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DESIGN, SYNTHESIS AND ANTIBACTERIAL EVALUATION OF 1-[(1R,2S)-2-FLUOROCYCLOPROPYL] CIPROFLOXACIN-(4-METHYL-3-ARYL)-1,2,4-TRIAZOLE-5(4*H*)-THIONE HYBRIDS

Yun-He GENG,[a,b,1] Zeng-Quan WEI,[b] Zhi XU,[c] Lu-Xin NA,[b] Shu ZHANG,[d,*] Hui-Yuan GUO,[b] Ming-Liang LIU,[b] Lian-Shun FENG,[b] and Xue-Fu YOU [b,*]

[a] College of Chemistry and Material Science, Hebei Normal University, Shijiazhuang 050024, China
[b] Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China
[c] Huanghuai University, Zhumadian, P. R. China;
[d] Pony testing group (WH), Wuhan, P. R. China

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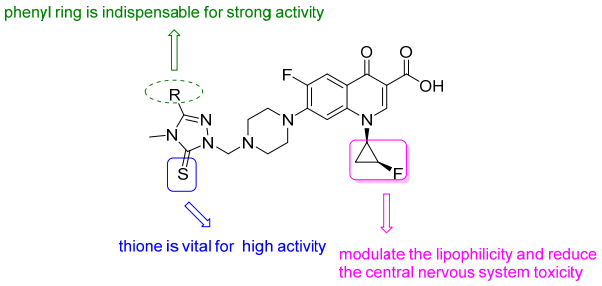
Fourteen novel 1-[(1R,2S)-2-Fluorocyclopropyl]ciprofloxacin-(4-methyl-3-aryl)-1,2,4-triazole-5(4*H*)-thione hybrids **6a-n** were designed, synthesized and assessed for their *in vitro* antibacterial activities against representative Gram-positive and Gram-negative bacteria. All hybrids **6a-n** exhibited promising antibacterial activity, especially against Gram-negative pathogens, warrant further investigation.



INTRODUCTION

Bacterial infections, caused by Gram-positive and -negative pathogens, are capable of leading various serious and even fatal diseases both in hospital and in the community.[1] Bacteria resistance to antibiotics which is associated with considerable mortality poses one of major threats to global public health.[2] Therefore, development of new antibiotics has become increasingly urgent.

Quinolones as the second widest used antibiotics, are mainstays of chemotherapy against various bacterial infections.[3,4] Besides their classical antibacterial activities, quinolone derivatives also endow with various atypical biological properties such as anti-malarial,[5,6] anti-tubercular,[7,8] anti-tumor[9,10] and anti-HIV activities,[11] and occupy an important position in drug development.

The structure-activity relationship (SAR) revealed that substituents at C-7 position influenced the antibacterial potency, spectrum, bioavailability, solubility, safety and pharmacokinetic profiles significantly, and this position is recognized as the most adaptable site for chemical modification.[1] Moreover, incorporation of large volume substituent at C-7 position of quinolone core is not a barrier to penetration, so various biological pharmacophores have been introduced into this position.[12]

Quinolone hybrids have the potential to provide novel drugs with a synergistic effect in terms of efficacy, lowered resistance selection propensity, activity against resistant bacteria, and reduced

* Corresponding authors: zhangshufsy@126.com or xuefuyou116@yahoo.cn

toxicity in comparison to a cocktail of the two drugs.[13,14] Great achievements have been obtained in searching quinolone hybrids as antibacterial agents in the past three decades, as evidenced by some of them which are exemplified by **Ro-23-9424** and **CBR-2092** are under pre-clinical or clinical evaluations for treatment of infections caused by various clinically relevant pathogens including fluoroquinolone-resistant, multidrug-resistant and difficult to treat bacteria.[1,2]

Plech *et al.* reported several series of ciprofloxacin-1,2,4-triazole-5(4*H*)-thione hybrids **1**, where R_1 is phenyl and R_2 is mainly phenyl or benzyl group, respectively, and some of the hybrids were more potent than the parent ciprofloxacin against the tested pathogens.[15-18] The SAR studies indicated that the substituents at C-3 position of 1,2,4-triazole-5(4*H*)-thione motif have great influence on the antibacterial activity, and phenyl ring is indispensable for the excellent antibacterial activity; For N-4 position of the 1,2,4-triazole-5(4*H*)-thione moiety, the length of alkyl substituent plays a pivotal role in the antibacterial activity, and shorter alkyl substituent preferred.

