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Design, Synthesis and Antibacterial Evaluation of 1-[(1R, 2S)-2-Fluorocyclopropyl]ciprofloxacin-1,2,4-triazole-5(4*H*)-thione Hybrids

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A new class of 1-[(1R, 2S)-2-fluorocyclopropyl]ciprofloxacin (CPFX)-1,2,4-triazole-5(4*H*)-thione hybrids **6a-o** was designed, synthesized and evaluated for their *in vitro* antibacterial activities against a panel of clinically important drug-sensitive and drug-resistant Gram-positive and Gram-negative pathogens. Our results revealed that all hybrids **6a-o** have great potency against the tested strains, especially Gram-negative pathogens. The synthesized hybrids were more potent than the parent 1-[(1R, 2S)-2-fluorocyclopropyl]CPFX **5**, and were comparable to CPFX and levofloxacin against the majority of the tested pathogens, worth to be further investigated.

Keywords: Quinolone; ciprofloxacin; 1,2,4-triazole-5(4*H*)-thione; Antibacterial; Structure-activity relationship

Introduction

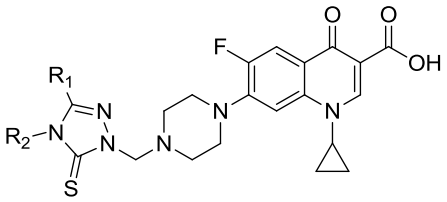
Bacterial infections are account for the majority of hospital-acquired infections, and place a heavy burden on global healthcare systems[1]. The antibiotics are common weapons to treat bacterial infections, but bacteria have already developed resistance towards almost all current therapeutic options duo to long-term, broad and inappropriate usage[2]. Therefore, new antibacterial agents are strongly sought and research on the agents has been carried out in the recent decades.

Fluoroquinolones, which play a pivotal role in drug discovery as they exhibit a variety of biological properties such as anti-tumor[3,4], anti-tubercular[5], anti-HIV[6], anti-malarial[7,8] and anti-bacterial activities[2], are the second widest used antibiotics in clinical practice for the treatment of various bacterial infections both in hospital and community settings[1]. Unfortunately, fluoroquinolone-resistant bacteria have already emerged and widely spread globally[2]. Therefore, it's imperative to develop new fluoroquinolone agents with great potency against both drug-sensitive and drug-resistant Gram-positive and Gram-negative pathogens.

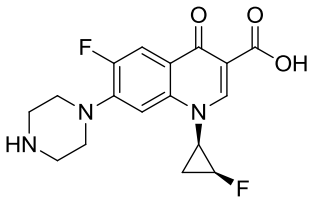
Molecular hybridization is a useful tool for the development of new drug candidates, and several fluoroquinolone hybrids which are exemplified by **MCB3681** and **CBR-2092** have already under clinical trials for treatment of infections caused by fluoroquinolone-resistant, multidrug-resistant and difficult to treat pathogens[9,10]. Obviously, fluoroquinolone hybrids are promising candidates with unique mechanism of action different from those of the currently used drugs.

The structure-activity relationship (SAR) demonstrated that the substituents at C-7 position of fluoroquinolone motif have significantly influence on antibacterial potency, spectrum and pharmacokinetic profiles, and the C-7 position is proven to be the most adaptable site for chemical modification[11,12]. Furthermore, the research results revealed that introduction of bulky substituent at C-7 position of fluoroquinolones is not a barrier to penetration, so various biological pharmacophores have been incorporated into this position[11,12].

Triazoles, one of the most important classes of nitrogen-containing heterocycles, possess diverse biological profiles including antibacterial activity which may due to their ability to exert various noncovalent interactions (π-π, electrostatic interactions, hydrogen bonds, metal coordination and van der Waals force etc.) with the various active sites in organisms[13]. Obviously, hybridization of triazoles with fluoroquinolones is a reasonable choice to develop new antibacterial agents.



