 (d, *J* = 31.6 Hz, 2H, furan-3,4-2*H*), 5.31 (dd, *J* = 23.6, 9.8 Hz, 1H, C*H*), 4.12–4.04 (m, 2H, OCH_2CH_3), 4.03–3.97 (m, 1H, OCH_2CH_3), 3.94–3.87 (m, 1H, OCH_2CH_3), 1.18 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.14 (t, *J* = 7.0 Hz, 3H,

OCH_2CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.4, 149.9, 143.3, 132.4, 127.4, 112.8, 111.1, 109.4, 63.1, 49.2, 48.1, 16.7; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.67; HRMS (ESI) calcd. for $C_{15}H_{21}N_2O_6PS$ $[M + H]^+$, 389.0936; found, 389.0928.

Compound (7a): Compound **7a** was prepared in the same way as compound **3a**, starting from sulfanilamide (250 mg, 1.45 mmol), 2,4-dichlorobenzaldehyde (382 mg, 2.18 mmol) and diethyl phosphite (600 mg, 4.34 mmol), the desired product **7a** (501 mg) was obtained as white solid. Yield: 73.9%; mp: 210–211 °C; ^1H NMR (600 MHz, DMSO-d_6) δ 7.66 (d, J = 6.9 Hz, 2H, Ph-3,5-2*H*), 7.49 (t, J = 8.7 Hz, 3H, Ph-3,5,6-3*H*), 7.38–7.29 (m, 1H, N*H*), 6.93 (s, 2H, SO_2NH_2), 6.75 (d, J = 8.6 Hz, 2H, Ph-2,6-2*H*), 5.25 (dd, J = 24.4, 9.5 Hz, 1H, C*H*), 4.18–4.05 (m, 2H, OCH_2CH_3), 3.92 (m, J = 14.6, 7.1 Hz, 1H, OCH_2CH_3), 3.84–3.73 (m, 1H, OCH_2CH_3), 1.22 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 1.06 (t, J = 7.0 Hz, 3H, OCH_2CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) δ 149.9, 134.8, 133.8, 132.9, 131.3, 129.1, 128.2, 127.7, 112.7, 63.6, 63.2, 51.3, 50.3, 16.8, 16.5; ^{31}P NMR (243 MHz, DMSO-d_6) δ 20.30; HRMS (ESI) calcd. for $C_{17}H_{21}Cl_2N_2O_5PS$ $[M + H]^+$, 467.0364; found, 467.0358.

Compound (7b): Compound **7b** was prepared in the same way as compound **3a**, starting from sulfanilamide (250 mg, 1.45 mmol), 4-nitrobenzaldehyde (218 mg, 1.44 mmol) and diethyl phosphite (612 mg, 4.43 mmol), the desired product **7b** (409 mg) was obtained as yellow solid. Yield: 63.6%; mp: 214–215 °C; ^1H NMR (600 MHz, DMSO-d_6) δ 8.23 (d, *J* = 8.5 Hz, 2H, 4-NO_2-Ph-3,5-2*H*), 7.80 (d, *J* = 7.4 Hz, 2H, Ph-3,5-2*H*), 7.46 (d, *J* = 8.7 Hz, 2H, 4-NO_2-Ph-2,6-2*H*), 7.31–7.24 (s, 1H, N*H*), 6.95–6.88 (m, 4H, 4-NO_2-Ph-3,5-2*H*, Ph-2,6-2*H*), 5.48 (dd, *J* = 25.7, 9.7 Hz, 1H, C*H*), 4.08 (m, *J* = 24.5, 12.3 Hz, 2H, $OCH_2CH_2CH_3$), 4.01–3.81 (m, 2H, $OCH_2CH_2CH_3$), 1.20 (t, *J* = 7.0 Hz, 3H, $OCH_2CH_2CH_3$), 1.10 (t, *J* = 7.0 Hz, 3H, $OCH_2CH_2CH_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.2, 147.5, 145.2, 132.6, 129.9, 127.5, 123.7, 113.0, 63.5, 63.1, 54.2, 53.2, 16.7, 16.5; ^{31}P NMR (243 MHz, DMSO-d_6) δ 20.72; HRMS (ESI) calcd. for $C_{17}H_{22}N_3O_7PS$ $[M + H]^+$, 444.0994; found, 444.0988.