The studies revealed that fluoroquinolones with 2-fluorocyclopropyl at N-1 position could modulate the lipophilicity and reduce the central nervous system (CNS) toxicity,[19] and sitafloxacin with a (1R, 2S)-2-fluorocyclopropyl group at the N1-position has already been approved for the treatment of various bacterial infections.[20]

Inspired by the above research results, fourteen novel 1-[(1R, 2S)-2-fluorocyclopropyl] ciprofloxacin-(4-methyl-3-aryl)-1,2,4-triazole-5(4*H*)-thione hybrids **6a-n** were designed, synthesized and evaluated for their *in vitro* antibacterial activities against representative Gram-positive and -negative bacteria in this study. The preliminary SAR study is also discussed to facilitate the further development of this kind of hybrids.

RESULTS AND DISCUSSION

Chemistry

The detailed synthetic route for 1-[(1R,2S)-2-fluorocyclopropyl]ciprofloxacin-(4-methyl-3-aryl)-1,2,4-triazole-5(4*H*)-thione hybrids **6a-n** is depicted in Scheme 1.[15-17] Treatment of substituted aryl hydrazides **1** with isothiocyanatomethane **2** in ethanol provided the corresponding thiosemicarbazides **3**, which were subsequently cyclized in 2% NaOH solution to give the key intermediates 1,2,4-triazole-5(4*H*)-thiones **4**. Finally, the Mannich reaction was performed with 1,2,4-triazole-5(4*H*)-thiones **4**, 1-[(1R, 2S)-2-fluorocyclopropyl] ciprofloxacin **5** and formaldehyde in ethanol to give the titled hybrids **6a-n**.

Antibacterial Activity

The 1-[(1R, 2S)-2-fluorocyclopropyl] ciprofloxacin-(4-methyl-3-aryl)-1,2,4-triazole-5(4*H*)-thione hybrids **6a-n** were evaluated for their *in vitro* antibacterial activity against representative strains using standard technique.[21,22] The minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give complete inhibition of bacterial growth, and the MIC values of **6a-n** along with those of vancomycin (VAN), ciprofloxacin (CPFX), levofloxacin (LVFX) and 8-methoxy ciprofloxacin (8-OMe CPFX) and the parent 1-[(1R, 2S)-2-fluorocyclopropyl] ciprofloxacin **5** for comparison, are listed in **Table 1** and Table 2, respectively.

From Table 1 and Table 2 it can be concluded that all 1-[(1R, 2S)-2-fluorocyclopropyl] ciprofloxacin-(4-methyl-3-aryl)-1,2,4-triazole-5(4*H*)-thione hybrids **6a-n** have broad-spectrum antibacterial activity. As shown in Table 1, all hybrids displayed promising potency in inhibiting the growth of some clinically important Gram-positive pathogens such as the methicillin-sensitive *Staphylococcus epidermidis* (MSSE), the methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA) and *Enterococcus faecalis* (two strains) with MIC in a range of 0.41 to 23.53 μM, which were more potent than the parent **5** (MIC: 2.86~>366.64 μM), and were no inferior to the references CPFX (MIC: 0.34~348.67 μM), LVFX (MIC: 0.35~177.22 μM) and 8-OMe CPFX (MIC: 0.35~88.61 μM). In particular, the most active hybrid **6m** (MIC: 0.41~6.53 μM) was ≥1.8 folds more potent than the parent **5** against MSSE, MSSA, MRSE, MRSA and *Enterococcus faecalis*, could act as a lead for further optimization.

The anti-Gram-negative activity of the target hybrids presented in Table 2 revealed that all hybrids **6a-n** endowed with excellent activity with MIC<1 μM against the majority of the tested Gram-negative strains. In general, their anti-Gram-negative activity was comparable to or better than the parent **5** as well as the references CPFX, LVFX and 8-OMe CPFX, and was far more potent than VAN (MIC: >88.32 μM). Amongst them, hybrid **6b** as found to be most active against the tested Gram-negative pathogens, and the MICs were ≤0.05 μM against most of the tested strains.

