Journal Pre-proofs

Design and biological evaluation of a novel type of potential multi-targeting antimicrobial sulfanilamide hybrids in combination of pyrimidine and azoles

Yan-Fei Sui, Di Li, Juan Wang, Rammohan R. Yadav Bheemanaboina, Mohammad Fawad Ansari, Lin-Ling Gan, Cheng-He Zhou

PII: S0960-894X(20)30042-1
DOI: https://doi.org/10.1016/j.bmcl.2020.126982
Reference: BMCL 126982

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 30 November 2019
Revised Date: 3 January 2020
Accepted Date: 17 January 2020

Please cite this article as: Sui, Y-F., Li, D., Wang, J., Yadav Bheemanaboina, R.R., Fawad Ansari, M., 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, *Bioorganic & Medicinal Chemistry Letters* (2020), doi: https://doi.org/10.1016/j.bmcl.2020.126982

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Design and biological evaluation of a novel type of potential multi-targeting antimicrobial sulfanilamide hybrids in combination of pyrimidine and azoles

Yan-Fei Sui,[a,§] Di Li,[a,§] Juan Wang,[a] Rammohan R. Yadav Bheemanaboina,[a,†] Mohammad Fawad Ansari,[a,‡] Lin-Ling Gan[b,**] and Cheng-He Zhou[a,*]

[a] *Institute of Bioorganic & Medicinal Chemistry, Key Laboratory of Applied Chemistry of Chongqing Municipality, School of Chemistry and Chemical Engineering, Southwest University, Chongqing, 400715, China*
[b] *Chongqing Engineering Research Center of Pharmaceutical Sciences, School of Pharmacy, Chongqing Medical University, Chongqing 401331, China*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Sulfanilamide
Pyrimidine
Azole
Antimicrobial
DNA
Mechanism

ABSTRACT

This work explored a novel type of potential multi-targeting antimicrobial three-component sulfanilamide hybrids in combination of pyrimidine and azoles. The hybridized target molecules were characterized by ^{1}H NMR, ^{13}C NMR and HRMS spectra. Some of the developed target compounds exerted promising antimicrobial activity in comparison with the reference drugs norfloxacin and fluconazole. Noticeably, sulfanilamide hybrid **5c** with pyrimidine and indole could effectively inhibit the growth of *E. faecalis* with MIC value of 1 μg/mL. The active molecule **5c** showed low cell toxicity and did not obviously trigger the development of resistance towards the tested bacteria strains. Mechanism exploration indicated that compound **5c** could not only exert efficient membrane permeability, but also intercalate into DNA of resistant *E. faecalis* to form **5c**-DNA supramolecular complex, which might be responsible for its antimicrobial action. The further investigation showed that this molecule could be effectively transported by human serum albumins through hydrogen bonds and *van der Waals* force.

Sulfonamides are the first class of artificial synthetic antibacterial agents, whose core skeleton is *para*-aminobenzene sulfonamide that can compete with dihydrofolate synthetase, hinder the synthesis of dihydrofolate, effectively prevent the synthesis of nucleic acids and proteins, and then inhibit the growth of various microorganisms. The hybridization of core antibacterial skeleton sulfanilamide with various types of aromatic heterocycles like six-membered pyrimidine and diazine as well as five-membered thiazole and oxazole produced a variety of sulfanilamide types of antibacterial drugs, such as sulfadiazine, sulfamethoxypyridazine, sulfamoxole and sulfathiazole. Especially, the hybrids of sulfanilamide and pyrimidine with simple substituents have achieved great success, and a large number of pyrimidine derivatives as clinical antibacterial drugs are being frequently employed in clinic for the treatment of bacterial infections (Figure 1).[1] However, this kind of antibacterial drugs were found to cause some allergic reactions and show high toxicity to liver, kidney and central nervous system of human body.[2] Moreover, their prevalent clinical use or abuse developed the resistance with a significant public threat to health. This highlighted our keen interest to develop new hybrids of sulfanilamide and pyrimidine as antimicrobial agents with lower toxicity, less bacterial resistance, higher safety and better activity.

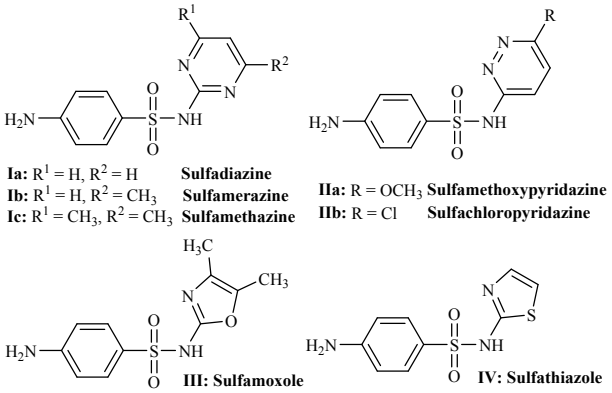


Figure 1. Sulfanilamide-aromatic heterocycle hybrids as clinical antimicrobial drugs.

Pyrimidine with a unique six-membered aromatic framework containing two nitrogen atoms is an important part in lots of biomolecules such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA),[3] and as a crucial block is prevalently employed in the design of new drug molecules. It has been extensively investigated towards pyrimidine in combination with a variety of functional groups and a lot of pyrimidine derived molecules such as antibacterial trimethoprim and brodimoprim[4]

** Corresponding author.
* Corresponding author.
E-mail addresses: ganlinling2012@163.com (L.-L. Gan), zhouch@swu.edu.cn (C.-H. Zhou).
† Postdoctoral researcher from CSIR-Indian Institute of Integrative Medicine (IIIM), Jammu, India.
‡ Postdoctoral researcher from Jamia Millia Islamia, New Delhi, India.
§ These authors contributed equally to this work.

as … acy marketed in currently treating microbial infections. More importantly, numerous pyrimidine derivatives were reported to be potential as promising antimicrobial agents.[7] Especially the combination of pyrimidine and heterocyclic azoles was revealed to result in strong bioactivity and high safety profile.[8]

Nitrogen-containing azole heterocycles and their analogs as antimicrobial agents[9] like metronidazole, ornidazole and fluconazole have been widely used in clinic to benefit human health. It has been well validated that nitrogen heterocyclic because this kind of heterocyclic fragment is able to make use of weak noncovalent interactions such as coordination bonds, hydrogen bonds, ion-dipole, cation-π, π-π stacking and hydrophobic effect as well as *van der Waals* force to bind with the enzymes and receptors in organisms by multiple sites and thus produce good biological potency.[10] However, so far novel three-component hybrids of sulfanilamide with pyrimidine and azole ring or its analogs have been rarely reported.