Compound (7c): Compound **7c** was prepared in the same way as compound **3a**, starting from sulfanilamide (253 mg, 1.47 mmol), 4-cyanobenzaldehyde (280 mg, 2.14 mmol) and diethyl phosphite (719 mg, 4.48 mmol), the desired product **7c** (542 mg) was obtained as white solid. Yield: 88.1%; mp: 120–121 °C; ^1H NMR (600 MHz, DMSO-d_6) δ 7.83 (d, *J* = 8.0 Hz, 2H, 4-CN-Ph-3,5-2*H*), 7.72 (d, *J* = 7.6 Hz, 2H, Ph-3,5-2*H*), 7.46 (d, *J* = 8.6 Hz, 2H, 4-CN-Ph-3,5-2*H*), 7.22 (dd, J = 6.8 Hz, 1H, N*H*), 6.91 (s, 2H, SO_2NH_2), 6.89 (d, *J* = 8.6 Hz, 2H, Ph-2,6-2*H*), 5.39 (dd, *J* = 25.5, 9.8 Hz, 1H, C*H*), 4.11–4.02 (m, 2H, OCH_2CH_3), 3.96 (m, *J* = 17.3, 7.3 Hz, 1H, OCH_2CH_3), 3.87–3.79 (m, 1H, OCH_2CH_3), 1.19 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.10–1.08 (t, 3H, OCH_2CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.3, 143.0, 132.5, 129.6, 127.5, 119.2, 113.0, 110.8, 65.4, 63.4, 63.0, 16.7, 16.7, 16.5, 16.5, 15.6;. ^{31}P NMR (243 MHz, DMSO-d_6) δ 20.95; HRMS (ESI) calcd. for $C_{18}H_{22}N_3O_5PS$ $[M + H]^+$, 424.1096; found, 424.1090.

Compound (7d): Compound **7d** was prepared in the same way as compound **3a**, starting from sulfanilamide (300 mg, 1.74 mmol), 4-hydroxybenzaldehyde (319 mg, 2.61 mmol) and diethyl phosphite (722 mg, 5.23 mmol), the desired product **7d** (101 mg) was obtained as white solid. Yield: 14.0%; mp: 111–112 °C; ^1H NMR (600 MHz, DMSO-d_6) δ 9.37 (s, 1H, O*H*), 7.44 (d, *J* = 8.8 Hz, 2H, Ph-3,5-2*H*), 7.31 (d, *J* = 7.1 Hz, 2H, Ph-2,6-2*H*), 7.00–6.94 (m, 1H, N*H*), 6.89 (s, 2H, SO_2NH_2), 6.86 (d, *J* = 8.8 Hz, 2H, 4-OH-Ph-2,6-2*H*), 6.70 (d, *J* = 8.4 Hz, 2H, 4-OH-Ph-3,5-2*H*), 4.99 (dd, *J* = 23.8, 9.6 Hz, 1H, C*H*), 4.02 (dd, *J* = 15.0, 7.6 Hz, 2H, OCH_2CH_3), 3.87 (dd, *J* = 17.3, 7.2 Hz, 1H, OCH_2CH_3), 3.71 (dd, *J* = 12.2, 4.9 Hz, 1H, OCH_2CH_3), 1.17 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.05 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) δ 157.3, 150.6, 131.8, 129.9, 129.9, 127.4, 126.6, 115.4, 112.9, 62.9, 62.8, 53.9, 16.8, 16.6; ^{31}P NMR (243 MHz, DMSO-d_6) δ 22.67; HRMS (ESI) calcd. for $C_{17}H_{23}N_2O_6PS$ $[M + H]^+$, 415.1093; found, 415.1087.