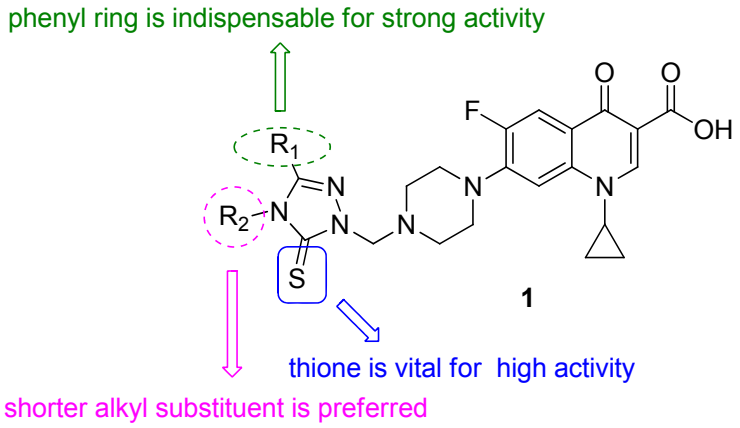
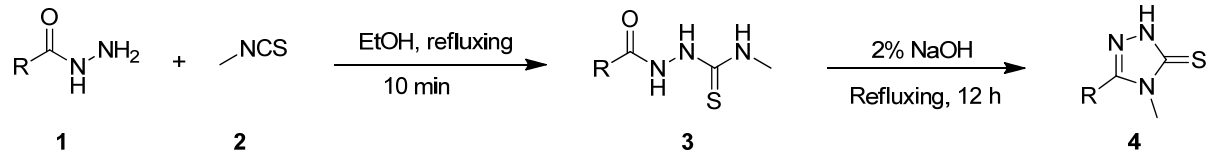
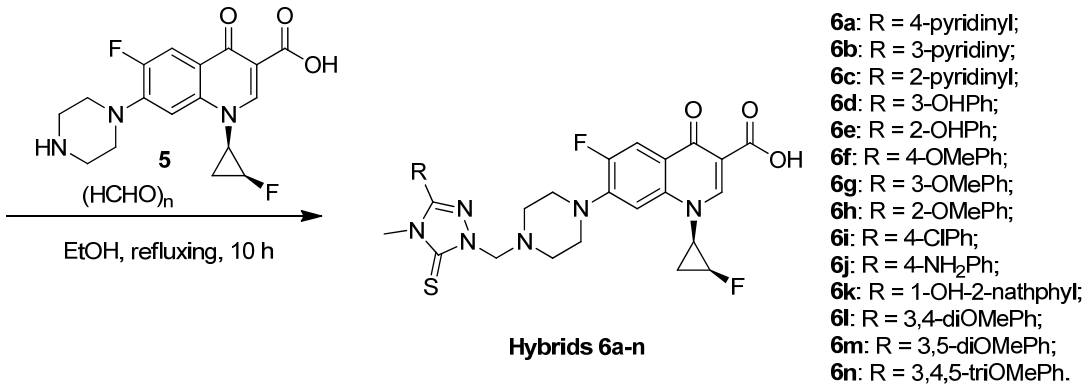


Fig. 1 – Chemical structures and structure-activity relationship of ciprofloxacin-1,2,4-triazole-5(4*H*)-thione hybrids **1**.





Scheme 1 – Synthetic route
for 1-[(1R, 2S)-2-fluorocyclopropyl] ciprofloxacin-(4-methyl-3-aryl)-1,2,4-triazole-5(4*H*)-thione hybrids **6a-n**.

Table 1

In vitro antibacterial activity of hybrids **6a-n** against Gram-positive strains

Compd.	MIC (μM)							
	MSSE	MRSE	MSSA	MRSA	E.fa.1	E.fa.2	E.fm.1	E.fm.2
6a	0.90	7.23	1.81	1.81	3.62	3.62	>231.39	28.92
6b	0.90	7.23	1.81	1.81	3.62	3.62	>231.39	14.46
6c	3.62	14.46	3.62	3.62	7.23	7.23	>231.39	115.69
6d	0.88	14.08	7.04	3.52	7.04	7.04	>225.28	28.16
6e	0.88	14.08	3.52	3.52	3.52	3.52	>225.28	28.16
6f	0.86	13.74	3.44	1.72	3.44	3.44	>219.86	27.48
6g	0.86	6.87	1.72	3.44	3.44	3.44	>219.86	27.48
6h	0.86	13.74	3.44	3.44	3.44	3.44	>219.86	27.48
6i	0.85	13.65	3.41	3.41	3.41	3.41	>218.38	27.3
6j	0.44	14.10	1.76	23.53	3.53	3.53	>225.67	28.21
6k	0.81	6.47	3.24	3.24	3.24	3.24	>207.06	25.88
6l	1.63	13.07	3.27	3.27	6.53	3.27	>209.08	52.27
6m	0.41	6.53	1.63	3.27	3.27	3.27	>209.08	26.14