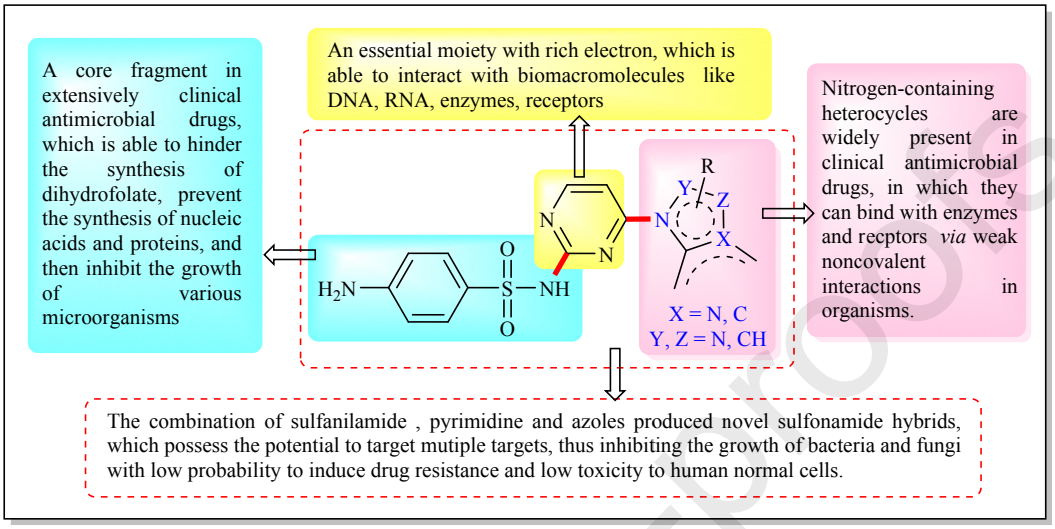
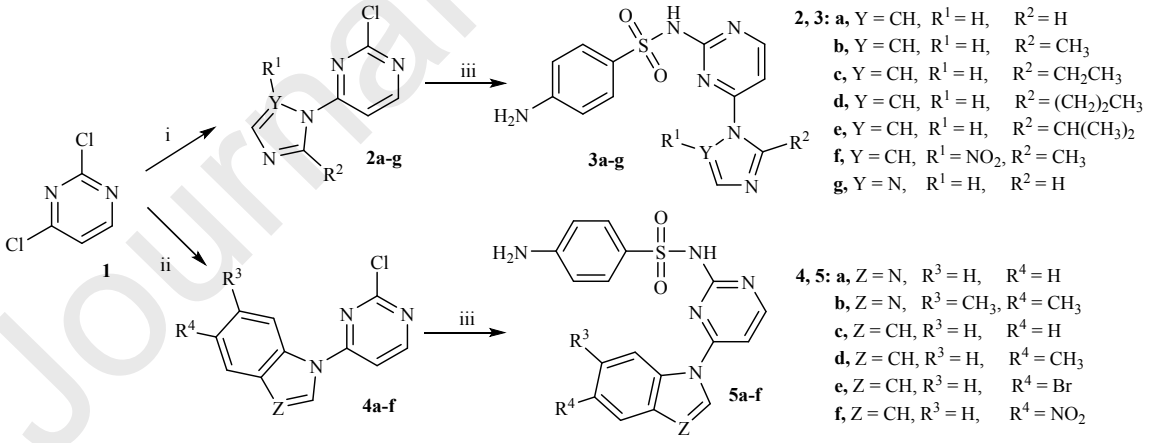


Figure 2. Design of novel three-component sulfanilamide hybrids with pyrimidine and azoles as potential multi-targeting antimicrobial agents.

In view of such stimulating overwhelming properties and as an extension of our studies towards novel antimicrobial agents, herein a new effort was explored towards a novel type of potential multi-targeting antimicrobial three-component sulfanilamide hybrids in combination of pyrimidine and azole ring or its analogs, the target azole derivatives and their analogs included imidazoles[11] and triazoles[12] as well as benzene-fused azoles like benzimidazoles[13] and their analog indoles[14] (Figure 2). These new structural compounds might exert multi-site bindings with biomolecules, regulate the interaction and affinity between small molecules and biomolecules, therefore the target molecules might be conducive to overcome increasingly severe drug resistance and broaden antimicrobial spectrum.



Scheme 1. Synthetic route of sulfanilamide derivatives **3** and **5**. Reagents and conditions: (i) imidazoles and triazole, potassium carbonate, acetonitrile, 80 °C; (ii) benzimidazoles and indoles, potassium carbonate, acetonitrile, 80 °C; (iii) *para*-aminobenzene sulfonamide, cesium carbonate, acetonitrile, 80 °C.

The desired target sulfanilamide hybrids were constructed *via* easy synthetic procedures from commercial *para*-aminobenzene sulfonamide, azoles or indoles and 2,4-dichloropyrimidine according to the preparative route in Scheme 1. The reaction of 2,4-dichloropyrimidine with azoles or indoles in the presence of potassium carbonate efficiently produced five-membered azoles **2a-g**, fused five-membered azoles and their analog indoles **4a-f** with good yields,[15] and then the coupling of intermediates **2a-g** and **4a-f** respectively with sulfanilamide in acetonitrile at 80 °C with cesium carbonate as base afforded the target three-component sulfanilamide hybrids **3a-g** and **5a-f** in moderate to good yields ranging from 43.8% to 75.1%.