Compound (7e): Compound **7e** was prepared in the same way as compound **3a**, starting from sulfanilamide (300 mg, 1.74 mmol), 4-fluorobenzaldehyde (432 mg, 3.48 mmol) and diethyl phosphite (722 mg, 5.23 mmol), the desired product **7e** (219 mg) was obtained as white solid. Yield: 30.2%; mp: 197–198 °C; ^1H NMR (600 MHz, DMSO) δ 7.57 (s, 2H, SO_2NH_2), 7.45 (d, *J* = 8.6 Hz, 2H, Ph-3,5-2*H*), 7.18 (t, *J* = 8.5 Hz, 2H, 4-F-Ph-3,5-2*H*), 7.15–7.09 (m, 1H, N*H*), 6.91 (s, 2H, 4-F-Ph-2,6-2*H*), 6.89 (d, *J* = 8.6 Hz, 2H, Ph-2,6-2*H*), 5.22 (dd, *J* = 24.4, 9.8 Hz, 1H, C*H*), 4.05 (dd, *J* = 14.5, 7.3 Hz, 2H, OCH_2CH_3), 3.91 (dd, *J* = 16.9, 7.3 Hz, 1H, OCH_2CH_3), 3.81–3.71 (m, 1H, OCH_2CH_3), 1.18 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.07 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.9, 161.2, 150.5, 150.4, 133.0, 132.2, 130.7, 127.4, 115.5, 112.9, 63.1, 62.9, 53.7, 52.7, 16.8, 16.5; ^{31}P NMR (243 MHz, DMSO-d_6) δ 22.00; HRMS (ESI) calcd. for $C_{17}H_{22}FN_2O_5PS$ $[M + H]^+$, 417.1049; found, 417.1043.

Compound (7f): Compound **7f** was prepared in the same way as compound **3a**, starting from sulfanilamide (300 mg, 1.74 mmol), 4-methoxybenzaldehyde (474 mg, 3.48 mmol) and diethyl phosphite (723 mg, 5.23 mmol), the desired product **7f** (329 mg) was obtained as white solid. Yield: 44.1%; mp: 181–182 °C; ^1H NMR (600 MHz, DMSO-d_6) δ 7.44 (d, *J* = 8.7 Hz, 4H, Ph-3,5-2*H*, 4-OCH_3-Ph-2,6-2*H*), 7.07–7.02 (m, 1H, N*H*), 6.90 (d, *J* = 3.1 Hz, 3H, Ph-2,6-2*H*, 4-OCH_3-Ph-3-*H*), 6.88 (s, 2H, SO_2NH_2), 6.87 (s, 1H, 4-OCH_3-Ph-5-*H*), 5.08 (dd, *J* = 24.1, 9.8 Hz, 1H, C*H*), 4.04 (dd, *J* = 14.9, 7.2 Hz, 2H, OCH_2CH_3), 3.89 (dd, *J* = 7.2, 2.9 Hz, 1H, OCH_2CH_3), 3.74 (d, *J* = 8.4 Hz, 1H, OCH_2CH_3), 3.71 (s, 3H, Ph-4-OCH_3), 1.18 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.06 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.2, 150.7, 150.6, 132.0, 129.9, 128.4, 127.4, 114.0, 112.9, 62.9, 62.8, 62.7, 55.5, 53.8, 52.8, 16.8, 16.6; ^{31}P NMR (243 MHz, DMSO-d_6) δ 22.48; HRMS (ESI) calcd. for $C_{18}H_{25}N_2O_6PS$ $[M + H]^+$, 429.1249; found, 429.1244.