Table 1 (continued)

6n	1.56	6.23	1.56	1.56	3.11	3.11	>199.31	49.83
LVFX	0.35	5.54	0.35	0.35	2.77	1.38	177.22	44.3
CPFX	0.34	10.9	0.68	0.68	1.36	1.36	348.67	21.79
5	2.86	22.88	2.86	5.73	5.73	5.73	>366.64	91.66
8-OMeCPFX	0.35	5.54	0.35	0.69	2.77	1.38	88.61	22.15
VAN	0.69	0.69	0.35	0.69	2.76	0.69	>88.32	0.69

Abbreviations: MSSE, methicillin-sensitive *Staphylococcus epidermidis* ATCC 12228; MRSE, methicillin- resistant *Staphylococcus epidermidis* 16-3; MSSA, methicillin-sensitive *Staphylococcus aureus* ATCC 29213; MRSA, methicillin-resistant *Staphylococcus aureus* ATCC 33591; E.fa.1, *Enterococcus faecalis* ATCC 29212; E.fa.2, *Enterococcus faecalis* ATCC 51575; E.fm.1, *Enterococcus faecium* ATCC 700221; E.fm.2, *Enterococcus faecium* 16-4; LVFX, levofloxacin; CPFX, Ciprofloxacin; 8-OMeCPFX, 8-methoxy ciprofloxacin; VAN, vancomycin.

Table 2

In vitro antibacterial activity of hybrids **6a-n** against Gram-negative strains

Compd.	MIC (μM)													
	E.co. 1	E.co.2	K.p.1	K.p.2	P.a.	A.c.	E.c.	E.a.	S.m.1	M.m.	P.r.	P.v.	P.m.	S.m.2	C.f.
6a	≤0.05	≤0.05	1.81	0.11	0.9	7.23	≤0.05	0.11	0.11	≤0.05	≤0.05	≤0.05	≤0.05	14.46	0.11
6b	≤0.05	≤0.05	0.9	≤0.05	0.9	7.23	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	14.46	≤0.05
6c	0.05	≤0.05	1.81	0.11	1.81	14.46	≤0.05	0.23	0.45	≤0.05	≤0.05	≤0.05	≤0.05	28.92	0.11
6d	0.11	≤0.05	1.76	0.11	3.52	14.08	≤0.05	0.22	0.44	≤0.05	≤0.05	≤0.05	0.11	28.16	0.11
6e	≤0.05	≤0.05	0.88	0.11	1.76	7.04	≤0.05	0.11	0.22	≤0.05	≤0.05	≤0.05	≤0.05	56.32	0.11
6f	≤0.05	≤0.05	0.86	≤0.05	0.86	6.87	≤0.05	≤0.05	0.21	≤0.05	≤0.05	≤0.05	≤0.05	27.48	≤0.05
6g	≤0.05	≤0.05	1.72	0.10	0.86	6.87	≤0.05	0.10	0.1	≤0.05	≤0.05	≤0.05	≤0.05	13.74	0.1
6h	≤0.05	≤0.05	1.72	0.10	1.72	13.74	≤0.05	0.21	0.43	≤0.05	≤0.05	≤0.05	≤0.05	27.48	≤0.05
6i	≤0.05	≤0.05	0.85	≤0.05	6.82	13.65	≤0.05	0.1	0.21	≤0.05	≤0.05	≤0.05	≤0.05	27.3	0.1
6j	≤0.05	≤0.05	0.88	0.11	0.88	7.05	≤0.05	≤0.05	0.22	≤0.05	≤0.05	≤0.05	≤0.05	28.2	0.11
6k	≤0.05	≤0.05	0.81	0.10	0.81	6.47	≤0.05	0.1	0.2	≤0.05	≤0.05	≤0.05	≤0.05	25.88	0.1
6l	0.10	0.10	1.63	0.10	1.63	13.07	≤0.05	0.1	0.2	≤0.05	≤0.05	≤0.05	≤0.05	26.14	0.1
6m	≤0.05	≤0.05	1.63	0.10	0.82	6.53	≤0.05	≤0.05	0.1	≤0.05	≤0.05	≤0.05	≤0.05	13.07	0.1