The spectral analysis from ^{1}H NMR, ^{13}C NMR and HRMS data supported the structures of synthesized target compounds. All the protons of NH groups gave downfield singlets at 11.61−11.91 ppm. The chemical shifts of 3-H and 5-H in benzene ring appeared at 6.56–6.62 ppm, while the 2-H and 6-H protons in benzene ring showed downfield signals at 7.59–7.69

ppm five
The electronegativity of nitrogen atom at 1-position in pyrimidine resulted in larger chemical values at 8.47–8.68 ppm for the 6-position proton in comparison with the 5-position proton (6.76–7.38 ppm) in pyrimidine. In ^{13}C NMR spectra, the chemical shifts of carbons in benzene ring gave expected signals of 130.3–129.6 ppm and 113.2–112.8 ppm, which should be assigned to the 2,6-C and 3,5-C, respectively. The 4-C in the pyrimidine ring gave expected signals at 159.7–159.0 ppm. The other carbons ideally gave the signals at the expected regions.

All the synthesized hybrids **3a-g** and **5a-f** in combination of pyrimidine and azoles were evaluated for their antimicrobial activities *in vitro* by two folds serial dilution technique according to Clinical and Laboratory Standards Institute (CLSI) with the positive control of clinical drugs. Minimum inhibitory concentration (MIC, μg/mL) is defined as the lowest concentration of new compounds that completely inhibit the growth of bacteria and fungi. The tested strains including clinical Gram-positive bacteria, six Gram-negative bacteria and five fungi.

As depicted in Table 1, sulfanilamide derivatives **3a-g** and **5a-f** showed inhibitory efficiency against some tested bacterial strains to some extent, while indolyl sulfanilamide **5c** displayed the most prominent efficiency. Imidazolyl compounds **3a-f** could not effectively inhibit the growth of all the tested bacteria, except for 2-propyl-imidazolyl derivative **3d**, which gave good activities with MIC value of 8 μg/mL against *S. aureus* and *S. aureus* ATCC 29213, and this might be beneficial from propyl group on the imidazole ring. The replacement of imidazolyl group in compound **3a** by triazolyl moiety yielded compound **3g**, resulting in decreased efficiency against most of the tested strains but increased activities towards *P. aeruginosa* (MIC = 4 μg/mL) and *P. aeruginosa* ATCC 27853 (MIC = 32 μg/mL), which might be attributed to the influence of nitrogen atom in triazolyl ring.

Table 1. The obtained antibacterial data as MIC (μg/mL) *in vitro* for sulfanilamide derivatives **3-5** [a,b,c]

Compds	Gram-positive bacteria					Gram-negative bacteria					
	MRSA	*S. A.*	*S. A.* 25923	*S. A.* 29213	*E. F.*	*K. P.*	*E.C.*	*E. C.* 25922	*P. A.*	*P. A* 27853	*A. B.*
3a	32	128	256	128	128	256	256	256	128	256	64
3b	128	256	256	256	128	512	128	256	128	256	256
3c	128	128	128	64	64	64	128	512	128	128	64
3d	16	8	64	8	64	64	16	128	128	128	32
3e	32	256	256	32	128	256	64	128	128	32	64
3f	64	128	128	128	128	64	128	256	256	128	64
3g	128	512	256	64	256	32	128	256	4	32	128
5a	8	256	512	256	256	256	128	256	512	256	512
5b	64	256	256	128	256	256	256	512	256	128	128
5c	2	32	4	8	1	32	32	32	8	1	16
5d	4	16	4	16	8	8	8	32	4	4	64
5e	4	64	2	16	4	32	4	2	4	4	128
5f	16	32	4	64	8	32	8	128	16	8	128
A	8	0.5	1	2	4	4	16	8	2	0.5	8

[a] Minimal inhibitory concentrations (MIC, μg/mL) were determined by micro broth dilution method for microdilution plates.

[b] MRSA, *methicillin-resistant Staphylococcus aureus*; *S. A., Staphylococcus aureus*; *S. A.* 25923, *Staphylococcus aureus* ATCC 25923; *S. A.* 29213, *Staphylococcus aureus* ATCC 29213; *E. F.*, *Enterococcus faecalis*; *K. P.*, *Klebsiella pneumonia*; *E. C.*, *Escherichia coli*; *E. C.* 25922, *Escherichia coli* ATCC 25922; *P. A.*, *Pseudomonas aeruginosa*; *P. A.* 27853, *Pseudomonas aeruginosa* ATCC 27853; *A. B.*, *Acinetobacter baumanii*.

[c] **A** = Norfloxacin.

It was shown that the benzimidazolyl sulfanilamide hybrids **5a-b** gave relatively lower inhibitory potencies with MIC values ranging from 64 to 512 μg/mL, except for towards MRSA strains. Benzimidazolyl sulfanilamide hybrid **5a** exhibited obvious suppression to the growth of MRSA with MIC value of 8 μg/mL, being more active than 5,6-dimethyl-benzimidazolyl derivative **5b**, which indicated that the methyl substitution might be unfavorable for the antibacterial activity.

In comparison with benzimidazolyl sulfanilamide derivatives **5a-b**, most of benzimidazolyl analogous indolyl derivatives **5c-f** showed relatively better activities in inhibiting the growth of the test bacterial strains and gave broader antibacterial spectrum. It was noticed that indolyl derivative **5c** showed apparent inhibition with the same MIC value of 1 μg/mL towards *E. faecalis* and *P. aeruginosa* ATCC 27853, which was superior or comparable to standard drug norfloxacin. In comparison to imidazolyl and benzimidazolyl sulfanilamides **3a** and **5a**, indolyl derivative **5c** gave more efficient potency against all the tested bacteria strains, manifesting that the indole ring played a crucial role in improving antibacterial activity.

Moreover, MRSA was more sensitive to compounds **5c-e** than norfloxacin (MIC = 8 μg/mL) with MIC values ranging from 2 to 4 μg/mL. Methylindolyl derivative **5d** gave good activities against MRSA, *S. aureus* ATCC 25923, *P. aeruginosa* and *P. aeruginosa* ATCC 27853 with MIC value of 4 μg/mL. Bromoindolyl derivative **5e** displayed relatively good inhibitory potency (MIC = 2 μg/mL) against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. The displacement of bromoindolyl moiety by nitroindolyl group would afford compound **5f** with good inhibitory efficiency (MIC = 4 μg/mL) against *S. aureus* ATCC 25923. Noticeably, Gram-negative *A. baumanii* was insensitive to sulfanilamide derivatives **3a-g** and **5a-f** with MIC values of 32–512 μg/mL, which showed that these substituent groups in

pyri ...fore, *baumanii* potentiality.