Compound (8): Compound **8** was prepared in the same way as compound **3a**, starting from sulfanilamide (100 mg, 0.58 mmol), 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carbaldehyde (226 mg, 0.84 mmol) and diethyl phosphite (245 mg, 1.10 mmol), the desired product **8** (211 mg) was obtained as white solid. Yield: 64.9%; mp: 197–198 °C; ^1H NMR (600 MHz, DMSO-d_6) δ 11.88 (s, 2H, O*H*), 7.97 (s, 1H, N*H*), 7.75 (d, *J* = 44.9 Hz, 2H, Ph-3,5-2*H*), 7.57 (s, 1H, anthraquinone-4-*H*), 7.48 (d, *J* = 8.7 Hz, 2H, Ph-2,6-2*H*), 7.37 (s, 1H, anthraquinone-2-*H*), 7.34–7.29 (m, 1H, anthraquinone-6-*H*), 6.96 (d, *J* = 8.8 Hz, 2H, anthraquinone-5,7-2*H*), 6.91 (s, 2H, SO_2NH_2), 5.53 (dd, *J* = 25.8, 9.0 Hz, 1H, C*H*), 4.16–4.07 (m, 2H, OCH_2CH_3), 4.03 (m, *J* = 10.2, 7.2 Hz, 1H, OCH_2CH_3), 3.91 (dd, *J* = 16.4, 9.0 Hz, 1H, OCH_2CH_3), 1.21 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.17–1.11 (m, 3H, OCH_2CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) δ 192.2 (C=O), 181.9 (C=O), 161.8, 161.6, 150.3, 147.9, 137.8, 127.5, 124.8, 123.9, 119.8, 116.5, 113.0, 63.6, 63.2, 54.4, 53.4, 16.7, 16.5; ^{31}P NMR (243 MHz, DMSO-d_6) δ 20.45; HRMS (ESI) calcd. for $C_{25}H_{25}N_2O_9PS$ $[M + H]^+$, 561.1097; found, 561.1093.

Supporting Information

The supporting information for this article is available on the WWW under https://doi.org/10.1002/cjoc.2021xxxxx.

Acknowledgement (optional)

This research was supported in part by grants from the National Natural Science Foundation of China (21971212), Postdoctoral Science Foundation Project of Chongqing Science and Technology Bureau (cstc2019jcyj-bshX0124), China Postdoctoral Science Foundation (2019M653821XB) and Chongqing Special Foundation for Postdoctoral Research Proposal (XmT2018082).

References

[1] Imai, Y.; Meyer, K. J.; Iinishi, A.; Favre-Godal, Q.; Green, R.; Manuse, S.; Caboni, M.; Mori, M.; Niles, S.; higlieri, M.; Honrao, C.; Ma, X.; Guo, J.

J.; Makriyannis, A.; Linares-Otoya, L.; Bohringer, N.; Wuisan, Z. G.; Kaur, H.; Wu, R.; Mateus, A.; Typas, A.; Savitski, M. M.; Espinoza, J. L.; O'Rourke, A.; Nelson, K. E.; Hiller, S.; Noinaj, N.; Schaberle, T. F.; D'Onofrio, A.; Lewis, K. A New Antibiotic Selectively Kills Gram-Negative Pathogens. *Nature*, **2019**, *576*, 459–464.

[2] Okasha, R. M.; Alsehli, M.; Ihmaid, S.; Althagfan, S. S.; El-Gaby, M. S. A.; Ahmed, H. E.; Afifi, T. H. First Example of Azo-Sulfa Conjugated Chromene Moieties: Synthesis, Characterization, Antimicrobial Assessment, Docking Simulation as Potent Class I Histone Deacetylase Inhibitors and Antitumor Agents. *Bioorg. Chem.* **2019**, *92*, 103262.

[3] Zhao, C.; Rakesh, K. P.; Ravidar, L.; Fang, W. Y.; Qin, H. L. Pharmaceutical and Medicinal Significance of Sulfur (S(VI))-Containing Motifs for Drug Discovery: A Critical Review. *Eur J. Med. Chem.* **2019**, *162*, 679–734.

[4] (a) Sui, Y. F.; Li, D.; Wang, J.; Bheemanaboina, R. R. Y.; Ansari, M. F.; Gan, L. L.; Zhou, C. H. Design and Biological Evaluation of A Novel Type of Potential Multi-Targeting Antimicrobial Sulfanilamide Hybrids in Combination of Pyrimidine and Azoles. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 126982; (b) Zhang, P. L.; Lv, J. S.; Ansari, M. F.; Battini, N.; Cai, G. X.; Zhou, C. H. Synthesis of A Novel Structural Framework of Naphthalimide Triazoles and Specific Anti-Aspergillus fumigatus Effects. *Sci. Sin. Chim.* **2021**, doi: 10.1360/SSC-2021-0003.

[5] Hu, Y. Y.; Bheemanaboina, R. R. Y.; Battini, N.; Zhou, C. H. Sulfonamide-Derived Four-Component Molecular Hybrids as Novel DNA-Targeting Membrane Active Potentiators against Clinical Escherichia coli. *Mol. Pharm.* **2019**, *16*, 1036–1052.