Table 2 (continued)

6n	≤0.05	≤0.05	0.78	≤0.05	0.78	6.23	≤0.05	≤0.05	0.19	≤0.05	≤0.05	≤0.05	≤0.05	49.83	≤0.05
LVFX	≤0.08	≤0.08	1.38	0.17	5.54	0.35	≤0.08	0.17	0.35	≤0.08	≤0.08	≤0.08	≤0.08	1.38	≤0.08
CPFX	≤0.08	≤0.08	1.36	≤0.08	0.68	1.36	≤0.08	≤0.08	0.16	≤0.08	≤0.08	≤0.08	≤0.08	5.45	1.38
5	≤0.09	≤0.09	2.86	≤0.09	2.86	11.46	≤0.09	0.17	0.36	≤0.09	≤0.09	≤0.09	≤0.09	45.83	≤0.09
8-OMeCPFX	≤0.08	≤0.08	2.77	0.17	5.54	1.38	≤0.08	0.35	0.69	≤0.08	≤0.08	≤0.08	0.35	5.54	0.17
VAN	>88.3	>88.3	>88.3	>88.3	>88.3	>88.3	>88.3	>88.3	>88.3	>88.3	>88.3	>88.3	>88.3	>88.3	>88.3

Abbreviations: E.co.1, *Escherichia coli* ATCC 25922 ESBLs(-); E.co.2*, Escherichia coli* ATCC 35218 ESBLs(+); K.p.1, *Klebsiella pneumoniae* ATCC 700603 ESBLs(+); K.p.2, *Klebsiella pneumonia* 7 ESBLs(-); P.a., *Pseudomonas aeruginosa* ATCC 27853; A.c., *Acinetobacter calcoacetious* ATCC 19606; E.c., *Enterobacter cloacae* ATCC 43560; E.a., *Enterobacter aerogenes* ATCC 13048; S.m.1, *Serratia marcescens* ATCC 21074; M.m., *Morganella morganii* ATCC 25830; P.r., *Providentia rettgeri* ATCC 31052; P.v., *Proteus vulgaris* ATCC 29905; P.m., *Proteus mirabilis* ATCC 49565; S.m.2, *Stenotrophomonas maltophilia* ATCC 13636; C.f., *Citrobacter freundii* ATCC 43864. ESBLs(+): Extended spectrum beta-lactamases (ESBLs)-producing; LVFX, levofloxacin; CPFX, ciprofloxacin; 8-OMeCPFX, 8-methoxy ciprofloxacin; VAN, vancomycin.

The SAR revealed that 1) pyridinyl and substituted phenyl ring at C'-3 position contributed equally to the antibacterial activity; 2) the hybrid **6e** was more potent than **6d** suggested that -OH at ortho-position of phenyl ring favored the activity; 3) the hybrid **6k** was as potent as **6e** indicated that introduction of nathphyl instead of phenyl couldn't improve the activity; 4) in general, introduction of neither electron-donating -OH and $-NH_2$ nor electron-withdrawing -Cl couldn't boost up the activity greatly.

EXPERIMENTAL

General

Melting points were determined in open capillaries and uncorrected. ^{1}H-NMR spectra were determined on a Varian Mercury-400 spectrometer (Varian Medical Systems Inc., Palo Alto, CA, USA) in DMSO-d_6 using tetra-methylsilane (TMS) as an internal standard. Unless otherwise noted, the reagents were obtained from a commercial supplier and were used without further purification.

Synthesis

1 mmol of the 1,2,4-triazole-5(4*H*)-thiones **4** was dissolved in 40 mL of anhydrous ethanol and then fluoroquinolone **5** (1 mmol) and formaldehyde solution (aq. 37%, 1 mmol) were added. The mixture was stirred at refluxing for 10 h, and then cooled to room temperature. The precipitate was filtered off, dried, and crystallized by ethanol (10 mL) to give desired hybrids **6a-n**.