Antifungal assay revealed that the sulfanilamide derivatives **3a-g** and **5a-f** showed weak to good antifungal activities *in vitro* against the tested fungi (Table S1). It was noticed that the antifungal abilities of these compounds displayed similar potency to their antibacterial efficiencies. Towards *C. albicans* ATCC 90023, 2-propylimidazolyl derivative **3d** (MIC = 4 μg/mL) was found to be more efficient than other imidazolyl compounds (MICs = 16─128 μg/mL). In addition, the triazolyl derivative **3g** exerted good antifungal efficacy with MIC value of 8 μg/mL against *C. albicans*. It was found that benzimidazolyl derivatives **5a-b** exhibited weak inhibitory activities against the tested fungi. Among the indolyl derivatives **5c-f**, the low MIC values of 2–8 μg/mL were observed towards *C. albicans*, *C. tropical* and *A. fumigatus*, which suggested that these compounds should be they might be potential to become new promising antifungal agents through deep investigation.

In order to identify the safety profile, the highly active indolyl pyrimidine hybridized sulfanilamide derivative **5c** was further evaluated for toxicities against lung cancer cell (A549) and human normal lung epithelium cells (BEAS-2B) with doxorubicin (0.5 μM) as a positive standard control using the colorimetric cell proliferation MTT assay. The results of these assays are depicted in Figure 3. The cell viability of hybrid **5c** towards these two cell lines was at least 70% after being incubated at the concentration of 64 μg/mL for 24 h, which manifested that compound **5c** displayed relatively low toxicity to A549 and BEAS-2B cell lines compared to standard drug doxorubicin.

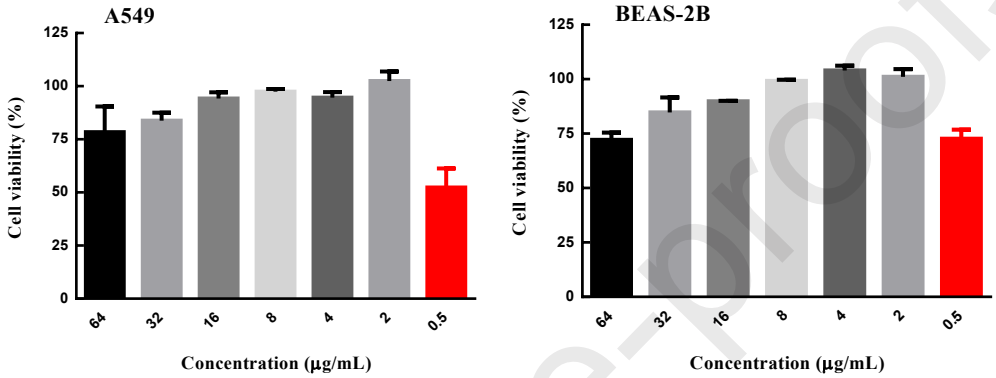


Figure 3. Cell viability of lung cancer cells (A549) and human normal lung epithelium cells (BEAS-2B) incubated with the active compound **5c** at different concentrations.

It is important for potentially antimicrobial agents that the development of resistance to their action is not readily attained.[16] To evaluate the probability of these molecules to elicit resistance in bacteria, the drug resistance study of indolyl pyrimidine hybridized sulfanilamide derivative **5c** against *E. faecalis* was carried out. As shown in Figure 4, the susceptibility of *E. faecalis* bacteria to indolyl derivative **5c** remained nearly unchanged even after 16 passages. By contrast, the drug resistance developing ability of *E. faecalis* against norfloxacin was obvious, indicating that sulfanilamide hybrid **5c** with pyrimidine and indole did not significantly trigger the development of drug resistance as easily as the reference drug norfloxacin.

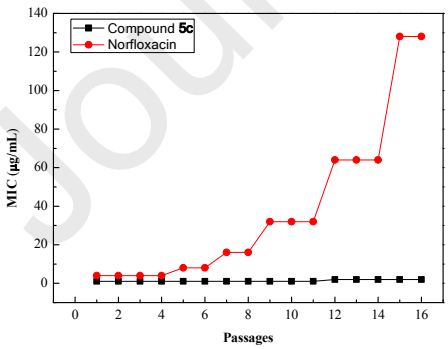


Figure 4. Development of *E. faecalis* resistance to compound **5c** and norfloxacin.

In order to study the bactericidal action of indolyl pyrimidine hybridized sulfanilamide derivative **5c**, a time-kill kinetics assay was performed against *E. faecalis* at different concentrations (MIC and 6 × MIC). As shown in Figure S1, there was more than 3 log (CFU/mL) reduction in the number of viable bacteria at concentrations of MIC and 6 × MIC against *E. faecalis*. These results clearly revealed that the active hybrid **5c** was capable of killing Gram-positive bacteria *E. faecalis* rapidly.

DNA-gyrase (PDB code: 2XCS) as an attractive target in antibacterial drug exploration was applied to be a flexible ligand-receptor docking investigation to further rationalize the observed antibacterial activity of indolyl derivative **5c**. The molecular simulation results (Figure 5) showed that nitrogen atom at 3-position in the pyrimidine ring and the hydrogen atom of the NH_2 group at the 4-position on benzene formed two hydrogen bonds with DC11 and Asp1083B residues, respectively. The value of binding energy is −6.9 kcal/mol. These non-covalent binding might be conducive to stabilize the **5c**-enzyme complex, which might contribute to the superior inhibitory efficacy.

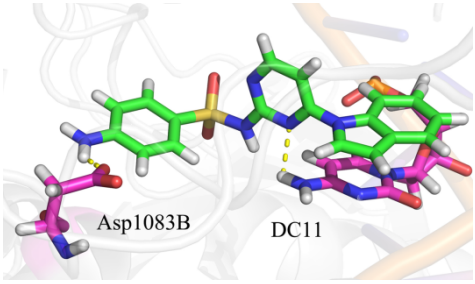


Figure 5. Supramolecular binding of compound **5c** docked in the bacterial DNA-gyrase (PDB code: 2XCS).