Ciprofloxacin-1,2,4-triazole-5(4H)-thione hybrids



1-[(1R, 2S)-2-fluorocyclopropyl]ciprofloxacin 1

Figure 1 Chemical structures of ciprofloxacin-1,2,4-triazole-5(4*H*)-thione hybrids and 1-[(1R, 2S)-2-fluorocyclopropyl]ciprofloxacin **1**

In recent year, several series of ciprofloxacin(CPFX)-1,2,4-triazole-5(4*H*)-thione hybrids (**Figure 1**) were assessed for their *in vitro* antibacterial activities against various clinically relevant bacteria, and some of them were more potent than the parent CPFX[14-17]. The SAR studies suggested that phenyl group at C-3 position of the 1,2,4-triazole moiety was crucial for the high antibacterial activity, and the substituents on the phenyl ring were one of the key factors.

Clinical data indicated that N1-cyclopropyl fluoroquinolones with outstanding antibacterial activity could cause serious central nervous system (CNS) toxicity[18], while the corresponding 2-fluorocyclopropyl counterparts could modulate the lipophilicity and reduce the CNS toxicity[19]. Sitafloxacin, bearing a (1R, 2S)-2-fluorocyclopropyl group at the N1-position, was approved in Japan for the treatment of a number of bacterial infections[20]. Moreover, many of 1-[(1R, 2S)-2-fluorocyclopropyl]fluoroquinolones with an oxime functional moiety at C-7 position were found to have a broad spectrum of antibacterial activity in our lab recently[21,22].

Inspired by the above research results and as a continuous program to develop new fluoroquinolone antibacterial agents, we intended to introduce 1,2,4-triazole-5(4*H*)-thione moieties to the piperazine ring of 1-[(1R, 2S)-2-fluorocyclopropyl]CPFX **1**. Thus, a series of novel hybrids were designed, synthesized and screened for their *in vitro* antibacterial activities against various clinically relevant pathogens in this study. A preliminary SAR study is also explored to facilitate the further development of this kind of hybrids.

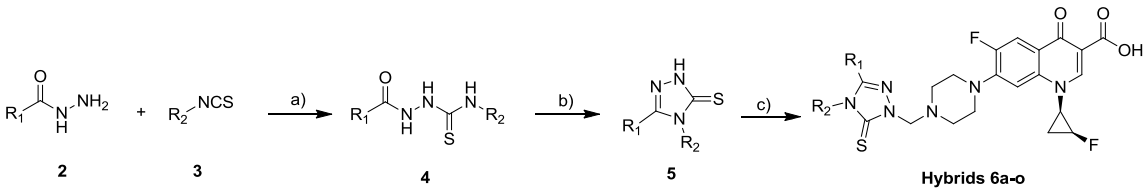
Results and Discussion

Chemistry

The desired 1-[(1R, 2S)-2-fluorocyclopropyl]CPFX-1,2,4-triazole-5(4*H*)-thione hybrids **6a-o** were prepared according to the literature reported procedure [15], and the detailed synthetic route was depicted in Scheme 1. Treatment of aromatic hydrazides **2** with substituted phenyl/benzyl isothiocyanates **3** yielded the corresponding thiosemicarbazides **4**, which were cyclized in 2% NaOH solution provided the key intermediates 1,2,4-triazole-5(4*H*)-thiones **5**. Finally, Mannich reaction of 1,2,4-triazole-5(4*H*)-thiones **5**, 1-[(1R,

2S)-2-fluorocyclopropyl]CPFX **1** and formaldehyde was performed in ethanol for 10 h to give the titled hybrids **6a-o**. The chemical structures of hybrids **6a-o** were presented in **Table 1**.

Scheme 1 Synthetic route for 1-[(1R, 2S)-2-fluorocyclopropyl]CPFX-1,2,4-triazole-5(4*H*)-thione hybrids **6a-o**



a) EtOH, reflux, 10 min. b) 2% NaOH, reflux, 12 h. c) 1-[(1R, 2S)-2-fluorocyclopropyl]CPFX **1**, $(HCHO)_n$, EtOH, reflux, 10 h.