Characteristics of synthesized compounds

7-(4-((4-methyl-3-(pyridin-4-yl)-5-thioxo-4,5-dihydro-1H- 1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6a)**

Yield: 46%. M.p.: 174-176 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.76-1.90 (2H, m, cyclopropyl-H, CH_2), 3.00 (4H, s, piperazine-4H), 3.00 (4H, s, piperazine-4H), 3.66-3.68 (4H, m, cyclopropyl-H and CH_3), 3.86-3.88 (1H, m, cyclopropyl-H), 5.27 (2H, s, CH_2), 7.49 (1H, d, J = 5.2 Hz, Ar-H), 7.82 (2H, d, J = 3.2 Hz, pyridinyl-2H), 7.91 (1H, d, J = 5.2 Hz, Ar-H), 8.74 (1H, s, Ar-H), 8.83 (2H, d, J = 3.2 Hz, pyridinyl-2H).

7-(4-((4-methyl-3-(pyridin-3-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6b)**

Yield: 41%. M.p.: 244-246 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.90-1.96 (2H, m, cyclopropyl-H, CH_2), 2.98 (4H, s, piperazine-4H), 3.35 (4H, s, piperazine-4H), 3.61 (3H, s, CH_3), 3.74-3.76 (1H, m, cyclopropyl-H), 3.86-3.88 (1H, m, cyclopropyl-H), 5.24 (2H, s, CH_2), 7.48 (1H, s, Ar-H), 7.63 (1H, s, Ar-H), 7.88 (1H, d, J = 12.8 Hz, pyridinyl-1H), 8.21 (1H, s, pyridinyl-1H), 8.73 (1H, s, Ar-H), 8.77 (1H, s, pyridinyl-1H), 8.95 (1H, s, pyridinyl-1H), 15.09 (1H, brs, COOH).

7-(4-((4-methyl-3-(pyridin-2-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6c)**

Yield: 34%. M.p.: 183-185 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.79-1.93 (2H, m, cyclopropyl-H, CH_2), 3.00 (4H, s,

piperazine-4H), 3.38 (4H, s, piperazine-4H), 3.81-3.93 (5H, m, 2×cyclopropyl-H and CH_3), 5.29 (2H, s, CH_2), 7.49 (1H, s, Ar-H), 7.63 (1H, s, Ar-H), 7.90 (1H, d, J = 10.4 Hz, pyridinyl-1H), 8.06 (2H, s, pyridinyl-1H), 8.74 (1H, s, Ar-H), 8.79 (1H, s, pyridinyl-1H).

6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-7-(4-((3-(3-hydroxyphenyl)-4-methyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6d)**

Yield: 27%. M.p.: 199-201 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.78-1.97 (2H, m, cyclopropyl-H, CH_2), 2.99 (4H, s, piperazine-4H), 3.37 (4H, s, piperazine-4H), 3.61 (3H, s, CH_3), 3.88-3.89 (1H, m, cyclopropyl-H), 4.36-4.37 (1H, m, cyclopropyl-H), 5.24 (2H, s, CH_2), 7.02 (1H, d, J = 6.0 Hz, Ar-H), 7.15 (1H, s, Ar-H), 7.20 (1H, d, J = 6.4 Hz, Ar-H), 7.39 (1H, t, J = 6.0 Hz, Ar-H), 7.50 (1H, d, J = 5.2 Hz, Ar-H), 7.92 (1H, d, J = 10.4 Hz, Ar-H), 8.76 (1H, s, Ar-H), 9.96 (1H, brs, OH).

6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-7-(4-((3-(2-hydroxyphenyl)-4-methyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6e)**

Yield: 21%. M.p.: 217-218 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.78-1.97 (2H, m, cyclopropyl-H, CH_2), 2.98 (4H, s, piperazine-4H), 3.37-3.42 (8H, m, piperazine-4H, cyclopropyl-H and CH_3), 3.88-3.89 (1H, m, cyclopropyl-H), 5.21 (2H, s, CH_2), 6.97 (1H, t, J = 5.6 Hz, Ar-H), 7.05 (1H, d, J = 5.6 Hz, Ar-H), 7.37-7.51 (3H, m, Ar-H), 7.93 (1H, d, J = 10.4 Hz, Ar-H), 8.76 (1H, s, Ar-H).

7-(4-((4-methyl-3-(4-methoxyphenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6f)**

Yield: 53%. M.p.: 168-169 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.77-1.93 (2H, m, cyclopropyl-H, CH_2), 2.99 (4H, s, piperazine-4H), 3.42-3.46 (5H, m, piperazine-4H and cyclopropyl-H), 3.61 (3H, s, CH_3), 3.87 (4H, s, cyclopropyl-H and OCH_3), 5.22 (2H, s, CH_2), 7.15 (1H, d, J = 6.4 Hz, Ar-H), 7.48 (2H, d, J = 4.8 Hz, Ar-H), 7.72 (2H, d, J = 6.4 Hz, Ar-H), 7.90 (1H, d, J = 10.4 Hz, Ar-H), 8.73 (1H, s, Ar-H).