Bacteria with biofilms are inherently insensitive to antimicrobial agents and they are much more resistant to conventional antibiotic treatment.[17] Permeabilization of the bacterial membrane of bacteria *E. faecalis* was carried out with propidium iodide (PI) as fluorescent indicator which can only pass through the membrane of compromised cells and then fluoresce red after binding with double-stranded DNA.

Fluc NR

treatment of cells with active molecule **5c**. The results of bacterial membrane permeabilization are shown in Figure 6. Obvious fluorescence enhancements at 617 nm of the mixtures of indolyl pyrimidine hybridized sulfanilamide derivative **5c** with PI-treated *E. faecalis* appeared and became steady after 60 min, which demonstrated that sulfanilamide hybrid **5c** with pyrimidine and indole could permeate the membranes of *E. faecalis* strains efficiently.

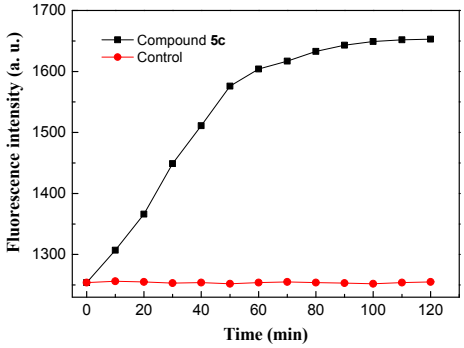


Figure 6. Bacterial membrane permeabilization of compound **5c** (12 × MIC) against *E. faecalis*.

The detection of hyperchromism and hypochromism by absorption spectroscopy, which are critical spectral features to distinguish the change of DNA double helical structure, is one of the most important and useful method in DNA binding studies. The observed strong hyperchromism might be attributed to the denaturation of DNA caused by the strong interaction between the electronic states of intercalating chromophore and that of the DNA base. As shown in Figure S2, the UV-vis spectra revealed that the maximum absorption peak of DNA at 260 nm showed proportional increase and slight blue shift with the increasing concentration of hybrid **5c**. Remarkably, the absorption values of the **5c**-DNA complex were lower than that of sum of free DNA and free molecule **5c**. This hyperchromism effect might be attributed to the formed binary complexes between **5c** and DNA bases. In general, the observed spectral properties could be explained by the potent interaction of indolyl derivative **5c** with *E. faecalis* DNA (Figure S3).

To further elucidate the supramolecular interaction between hybrid **5c** and *E. faecalis* DNA, neutral red (NR, a planar phenazine dye with a confirmed intercalative binding mode with DNA) was employed as a spectral probe due to its lower toxicity and higher stability. Therefore, NR was chosen to investigate the binding mode of compound **5c** with DNA in present work, and the absorption spectra of the NR dye after the addition of DNA were shown in Figure S4.[18] The absorption peak of NR at around 460 nm gradually decreased with the increasing concentration of DNA, and a new band at around 530 nm appeared, which could be attributed to the formation of the new DNA-NR complex. The isosbestic point at 500 nm provided the evidence for the formation of DNA-NR complex.

The absorption spectra of a competitive binding between NR and **5c** with DNA in Figure S5 showed that with the increasing concentration of hybrid **5c**, the maximum absorption around 530 nm of the DNA-NR complex decreased, but a slight intensity increase was observed in the developing band around 460 nm. In comparison with the absorption band of the free NR in the presence of the increasing concentrations of DNA (Figure S4), the spectra in the inset of Figure S5 exhibited the reverse process. The apparent changes of spectra suggested that indolyl pyrimidine hybridized sulfanilamide derivative **5c** could in the DNA-NR complex.

Many promising new drugs have been rendered ineffective because of their unusually high affinity to the protein human serum albumins (HSA), which is the principal extracellular protein of the circulatory system. As a result, the investigations of interactions between drugs or bioactive small molecules and HSA are not only beneficial to provide a proper understanding of the absorption, transportation, distribution, metabolism and excretion properties of drugs, but also significant to design, modify and screen drug molecules.[19]

The binding mode between HSA and the highly active molecule **5c** was investigated with UV-vis spectroscopy on the molecular level. In the binding experiment (Figure S6), the aromatic rings in Tryptophan (Trp-214), Tyrosine (Tyr-411) and Phenylalanine (Phe) residues in HSA gave absorption peak at 278 nm and the peak intensity increased with the addition of hybrid **5c**, which indicated the supramolecular interaction of indolyl pyrimidine hybridized sulfanilamide derivative **5c** with HSA and the extension of HSA peptide strands (Figure S6).

The unchanged maximum absorption wavelength implied that there was a non-covalent interaction between indolyl derivative **5c** and HSA. The fluorescence intensity of Trp-214 may change when HSA interacts with other small molecules, which could be reflected in the fluorescence spectra of HSA in the UV region. The different mechanisms of quenching are usually classified as either dynamic quenching or static quenching, which can be distinguished by their different dependence on temperature (Figure S7, Table S2).

The Modified Stern-Volmer plots were showed in Figure S8 and the calculated results were depicted in Table S3. As shown in Figure S9 and Table S3, with the increase of temperature, the diffusion coefficient increased while the stability of the **5c**-HSA complex reduced. The results suggested that there was a single class of binding sites on HSA for sulfanilamide hybrid **5c**. In addition, the thermodynamic parameters were obtained in Table S4. Both hydrophobic interactions and hydrogen bonds played vital roles in the binding of molecule **5c** to HSA and seemed to contribute to the stability of the **5c**-HSA complex.[20]

In conclusion, this work employed a facile synthetic procedure to develop a novel type of three-component sulfanilamide hybrids with pyrimidine and azoles. Some prepared new sulfanilamide derivatives could significantly inhibit the growth of the tested strains with equipotent or superior activities to the current clinical drugs norfloxacin and fluconazole. Notably, the indolyl pyrimidine hybridized sulfanilamide derivative **5c** not only showed superior inhibitory behaviors against *E. faecalis* (MIC = 1 μg/mL) to norfloxacin, but also exhibited low toxicity towards A549 and BEAS-2B cell lines and no obvious propensity to trigger development of resistance towards *E. faecalis*. Moreover, the active sulfanilamide hybrid **5c** was also membrane active and could kill bacteria in short time. The specific interaction of indoyl derivative **5c** with *E. faecalis* DNA displayed that it could intercalate into DNA to form **5c**-DNA complex which might further block DNA replication to exert their powerful antimicrobial activities. Binding investigations revealed that compound **5c** was able to be carried by HSA, which could generate fluorescent quenching by the active hybrid **5c** as a result of the formation of ground-state, and the calculated parameters indicated that the binding process was spontaneous. These results revealed that indolyl pyrimidine hybridized sulfanilamide derivative **5c** could be regarded as a promising lead compound for the further development as potential