[6] Ye, J. Q.; Chu, A. J.; Harper, R.; Chan, S.T.; Shek, T. L.; Zhang, Y. F. M.; Sambir, Ip. M.; Artsimovitch, I.; Zuo, Z.; Yang, X.; Ma, C. Discovery of Antibacterials that Inhibit Bacterial RNA Polymerase Interactions with Sigma Factors. *J. Med. Chem.* **2020**, *63*, 7695−7720.

[7] Hu, Y. Y.; Wang, J.; Li, T. J.; Bheemanaboina, R. R. Y.; Ansari, M. F.; Cheng, Y.; Zhou, C. H. An Unexpected Discovery toward Novel Membrane Active Sulfonyl Thiazoles as Potential MRSA DNA Intercalators, *Future Med. Chem.* **2020**, *12*, 1709–1727.

[8] He, S. C.; Ponmani, J.; Avula, S.; Wang, X. L.; Zhang, H. Z.; Zhou, C. H. Recent Advance in Sulfonamide-Based Medicinal Chemistry. *Sci. Sin. Chim.* **2016**, *46*, 823–847; (b) Wang, J.; Ansari, M. F.; Zhou, C. H. Unique para-Aminobenzenesulfonyl Oxadiazoles as Novel Structural Potential Membrane Active Antibacterial Agents towards Drug-Resistant Methicillin Resistant Staphylococcus aureus. *Bioorg. Med. Chem. Lett.* **2021**, *41*, 127995.

[9] [illegible]aid, M. A.; Eldehna, W. M.; Nocentini, A.; Bonardi, A.; Fahim, S. H.; Bua, S.; Soliman, D. H.; Abdel-Aziz, H. A.; Gratteri, P.; Abou-Seri, S. M.; Supuran, C. T. Synthesis, Biological and Molecular Dynamics Investigations with A Series of Triazolopyrimidine/Triazole-Based Benzenesulfonamides as Novel Carbonic Anhydrase Inhibitors. *Eur. J. Med. Chem.* **2020**, *185*, 111843.

[10] Shah, F.; Bell, I. M. Cutaneous Adverse Events Caused by Sulfonamide-Containing Drugs: Reality or Perception. *J. Med. Chem. 63*, **2020**, 7447–7457.

[11] Ewies, E. F.; Hussieny, M. E.; Sayed, N. F. E.; Fouad, M. A. Design, Synthesis and Biological Evaluationof Novel α-Aminophosphonate Oxadiazoles *via* Optimized Iron Triflate Catalyzed Reaction as Apoptotic Inducers. *Eur. J. Med. Chem.* **2020**, *180*, 310–320.

[12] Li, D.; Bheemanaboina, R. R. Y.; Battini N.; Tangadanchu, V. K. R.; Fang, X. F.; Zhou, C. H. Novel Organophosphorus Aminopyrimidines as Unique Structural DNA-Targeting Membrane Active Inhibitors towards Drug-Resistant Methicillin Resistant Staphylococcus aureus. *Med. Chem. Commun*. **2018**, *9*, 1529–1537.

[13] Maestro, A.; Martin-Encinas, E.; Alonso, C.; Martinez de Marigorta, E.; Rubiales, G.; Vicario, J.; Palacios, F. Synthesis of Novel Antiproliferative Hybrid Bis-(3-Indolyl)Methane Phosphonate Derivatives. *Eur. J. Med. Chem.* **2018**, *158*, 874–883.

[14] Finkbeiner, P.; Hehn, J. P.; Gnamm, C. Phosphine Oxides From A Medicinal Chemist'S Perspective: Physicochemical and *in vitro* Parameters Relevant for Drug Discovery. *J. Med. Chem.* **2020**, *63*, 7081−7107.

[15] Zhang, L.; Peng, X. M.; Damu, G. L.; Geng, R. X.; Zhou, C. H. Comprehensive Review in Current Developments of Imidazole-Based Medicinal Chemistry. *Med. Res. Rev.* **2014**, *34*, 340–437.