7-(4-((4-methyl-3-(3-methoxyphenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6g)**

Yield: 53%. M.p.: 154-156 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.77-1.96 (2H, m, cyclopropyl-H, CH_2), 2.99 (4H, s, piperazine-4H), 3.37 (4H, s, piperazine-4H), 3.62-3.64 (4H, m, cyclopropyl-H and CH_3), 3.85 (4H, s, cyclopropyl-H and OCH_3), 5.24 (2H, s, CH_2), 7.21 (1H, d, J = 6.4 Hz, Ar-H), 7.30-7.34 (2H, m, Ar-H), 7.49-7.53 (2H, m, Ar-H), 7.91 (1H, d, J = 10.8 Hz, Ar-H), 8.75 (1H, s, Ar-H).

7-(4-((4-methyl-3-(2-methoxyphenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6h)**

Yield: 37%. M.p.: 177-179 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.76-1.96 (2H, m, cyclopropyl-H, CH_2), 2.97 (4H, s, piperazine-4H), 3.35-3.37 (5H, m, piperazine-4H and cyclopropyl-H), 3.79-3.89 (7H, m, cyclopropyl-H, CH_3 and OCH_3), 5.21 (2H, s, CH_2), 7.16 (1H, t, J = 6.0 Hz, Ar-H), 7.28 (1H, d, J = 7.2 Hz, Ar-H), 7.45-7.49 (2H, m, Ar-H), 7.76 (1H, t, J = 6.4 Hz, Ar-H), 7.88 (1H, d, J = 10.4 Hz, Ar-H), 8.65 (1H, s, Ar-H).

7-(4-((3-(4-chlorophenyl)-4-methyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6i)**

Yield: 31%. M.p.: 161-163 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.77-1.95 (2H, m, cyclopropyl-H, CH_2), 2.99 (4H, s, piperazine-4H), 3.34-3.37 (5H, m, piperazine-4H and cyclopropyl-H), 3.61 (3H, s, CH_3), 3.86-3.87 (1H, m, cyclopropyl-H), 5.21 (2H, s, CH_2), 7.49 (1H, d, J = 4.8 Hz, Ar-H), 7.69 (2H, d, J = 6.4 Hz, Ar-H), 7.82 (2H, d, J = 6.4 Hz, Ar-H), 7.91 (1H, d, J = 10.8 Hz, Ar-H), 8.75 (1H, s, Ar-H).

7-(4-((3-(4-aminophenyl)-4-methyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6i)**

Yield: 13%. M.p.: 233-235 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.76-1.97 (2H, m, cyclopropyl-H, CH_2), 3.00 (4H, s, piperazine-4H), 3.43 (4H, s, piperazine-4H), 3.47-3.60 (4H, m, cyclopropyl-H and CH_3), 3.88-3.89 (1H, m, cyclopropyl-H), 5.24 (2H, s, CH_2), 7.49-7.52 (3H, m, Ar-H), 7.86-7.93 (3H, m, Ar-H), 8.76 (1H, s, Ar-H).

6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-7-(4-((3-(2-hydroxynaphth-1-yl)-4-methyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6k)**

Yield: 33%. M.p.: 241-243 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.77-1.96 (2H, m, cyclopropyl-H, CH_2), 3.05 (4H, s, piperazine-4H), 3.41 (4H, s, piperazine-4H), 3.47 (3H, s, CH_3), 3.87-3.88 (1H, m, cyclopropyl-H), 4.34-4.35 (1H, m, cyclopropyl-H), 5.33 (2H, q, CH_2), 7.57-7.88 (7H, m, Ar-H and OH), 8.12 (1H, d, J = 6.4 Hz, Ar-H), 8.24 (1H, d, J = 6.4 Hz, Ar-H), 8.76 (1H, s, Ar-H).