Acknowledgments

This work was partially supported by National Natural Science Foundation of China (Nos. 21672173, 21971212), China Postdoctoral Science Foundation (2019M653821XB, 2018M633313), Chongqing Special Foundation for Postdoctoral Research Proposal (Xm2016039, XmT2018082), Natural Science Foundation Project of the Chongqing Science, Chongqing Natural Science Foundation Postdoctoral Science Foundation Project (cstc2019jcyj-bshX0124) and Technology Committee (cstc2019jcyj-msxmX0236) and Program of Chongqing Medicinal and Pharmaceutical College (YGZ 2019102).

References

1. (a) Brown ED, Wright GD. Antibacterial drug discovery in the resistance era. *Nature.* 2016; 529: 336–343. (b) He SC, Ponmani J, Avula SR, Wang XL, Zhang HZ, Zhou CH. Recent advance in sulfomide-based medicinal chemistry. *Sci Sin Chem.* 2016; 46(9): 823–847. (c) Hu YY, Bheemanaboina RRY, Battini N, Zhou CH. Sulfonamide-derived four-component molecular hybrids as novel DNA-targeting membrane active potentiators against clinical *Escherichia coli*. *Mol Pharm.* 2019; 16: 1036–1052.
2. Li H, Song HL, Xu H, Lu Y, Zhang S, Yang YL, Yang XL, Lu YX. Effect of the coexposure of sulfadiazine, ciprofloxacin and zinc on the fate of antibiotic resistance genes, bacterial communities and functions in three-dimensional biofilm-electrode reactors. *Bioresour Technol.* 2019; 296: 122290.
3. (a) Liu HB, Gao WW, Tangadanchu VKR, Zhou CH, Geng RX. Novel aminopyrimidinyl benzimidazoles as potentially antimicrobial agents: Design, synthesis and biological evaluation. *Bioorg Med Chem.* 2018; 26: 3707–3715. (b) Fang XF, Li D, Tangadanchu VKR, Gopala L, Gao WW, Zhou CH. Novel potentially antifungal hybrids of 5-flucytosine and fluconazole: Design, synthesis and bioactive evaluation. *Bioorg Med Chem Lett.* 2017; 27: 4964–4969.
4. Jain KS, Arya N, Inamdar NN, Auti PB, Unawane SA, Puranik HH, Sanap MS, Inamke AD, Mahale VJ, Prajapati CS, Shishoo CJ. The chemistry and bio-medicinal significance of pyrimidines & condensed pyrimidines. *Curr Top Med Chem.* 2016; 16: 3133–3174.
5. Mourad A, Stiber JA, Perfect JR, Johnson MD. Real-world implications of QT prolongation in patients receiving voriconazole and amiodarone. *J Antimicrob Chemother.* 2019; 74(1): 228–233.
6. Tang R, Tang T, Tang G, Liang Y, W WC, Yang JL, Niu JF; Tang JY, Zhou ZY, Cao YS. Pyrimethanil ionic liquids paired with various natural organic acid anions for reducing its adverse impacts on the environment. *J Agr Food Chem.* 2019; 67(40): 11018–11024.
7. Hagras M, Abutaleb NS, Ali AO, Abdel-Aleem JA, Elsebaei MM, Seleem MN, Mayhoub AS. Naphthylthiazoles: targeting multidrug-resistant and intracellular *Staphylococcus aureus* with biofilm disruption activity. *ACS Infect Dis.* 2018; 4: 1679–1691.
8. (a) Liu HB, Gao WW, Tangadanchu VKR, Zhou CH, Geng RX. Novel aminopyrimidinyl benzimidazoles as potentially antimicrobial agents: Design, synthesis and biological evaluation. *Eur J Med Chem.* 2018; 143: 66−84. (b) Li D, Bheemanaboina RRY, Battini N, Tangadanchu VKR, Fang XF, Zhou CH. Novel organophosphorus aminopyrimidines as unique structural DNA-targeting membrane active inhibitors towards drug-resistant methicillinresistant *Staphylococcus aureus*. *Med Chem Commun.* 2018; 9: 1529–1537.
9. (a) Zhang L, Peng XM, Damu GLV, Geng RX, Zhou CH. Comprehensive review in current developments of imidazole-based medicinal chemistry. *Med Res Rev.* 2014; 34(2): 340−437. (b) Peng XM, Cai GX, Zhou CH. Recent developments in azole compounds as antibacterial and antifungal agents. *Curr Top Med Chem.* 2013; 13(16): 1963−2010. (c) Zhang HZ, Damu GLV, Cai GX, Zhou CH. Current developments in the syntheses of 1,2,4-triazole compounds. *Curr Org Chem.* 2014; 18(3): 359−406. (d) Zhang HZ, Gan LL, Wang H, Zhou CH. New progress in azole compounds as antimicrobial agents. *Mini-Rev Med Chem*. 2017; 17(2): 122–166.