[16] Fan, Y. L.; Jin, X. H.; Huang, Z. P.; Yu, H. F.; Zeng, Z. G.; Gao, T.; Feng, L. S. Recent Advances of Imidazole-Containing Derivatives as Anti-Tubercular Agents. *Eur. J. Med. Chem.* **2018**, *150*, 347–365.

[17] Zheng, X.; Ma, Z.; Zhang, D. Synthesis of Imidazole-Based Medicinal Molecules Utilizing the Van Leusen Imidazole. *Pharmaceuticals* **2020**, *13*, 37–56.

[18] Gunduz, M. G.; Ugur, S. B.; Guney, F.; Ozkul, C.; Krishna, V. S.; Kaya, S.; Sriram, D.; Dogan, S. D. 1,3-Disubstituted Urea Derivatives: Synthesis, Antimicrobial Activity Evaluation and in silico Studies. *Bioorg. Chem.* **2020**, *102*, 104104.

[19] Shabalin, D. A.; Camp, J. E. Recent Advances in The Synthesis of Imidazoles. *Org. Biomol. Chem.* **2020**, *18*, 3950–3964.

[20] Wang, L. L.; Battini, N.; Bheemanaboina, R. R. Y.; Mohammad, F. A.; Chen, J. P.; Xie, Y. P.; Cai, G. X.; Zhang, S. L.; Zhou, C. H. A New Exploration towards Aminothiazolquinolone Oximes as Potentially Multi-Targeting Antibacterial Agents: Design, Synthesis and Evaluation Acting on Microbes, DNA, HSA and Topoisomerase IV. *Eur. J. Med. Chem.* **2019**, *179*, 166–181.

[21] Cui, Y.; Chen, L. J.; Huang, T.; Ying, J. Q.; Li, J. The Pharmacology, Toxicology and Therapeutic Potential of Anthraquinone Derivative Emodin. *Chin. J. Nat. Medicines,* **2020**, *18*, 425–435.

[22] Chen, R.; Zhang, J.; Hu, Y.; Wang, S.; Chen, M.; Wang, Y. Potential Antineoplastic Effects of Aloe-Emodin: A Comprehensive Review. *Am. J. Chin. Med.* **2014**, *42*, 275–288.

[23] Konai, M. M.; Haldar, J. Lysine-Based Small Molecule Sensitizes Rifampicin and Tetracycline against Multidrug-Resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *ACS Infect. Dis.* **2020**, *6*, 91−99; (b) Xie, Y. P.; Ansari, M. F.; Zhang, S. L.; Zhou, C. H. Novel Carbazoleoxadiazoles as Potential Staphylococcus aureus Germicides. *Pestic. Biochem. Phys.* **2021**, https://doi.org/10.1016/j.pestbp.2021.104849.

[24] Song, R. H.; Yu, B.; Friedrich, D.; Li, J. F.; Shen, H.; Krautscheid, H.; Huang, S. P. D.; Kim, M. H. Naphthoquinone-Derivative as A Synthetic Compound to Overcome the Antibiotic Resistance of Methicillin-Resistant S. aureus. *Commun. Biol.* **2020**, *3*, 529.

[25] Lam, A. K.; Panlilio, H.; Pusavat, J.; Wouters, C. L.; Moen, E. L.; Neel, A. J.; Rice, C. V. Low-Molecular-Weight Branched Polyethylenimine Potentiates Ampicillin against MRSA Biofilms, *ACS Med. Chem. Lett.* **2020**, *11*, 473–478.

[26] Hagras, M.; Abutaleb, N. S.; Ali, A. O.; Abdel-Aleem, J. A.; Elsebaei, M. M.; Seleem, M. N.; Mayhoub, A. S. Naphthylthiazoles: Targeting Multidrug-Resistant and Intracellular Staphylococcus aureus with Biofilm Disruption Activity. *ACS Infect. Dis.* **2018**, *4*, 1679–1691; (b) Sui, Y. F.; Ansari, M. F.; Zhou, C. H. Pyrimidinetrione-Imidazoles as A Unique Structural Type of Potential Agents towards Candida Albicans: Design, Synthesis and Biological Evaluation. *Chem. Asian J.* **2021**, 10.1002/asia.202100146R1.