Table 1. Chemical structures of hybrids **6a-o**

Compd.	R_1	R_2
6a	4-pyridinyl	-Bn
6b	3-pyridinyl	-Bn
6c	2-pyridinyl	-Bn
6d	2-naphthyl	-Bn
6e	3,5-diOMePh	-Bn
6f	4-pyridinyl	-Ph
6g	3-pyridinyl	-Ph
6h	2-pyridinyl	-Ph
6i	3-OHPh	-Ph
6j	2-OHPh	-Ph
6k	3-OMePh	-Ph
6l	2-OMePh	-Ph
6m	3,4-diOMePh	-Ph
6n	3,5-diOMePh	-Ph
6o	3,4,5-triOMePh	-Ph

Antibacterial Activity

The 1-[(1R, 2S)-2-fluorocyclopropyl]CPFX-1,2,4-triazole-5(4*H*)-thione hybrids **6a-o** were evaluated for their *in vitro* antibacterial activity against a panel of clinically important drug-sensitive and drug-resistant Gram-positive and Gram-negative strains using standard techniques[22]. The minimum inhibitory concentration (MIC) defined as the concentration of the compound required to give complete inhibition of bacterial growth, and the MIC values of **6a-o** against Gram-positive and Gram-negative strains, along with those of the references vancomycin (VAN), CPFX, levofloxacin (LVFX) and 8-methoxy CPFX (8-OMe CPFX) and the parent 1-[(1R, 2S)-2-fluorocyclopropyl]CPFX **1** for comparison, are listed in **Table 2** and **Table 3**, respectively.

The *in vitro* antibacterial data suggested that all of the target hybrids **6a-o** have a similar antibacterial spectrum with the references VAN, CPFX, LVFX and 8-OMe CPFX. Further analysis indicated that all hybrids exhibited considerable potency in inhibiting the growth of some tested Gram-positive strains including methicillin-sensitive *Staphylococcus epidermidis* (MSSE), methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA) and *Enterococcus faecalis* (two strains) as well as Gram-negative strains, and the MICs for these hybrids against Gram-positive and Gram-negative pathogens were 0.44~>208.06 μM and ≤0.04~92.99 μM, respectively. The antibacterial activity of hybrids **6a-o** was found to be more potent than the parent 1-[(1R, 2S)-2-fluorocyclopropyl]CPFX **1** (MIC: ≤0.09~>366.64 μM), and was comparable to CPFX (MIC: ≤0.08~>348.67 μM) and LVFX (MIC: ≤0.08~>177.22 μM) against the majority of the tested pathogens. Moreover, the anti-Gram-negative bacteria activity of hybrids **6a-o** was far more potent than VAN (MIC: >88.32 μM).

Table 2. *In vitro* antibacterial activity of hybrids **6a-o** against Gram-positive strains.