6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-7-(4-((3-(3,4-dimethoxyphenyl)-4-methyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6l)**

Yield: 43%. M.p.: 215-217 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.78-1.97 (2H, m, cyclopropyl-H, CH_2), 2.99 (4H, s, piperazine-4H), 3.34 (4H, s, piperazine-4H), 3.47-3.48 (1H, m, cyclopropyl-H), 3.61 (3H, s, CH_3), 3.84 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 4.36-4.37 (1H, m, cyclopropyl-H), 5.23 (2H, s, CH_2), 7.16 (1H, d, J = 6.4 Hz, Ar-H), 7.36 (2H, s, Ar-H), 7.50 (1H, d, J = 4.4 Hz, Ar-H), 7.92 (1H, d, J = 10.0 Hz, Ar-H), 8.76 (1H, s, Ar-H).

6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-7-(4-((3-(3,5-dimethoxyphenyl)-4-methyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6m)**

Yield: 39%. M.p.: 237-238 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.78-2.00 (2H, m, cyclopropyl-H, CH_2), 2.99 (4H, s, piperazine-4H), 3.37 (4H, s, piperazine-4H), 3.47-3.49 (1H, m, cyclopropyl-H), 3.61 (3H, s, CH_3), 3.86 (6H, s, 2×OCH_3), 4.36-4.37 (1H, m, cyclopropyl-H), 5.24 (2H, s, CH_2), 6.77 (1H, s, Ar-H), 6.90 (2H, s, Ar-H), 7.51 (1H, d, J = 4.4 Hz, Ar-H), 7.93 (1H, d, J = 10.4 Hz, Ar-H), 8.76 (1H, s, Ar-H).

6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-7-(4-((4-methyl-5-thioxo-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **(6n)**

Yield: 23%. M.p.: 212-213 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.76-1.96 (2H, m, cyclopropyl-H, CH_2), 2.99 (4H, s, piperazine-4H), 3.36 (4H, s, piperazine-4H), 3.55-3.56 (1H, m, cyclopropyl-H), 3.61 (3H, s, CH_3), 3.76 (3H, s, OCH_3), 3.86 (6H, s, 2×OCH_3), 4.356-4.36 (1H, m, cyclopropyl-H), 5.21 (2H, s, CH_2), 7.03-7.05 (2H, m, Ar-H), 7.50 (1H, d,

J = 6.4 Hz, Ar-H), 7.92 (1H, d, *J* = 10.4 Hz, Ar-H), 8.76 (1H, s, Ar-H).

Organisms

A total of 23 organisms were tested *in vitro*, including methicillin-sensitive *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus epidermidis*, methicillin-sensitive *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 33591, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecalis* ATCC 51575, *Enterococcus faecium* ATCC 700221, *Enterococcus faecium* 16-4, Escherichia coli ATCC 25922, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, *Klebsiella pneumonia* 7, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter calcoacetious* ATCC 19606, *Enterobacter cloacae*, *Enterobacter aerogenes* ATCC 13048, *Serratia marcescens* ATCC 21074, *Morganella morganii* ATCC 25830, *Providentia rettgeri* ATCC 31052, *Proteus vulgaris* ATCC 29905, *Proteus mirabilis* ATCC 49565, *Stenotrophomonas maltophilia* ATCC 13636 and *Citrobacter freundii* ATCC 43864. All bacterial strains were obtained from Chinese Center For Disease Control And Prevention or collected from hospitals in Beijing. All of the isolates were frozen at 70°C until they were used.

Anti-Bacterial MIC Determination

All hybrids were screened for their *in vitro* antibacterial activity against representative Gram-positive and Gram-negative strains, by means of standard two-fold serial dilution method using agar media [21,22]. Hybrids (10.0 mg) were dissolved in 0.1 N NaOH solution and water (10 mL). Further progressive two folds serial dilution with melted Mueller-Hinton agar was performed to obtain the required concentrations of 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06, 0.03, 0.015 and 0.008 mg/mL. Petri dishes were incubated with 10^4 colony-forming units (cfu) and incubated at 35 °C for 18-24 h.

CONCLUSIONS

In summary, a series of novel 1-[(1R,2S)-2-fluorocyclopropyl] ciprofloxacin-(4-methyl-3-aryl)-1,2,4- triazole-5(4*H*)-thione hybrids **6a-n** were designed, synthesized, and assessed for their antibacterial activity against a panel of clinically important pathogens. The results show that all of the hybrids were more potent than the parent 1-[(1R, 2S)-2-fluorocyclopropyl] ciprofloxacin **5**, and were no inferior to CPFX, LVFX and 8-OMe CPFX against the majority of the tested pathogens. Moreover, the anti-Gram-negative bacteria activity of hybrids was far more potent than VAN.

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