[27] Guo, Y.; Bao, C. N.; Li, F.; Hou, E. H.; Qin, S. S.; Zhang, Q. R.; Liu, J. F. Discovery, Synthesis, and Biological Evaluation of Dunnianol-Based Mannich Bases against Methicillin-Resistant Staphylococcus aureus (MRSA), *ACS Infect. Dis.* **2020**, *6*, 2478−2489.

[28] Cui, S. F.; Addla, D.; Zhou, C. H. Novel 3-Aminothiazolquinolones: Design, Synthesis, Bioactive Evaluation, SARs, and Preliminary Antibacterial Mechanism. *J. Med. Chem.* **2016**, *59*, 4488–4510; (b) Chen, J. P.; Battini, N.; Ansari, M. F.; Zhou C. H. Membrane Active 7-Thiazoxime Quinolones as Novel DNA Binding Agents to Decrease the Genes Expression and Exert Potent Anti-Methicillin-Resistant Staphylococcus aureus Activity. *Eur. J. Med. Chem.* **2021**, *217*, 113340.

[29] Luther, A.; Urfer, M.; Zahn, M.; Muller, M.; Wang, S. Y.; Mondal, M.; Vitale, A., Hartmann, J. B., Sharpe, T., Monte, F. L., Kocherla, H.; Cline, E., Pessi, G.; Rath, P., Modaresi, S. M., Chiquet, P., Stiegeler, S.; Verbree, C., Remus, T., Schmitt, M., Kolopp, C., Westwood, M. A.; Desjonqueres, N., Brabet, E., Hell, S., LePoupon, K., Vermeulen, A., Jaisson, R., Rithie, V., Upert, G., Lederer, A.; Zbinden, P.; Wach, A.; Moehle, K.; Zerbe, K.; Locher, H. H.; Bernardini, F.; Dale, G. E.; Eberl, L.; Wollscheid, B.; Hiller,

S.; Robinson, J. A.; Obrecht, D. Chimeric Peptidomimetic Antibiotics against Gram-Negative Bacteria. *Nature* **2019**, *576*, 452–458.

[30] Rojas, E. R.; Billings, G.; Odermatt, P. D.; Auer, G. K.; Zhu, L.; Miguel, A.; Chang, F.; Weibel, D. B.; Theriot, J. A.; Huang, K. C. The Outer Membrane is an Essential Load-Bearing Element in Gram-Negative Bacteria. *Nature,* **2018**, *559*, 617–621.

[31] Sun, B.; Dong, Y.; An, Y. F.; Liu, M.; Han, J.; Zhao, L. Y.; Liu, X. Y. Design, Synthesis and Bioactivity Evaluation of Novel Arylalkene-Amide Derivatives as Dual-Target Antifungal Inhibitors. *Eur. J. Med. Chem.* **2020**, *205,* 112645.

[32] Shounak, R.; Anupam, M.; Varnika, Y.; Ankita, S.; Ruptanu, B.; Pallab, S.; Amit, J. Mechanistic Insight into the Antibacterial Activity of Chitosan Exfoliated MOS_2 Nanosheets: Membrane Damage, Metabolic Inactivation, and Oxidative Stress. *ACS Appl. Bio. Mater*. **2019**, *2,* 2738−2755.

[33] Zhang, E.; Bai, P. Y.; Cui, D. Y.; Chu, W. C.; Hua, Y. G.; Liu, Q.; Yin, H. Y.; Zhang, Y. J.; Qin, S.; Liu, H. M. Synthesis and Bioactivities Study of New Antibacterial Peptide Mimics: The Dialkyl Cationic Amphiphiles. *Eur. J. Med. Chem.* **2018**, *143*, 1489–1509.

[34] Chen, L.; Gong, M. W.; Peng, Z. F.; Zhou, T.; Ying, M. G.; Zheng, Q. H.; Liu, Q. Y.; Zhang, Q. Q. The Marine Fungal Metabolite, Dicitrinone B, Induces A375 Cell Apoptosis through the ROS-Related Caspase Pathway. *Mar. Drugs* **2014**, *12*, 1939–1958; (b) Wang, J.; Battini, N.; Ansari, M. F.; Zhou, C. H. Synthesis and Biological Evaluation of Quinazolonethiazoles as New Potential Conquerors towards Pseudomonas aeruginosa. *Chin. J. Chem.* **2020**, *39*, http://dx.doi.org/10.1002/cjoc.202000627.