Compd.	MIC (μM)							
	MSSE	MRSE	MSSA	MRSA	E.fa.1	E.fa.2	E.fm.1	E.fm.2
6a	0.79	6.36	0.79	0.79	6.36	6.36	>203.43	50.86
6b	0.79	6.36	1.59	1.59	3.18	3.18	>203.43	25.43
6c	0.44	6.36	1.59	1.59	3.18	1.59	>203.43	50.86
6d	0.74	5.9	1.47	1.47	11.8	5.9	>188.73	47.18
6e	0.76	11.62	0.73	1.45	5.81	2.91	>185.98	46.50
6f	1.63	26.01	3.25	6.5	6.5	13	>208.06	104.03
6g	3.25	13	3.25	3.25	3.25	6.5	>104.03	26.11
6h	1.63	6.5	1.63	1.63	3.25	3.25	>208.06	26.01
6i	0.79	12.69	3.17	3.17	6.35	6.35	>203.11	25.39
6j	0.79	12.69	1.59	1.59	3.17	3.17	>203.11	50.78
6k	0.78	24.84	3.1	3.10	6.21	6.21	>99.35	24.84
6l	1.55	6.21	1.55	1.55	3.1	6.21	>198.69	24.84
6m	1.48	8.00	2.97	2.97	5.93	11.87	>189.85	23.73
6n	1.48	11.87	2.97	2.97	2.97	5.93	>94.93	94.93
6o	1.42	11.36	1.42	1.42	2.84	2.84	>181.76	45.44
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
1	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 3. *In vitro* antibacterial activity of hybrids **6a-o** 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	1.59	≤0.05	0.79	12.71	≤0.05	0.1	0.2	≤0.05	≤0.05	≤0.05	≤0.05	50.86	≤0.05
6b	≤0.05	≤0.05	0.4	≤0.05	1.59	6.36	≤0.05	0.1	0.2	≤0.05	≤0.05	≤0.05	≤0.05	25.43	≤0.05
6c	≤0.05	≤0.05	0.79	≤0.05	0.79	6.36	≤0.05	0.1	0.2	≤0.05	≤0.05	≤0.05	≤0.05	12.71	≤0.05
6d	≤0.04	≤0.04	1.47	0.09	1.47	11.8	≤0.04	0.18	0.37	≤0.04	≤0.04	≤0.04	≤0.04	47.18	≤0.04
6e	≤0.04	≤0.04	1.45	0.09	1.45	5.81	≤0.04	0.18	0.36	0.09	≤0.04	≤0.04	0.09	92.99	≤0.04
6f	0.1	0.1	3.25	0.2	3.25	13	≤0.05	0.2	0.41	≤0.05	≤0.05	≤0.05	0.10	52.02	0.10
6g	≤0.05	≤0.05	1.63	0.10	1.63	13	≤0.05	0.1	0.2	≤0.05	≤0.05	≤0.05	≤0.05	26.01	≤0.05
6h	≤0.05	≤0.05	1.63	≤0.05	1.63	6.5	≤0.05	0.1	0.2	≤0.05	≤0.05	≤0.05	≤0.05	26.01	≤0.05
6i	≤0.05	≤0.05	1.59	≤0.05	1.59	6.35	≤0.05	0.1	0.2	≤0.05	≤0.05	≤0.05	≤0.05	25.39	0.1
6j	0.10	0.1	1.59	0.10	1.59	12.69	≤0.05	0.2	0.2	≤0.05	≤0.05	≤0.05	≤0.05	25.39	≤0.05
6k	≤0.05	≤0.05	1.55	≤0.05	6.21	12.42	≤0.05	0.19	0.19	≤0.05	≤0.05	≤0.05	≤0.05	24.84	0.09
6l	≤0.05	≤0.05	0.78	≤0.05	3.1	6.21	≤0.05	0.09	0.19	≤0.05	≤0.05	≤0.05	≤0.05	24.84	≤0.05
6m	≤0.04	≤0.04	2.97	0.19	1.48	23.73	≤0.04	0.19	0.37	0.09	≤0.04	≤0.04	0.09	23.73	0.09
6n	≤0.04	≤0.04	1.48	0.09	1.48	5.93	≤0.04	0.09	0.19	≤0.04	≤0.04	≤0.04	≤0.04	23.73	0.09
6o	≤0.04	≤0.04	1.42	0.09	0.71	5.68	≤0.04	≤0.04	0.18	≤0.04	≤0.04	≤0.04	≤0.04	22.72	0.09
LVFX	88.61	≤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
1	≤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., *Acinetobactercal coacetious* 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) hybrids were more active against Gram-negative pathogens than against Gram-positive bacteria; 2) the substituents on the N'-4 position of 1,2,4-triazole-5(4*H*)-thione have great influence on the antibacterial activity, and hybrids with substituted phenyl groups were more potent than the corresponding benzyl substituted analogs; 3) for the substituents at C'-3 position, -OMe on the phenyl ring was more favorable than -OH; 4) in general, introduction of the second and the third -OMe was detrimental to the activity; 5) hybrids with pyridine-2-yl at C'-3 position were more active than the pyridine-3-yl and pyridine-4-yl analogs. It is worth noting that hybrid **6a** exhibited promising activity against all tested pathogens, could act as a starting point for further optimization.