10. (a) Gong HH, Addla D, Lv JS, Zhou CH. Synthesis and biological evaluation of berberine-benzimidazole hybrids as new type of potentially DNA-targeting antimicrobial agents. *Eur J Med Chem.* 2016; 122: 205–215. (b) Chen YY, Gopala L, Bheemanaboina RRY, Liu HB, Cheng Y, Geng RX, Zhou CH. Novel naphthalimide aminothiazoles as potential multitargeting antimicrobial agents. *ACS Med Chem Lett.* 2017; 8: 1331–1335; (c) Wang LL, Battini N, Bheemanaboina RRY, Ansari MF, Chen JP, Xie YP, Cai GX, Zhang SL, Zhou CH. 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. (d) Gong HH, Addla D, Lv JS, Zhou CH. Heterocyclic naphthalimides as new skeleton structure of compounds with increasingly expanding relational medicinal applications. *Curr Top Med Chem.* 2016; 16: 3303–3364.
11. (a) Walton T, Zhang W, Li L, Tam CP, Szostak, JW. The mechanism of nonenzymatic template copying with imidazole-activated nucleotides. *Angew Chem Int Ed.* 2019; 58(32): 10812–10819 (b) Li ZZ, Maddili SK, Tangadanchu VKR, Bheemanaboina RRY, Lin JM, Yang RG, Cai gx, Zhou CH. Progress in medicinal chemistry of nitroimidazole heterocycle. *Sci China Chem.* 2019; 49(2): 230–255. (c) Li ZZ, Tangadanchu VKR, Battini N, Bheemanaboina RRY, Zang ZL, Zhang SL, Zhou CH. Indole-nitroimidazole conjugates as efficient manipulators to decrease the genes expression of methicillin-resistant *Staphylococcus aureus.* *Eur J Med Chem.* 2019; 179: 723–735. (d) Li ZZ, Gopala L, Tangadanchu VKR, Gao WW, Zhou CH. Discovery of novel nitroimidazole enols as *Pseudomonas aeruginosa* DNA cleavage agents. *Bioorg Med Chem.* 2017; 25: 6511–6522.
12. (a) Zhang Y, Tangadanchu VKR, Bheemanaboina RRY, Cheng Y, Zhou CH. Novel carbazole-triazole conjugates as DNA-targeting membrane active potentiators against clinical isolated fungi. *Eur J Med Chem.* 2018; 155: 579–589. (b) Zhang Y, Damu GLV, Cui SF, Mi JL, Tangadanchu VKR, Zhou CH. Discovery of potential antifungal triazoles: design, synthesis, biological evaluation, and preliminary antifungal mechanism exploration. *Med Chem Commun.* 2017; 8: 1631–1639. (c) Duan JR, Liu HB, Jeyakkumar P, Gopala L, Li S, Geng RX, Zhou CH. Design, synthesis and biological evaluation of novel Schiff base-bridged tetrahydroproto berberine triazoles as a new type of potential antimicrobial agents. *Med Chem Commun.* 2017; 8: 907–916. (d) Cheng Y, Avula SR, Gao WW, Addla D, Tangadanchu VKR, Zhang L, Lin JM, Zhou CH. Multi-targeting exploration of new 2-aminothiazolyl quinolones: Synthesis, antimicrobial evaluation, interaction with DNA, combination with topoisomerase IV and penetrability into cells. *Eur J Med Chem.* 2016; 124: 935–945.
13. (a) Li ZY, Dong JN, Wang JW, Yang DY, Weng ZQ. Elemental sulfur-promoted one-pot synthesis of 2-(2,2,2-trifluoroethyl)benzoxazoles and their derivatives. *Chem Commun.* 2019; 55(87): 13132–13135. (b) Zhang L, Addla D, Ponmani J, Wang A, Xie D, Wang YN, Zhang SL, Geng RX, Cai GX, Li S, Zhou CH. Discovery of membrane active benzimidazole quinolones-based topoisomerase inhibitors as potential DNA-binding antimicrobial agents. *Eur J Med Chem.* 2016; 111: 160–182. (c) Wang YN, Bheemanaboina RRY, Cai GX, Zhou CH. Novel purine benzimidazoles as antimicrobial agents by regulating ROS generation and targeting clinically resistant *Staphylococcus aureus* DNA groove. *Bioorg Med Chem Lett.* 2018; 28: 1621–1628. (d) Jeyakkumar P, Liu HB, Gopala L, Cheng Y, Peng XM, Geng RX, Zhou CH. Novel benzimidazolyl tetrahydroprotoberberines: Design, synthesis, antimicrobial evaluation and multi-targeting exploration. *Bioorg Med Chem Lett.* 2017; 27: 1737–1743.
14. (a) Veeraveedu P Thanikachalam, Maurya RK, Garg V, Monga V. An insight into the medicinal perspective of synthetic analogs of indole: A review. *Eur J Med Chem.* 2019; 180: 562–612. (b) Aggarwal T, Sushmita, Verma A K. Recent advances in the synthesis of carbazoles from indoles. *Org Biomol Chem.* 2019; 17(36): 8330–8342.
15. Xiang JB, Leung C, Zhang ZQ, Hu C, Geng D, Liu LL, Yi L, Li ZW, Berenson J, Bai X. Synthesis and evaluation of 2-alkylthio-4-(N-substituted sulfonamide)pyrimidine hydroxamic acids as anti-myeloma agents. *Chem Biol Drug Des.* 2016; 87: 472–477.
16. (a) Cui SF, Addla D, Zhou CH. Novel 3-aminothiazolquinolones: design, synthesis, bioactive evaluation, SARs, and preliminary antibacterial mechanism. *J Med Chem.* 2016; 59: 4488–4510. (b) Gao WW, Gopala L, Bheemanaboina RRY, Zhang GB, Li S, Zhou