[35] Narva, S.; Chitti, S.; Bala, B. R.; Alvala, M.; Jain, N.; Kondapalli, V. G. C. S. Synthesis and Biological Evaluation of Pyrrolo[2,3-B]Pyridine Analogues as Antiproliferative Agents and Their Interaction with Calf Thymus DNA, *Eur. J. Med. Chem.* **2016**, *114*, 220–231.

[36] Sun, H.; Ansari, M. F.; Battini, N.; Bheemanaboina, R. R. Y.; Zhou, C. H. Novel Potential Artificial MRSA DNA Intercalators: Synthesis and Biological Evaluation of Berberine-Derived Thiazolidinediones. *Org. Chem. Front* **2019**, *6,* 319–334.

[37] Konai, M. M.; Ghosh, C.; Yarlagadda, V.; Samaddar, S.; Haldar, J. Membrane Active Phenylalanine Conjugated Lipophilic Norspermidine Derivatives with Selective Antibacterial Activity. *J. Med. Chem.* **2014**, *57*, 9409–9423.

[38] Gong, H. H.; Baathulaa, K.; Lv, J. S.; Cai, G. X.; Zhou, C. H. Synthesis and Biological Evaluation of Schiff Base-Linked Imidazolyl Naphthalimides as Novel Potential Anti-MRSA Agents. *Med. Chem. Comm.* **2016**, *7*, 924–931.

(The following will be filled in by the editorial staff)

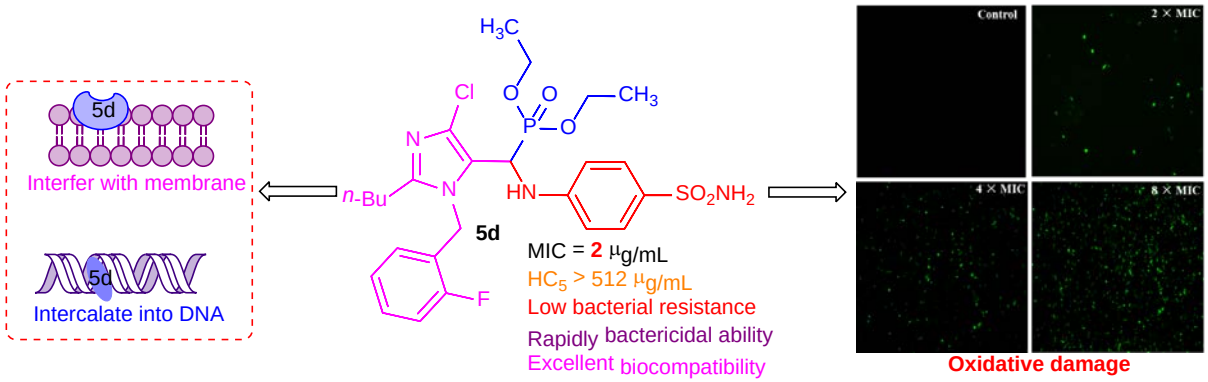
Manuscript received: XXXX, 2021
Manuscript revised: XXXX, 2021
Manuscript accepted: XXXX, 2021
Accepted manuscript online: XXXX, 2021
Version of record online: XXXX, 2021

Entry for the Table of Contents

Design and Synthesis of Sulfanilamide Aminophosphonates as Novel Antibacterial Agents towards *Escherichia Coli*

Juan Wang,[a] Mohammad Fawad Ansari,[a] Jian-Mei Lin,[*,b] and Cheng-He Zhou[*,a]

Chin. J. Chem. **2021**, *39*, XXX—XXX. **DOI: 10.1002/cjoc.202100XXX**



A class of unique sulfanilamide aminophosphonates as new regulators against microbes was synthesized by one-pot three-component reaction and their biological evaluations indicated that sulfanilamide aminophosphonates would shed light on developing novel potential antibacterial agents to combat with microbial infection.