Experimental Section

1 mmol of the 1,2,4-triazole-5(4*H*)-thiones **5** was dissolved in 40 mL of anhydrous ethanol and then 1-[(1R, 2S)-2-fluorocyclopropyl]CPFX **1** (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-o**.

*7-(4-((4-benzyl-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: 52%. M.p.: 167-169 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.77-1.98 (2H, m, cyclopropyl-H, CH_2), 3.04 (4H, s, piperazine-4H), 3.40-3.46 (5H, m, piperazine-4H and cyclopropyl-H), 3.89-3.90 (1H, m, cyclopropyl-H), 5.31-5.38 (2H, m, CH_2), 5.51-5.58 (2H, m, CH_2), 7.08-7.09 (2H, m, Ar-H), 7.28-7.29 (3H, m, Ar-H), 7.51-7.52 (1H, m, Ar-H), 7.61-7.62 (2H, m, Ar-H), 7.92-7.95 (1H, s, Ar-H), 8.71-8.77 (3H, m, Ar-H).

*7-(4-((4-benzyl-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: 56%. M.p.: 239-240 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.78-2.04 (2H, m, cyclopropyl-H, CH_2), 3.05 (4H, s, piperazine-4H), 3.40-3.50 (5H, m, piperazine-4H and cyclopropyl-H), 3.88-3.90 (1H, m, cyclopropyl-H), 5.34 (2H, s, CH_2), 5.48 (2H, s, CH_2), 7.05-7.06 (2H, m, Ar-H), 7.27-7.28 (3H, m, Ar-H), 7.53-7.54 (2H, m, Ar-H), 7.92-7.95 (1H, m, Ar-H), 8.00-8.02 (1H, m, Ar-H), 8.74-8.77 (3H, m, Ar-H).

*7-(4-((4-benzyl-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: 53%. M.p.: 165-167 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.77-2.00 (2H, m, cyclopropyl-H, CH_2), 3.03 (4H, s, piperazine-4H), 3.40-3.45 (5H, m, piperazine-4H and cyclopropyl-H), 3.89-3.90 (1H, m, cyclopropyl-H), 5.35 (2H, s, CH_2), 5.93 (2H, s, CH_2),

7.19-7.25 (5H, m, Ar-H), 7.50-7.51 (2H, m, Ar-H), 7.90 (1H, s, Ar-H), 7.93-8.01 (2H, m, Ar-H), 8.71-8.80 (2H, m, Ar-H).

7-(4-((4-benzyl-3-(naphthalen-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 ***(6d)***

Yield: 63%. M.p.: 194-196 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.80-1.86 (2H, m, cyclopropyl-H, CH_2), 3.07 (4H, s, piperazine-4H), 3.45-3.49 (5H, m, piperazine-4H and cyclopropyl-H), 3.83-3.85 (1H, m, cyclopropyl-H), 5.18-5.20 (2H, m, CH_2), 5.38-5.44 (2H, m, CH_2), 6.80-6.81 (2H, m, Ar-H), 7.06-7.08 (3H, m, Ar-H), 7.47-7.65 (6H, m, Ar-H), 7.88-7.90 (1H, m, Ar-H), 8.04-8.05 (1H, m, Ar-H), 8.17-8.19 (1H, m, Ar-H), 8.70 (1H, s, Ar-H).