effectively antibacterial agents toward clinically drug-resistant Gram-negative *Acinetobacter baumanii*. *Eur J Med Chem.* 2018; 146: 15–37. (c) Wang LL, Battini N, Bheemanaboina RRY, Zhang SL, Zhou CH. Design and synthesis of aminothiazolyl norfloxacin analogues as potential antimicrobial agents and their biological evaluation. *Eur J Med Chem.* 2019; 167: 105–123. (d) Maddili SK, Li ZZ, Kannekanti VK, Bheemanaboina RRY, Tuniki B, Tangadanchu VKR, Zhou CH. Azoalkyl ether imidazo[2,1-*b*]benzothiazoles as potentially antimicrobial agents with novel structural skeleton. *Bioorg Med Chem Lett.* 2018; 28: 2426–2431.

17. (a) Benoit DSW, Sims KRJ, Fraser D. Nanoparticles for oral biofilm treatments. *ACS Nano.* 2019; 13(5): 4869–4875. (b) Sun H, Ansari MF, Battini N, Bheemanaboina RRY, Zhou CH. Novel potential artificial mrsa DNA intercalators: Synthesis and biological evaluation of berberine-derived thiazolidinediones. *Org Chem Front.* 2019; 6: 319–334. (c) Wang YN, Bheemanaboina RRY, Gao WW, Kang J, Cai GX, Zhou CH. Discovery of benzimidazole–quinolone hybrids as new cleaving agents toward drug-resistant *Pseudomonas aeruginosa* DNA. *ChemMedChem.* 2018; 13: 1004–1017. (d) Gao WW, Zhou CH. Antimicrobial 2-aminothiazolyl quinolones: what is their potential in the clinic? *Future Med Chem.* 2017; 9(13): 1461–1464.
18. (a) Zhang GB, Maddili SK, Tangadanchu VKR, Gopala L, Gao WW, Cai GX, Zhou CH. Discovery of natural berberine-derived nitroimidazoles as potentially multi-targeting agents against drug-resistant *Escherichia coli*. *Sci China Chem.* 2018; 61(5): 557–568. (b) Kang J, Gopala L, Tangadanchu VKR, Gao WW, Zhou CH. Novel naphthalimide nitroimidazoles as multitargeting antibacterial agents against resistant *Acinetobacter baumannii*. *Future Med Chem.* 2018; 10(7): 711–724. (c) Zhang Y, Tangadanchu VKR, Cheng Y, Yang RG, Lin JM, Zhou CH. Potential antimicrobial isopropanol-conjugated carbazole azoles as dual targeting inhibitors of *Enterococcus faecalis*. *ACS Med Chem Lett.* 2018; 9: 244–249. (d) Cao QC, Wang H, Tangadanchu VKR, Gopala L, Cai GX, Zhou CH. Design, synthesis, antimicrobial activity and targeted DNA study of quinazolone imidazoles. *Sci China Chem.* 2017; 47(7): 844–858. (e) Liu HB, Gopala L, Avula SR, Jeyakkumar P, Peng XM, Zhou CH, Geng RX. Chalcone-benzotriazole conjugates as new potential antimicrobial agents: design, synthesis, biological evaluation and synergism with clinical drugs. *Chin J Chem.* 2017; 35: 483–496.
19. (a) Yin BT, Yan CY, Peng XM, Zhang SL, Rasheed S, Geng RX, Zhou CH. Synthesis and biological evaluation of α-triazolyl chalcones as a new type of potential antimicrobial agents and their interaction with calf thymus DNA and human serum albumin. *Eur J Med Chem.* 2014; 71: 148–159. (b) Gao WW, Rasheed S, Tangadanchu VKR, Sun Y, Peng XM, Cheng Y, Zhang FX, Lin JM, Zhou CH. Design, synthesis and biological evaluation of amino organophosphorus imidazoles as a new type of potential antimicrobial agents. *Sci China Chem.* 2017; 6: 769–785. (c) Kang J, Tangadanchu VKR, Gopala L, Gao WW, Cheng Y, Liu HB, Geng RX, Li S, Zhou CH. Novel potentially antibacterial naphthalimide-derived metronidazoles: Design, synthesis, biological evaluation and supramolecular interactions with DNA, human serum albumin and topoisomerase II. *Chin Chem Lett.* 2017; 28: 1369–1374.
20. (a) Zhou CH, Sui YF. Supermolecules as medicinal drugs. In: Liu Y, Chen Y, Zhang HY, eds. Handbook of macrocyclic supramolecular assembly. *Springer.* Singapore; 2019. https://doi.org/10.1007/978-981-13-1744-6_66-1. (b) Zhang HZ, He SC, Peng YJ, Zhang HJ, Gopala L, Tangadanchu VKR, Gan LL, Zhou CH. Design, synthesis and antimicrobial evaluation of novel benzimidazole-incorporated sulfonamide analogues. *Eur J Med Chem.* 2017; 136: 165–183. (c) Zhang HZ, Jeyakkumar P, Kumar KV, Zhou CH. Synthesis of novel sulfonamide azoles *via* C–N cleavage of sulfonamides by azole ring and relational antimicrobial study. *New J Chem.* 2015; 39: 5776–5796. (d) Cheng Y, Wang H, Dinesh A, Zhou CH. Current researches and applications of azole-based supermolecules as medicinal agents. *Chin J Org Chem.* 2016; 36, 1–42.

- Multi-targeting antimicrobial sulfanilamide hybrids in combination of pyrimidine and azoles were developed.
- Indolyl sulfanilamide derivative **5c** displayed higher potency against *E. faecalis* than norfloxacin.
- Hybrid **5c** exhibited low toxicity and no obvious propensity to trigger development of resistance.
- The active molecule **5c** could disturb the cell membrane, intercalate into *E. faecalis* DNA.
- Compound **5c** could be effectively transported by human serum albumins.

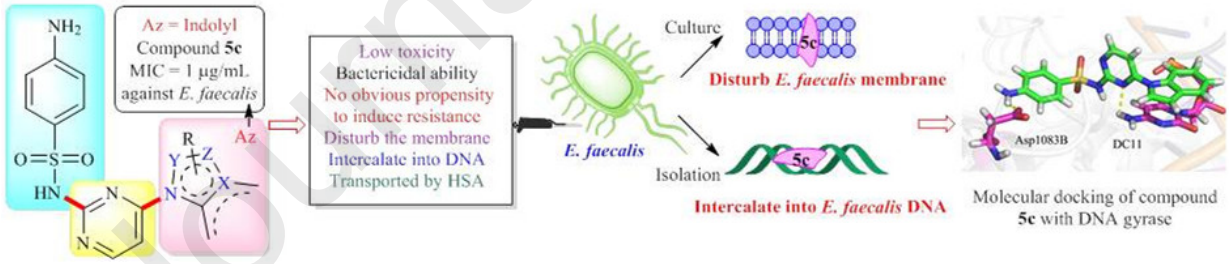
☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical Abstract

Design and biological evaluation of a novel type of potential multi-targeting antimicrobial sulfanilamide hybrids in combination of pyrimidine and azoles

Yan-Fei Sui, Di Li, Juan Wang, Rammohan R. Yadav Bheemanaboina, Mohammad Fawad Ansari, Lin-Ling Gan** and Cheng-He Zhou*



** Corresponding author.
* Corresponding author.
E-mail addresses: ganlinling2012@163.com (L.-L. Gan), zhouch@swu.edu.cn (C.-H. Zhou).
† Postdoctoral researcher from Jamia Millia Islamia, India.
§ These authors contributed equally to this work.