7-(4-((4-benzyl-3-(3,5-dimethoxyphenyl)-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 ***(6e)***

Yield: 66%. M.p.: 164-166 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.86-2.09 (2H, m, cyclopropyl-H, CH_2), 2.95-3.09 (4H, s, piperazine-4H), 3.35-3.45 (4H, m, piperazine-4H), 3.61-3.63 (1H, m, cyclopropyl-H), 3.75 (6H, s, 2×OCH_3), 3.83-3.85 (1H, m, cyclopropyl-H), 5.25-5.35 (2H, m, CH_2), 5.36-5.52 (2H, m, CH_2), 6.61-6.71 (1H, m, Ar-H), 7.05-7.15 (3H, m, Ar-H), 7.25-7.41 (4H, m, Ar-H), 7.48-7.62 (1H, m, Ar-H), 7.85-8.01 (1H, m, Ar-H), 8.71-8.78 (1H, m, Ar-H).

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

Yield: 66%. M.p.: 274-276 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.79-1.93 (2H, m, cyclopropyl-H, CH_2), 3.10 (4H, s, piperazine-4H), 3.42-3.62 (5H, m, piperazine-4H and cyclopropyl-H), 3.90-3.91 (1H, s, cyclopropyl-H), 5.35 (2H, s, CH_2), 7.30 (2H, s, Ar-H), 7.50-7.57 (6H, m, Ar-H), 7.94 (1H, d, Ar-H), 8.62 (2H, d, Ar-H), 8.77 (1H, s, Ar-H).
6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-7-(4-((4-phenyl-3-(pyridin-3-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid ***(6g)***

Yield: 63%. M.p.: 256-258 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.71-2.01 (2H, m, cyclopropyl-H, CH_2), 3.05-3.21 (4H, m, piperazine-4H), 3.41-3.50 (5H, m, piperazine-4H and cyclopropyl-H), 3.85-3.87 (1H, m, cyclopropyl-H), 5.28 (2H, s, CH_2), 7.41-7.61 (7H, m, Ar-H), 7.71-7.79 (1H, m, Ar-H), 7.91-8.01 (1H, m, Ar-H), 8.55 (1H, s, Ar-H), 8.62 (1H, s, Ar-H), 8.77 (1H, s, Ar-H).

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

Yield: 66%. M.p.: 272-273 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.78-1.99 (2H, m, cyclopropyl-H, CH_2), 3.10 (4H, s, piperazine-4H), 3.42 (5H, s, piperazine-4H and cyclopropyl-H), 3.89-3.91 (1H, m, cyclopropyl-H), 5.35 (2H, s, CH_2), 7.37-7.38 (2H, m, Ar-H), 7.42-7.47 (4H, m, Ar-H), 7.53-7.54 (1H, m, Ar-H), 7.82-7.84 (1H, m, Ar-H), 7.92-7.94 (2H, m, Ar-H), 8.40 (1H, s, Ar-H), 8.78 (1H, s, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.29, 13.40, 37.25, 37.33, 49.99, 50.07, 69.41, 69.74, 71.94, 111.37, 111.61, 124.77, 125.69, 128.90, 129.29, 129.46, 136.05, 137.82, 139.73, 145.26, 148.67, 149.53, 149.77, 152.22, 166.17, 170.56, 176.89.

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

Yield: 65%. M.p.: 227-229 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.77-1.94 (2H, m, cyclopropyl-H, CH_2), 3.10 (4H, s, piperazine-4H), 3.40 (5H, s, piperazine-4H and cyclopropyl-H), 3.88-3.89 (1H, m, cyclopropyl-H), 5.31 (2H, s, CH_2), 6.69 (1H, d, Ar-H), 6.84-6.85 (2H, m, Ar-H), 7.12-7.15 (1H, m, Ar-H), 7.42-7.43 (2H, m, Ar-H), 7.53 (4H, m, Ar-H), 7.91 (1H, d, Ar-H), 8.74 (1H, s, Ar-H).

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

Yield: 60%. M.p.: 251-252 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.71-2.01 (2H, m, cyclopropyl-H, CH_2), 3.11 (4H, s, piperazine-4H), 3.25-3.38 (5H, m, piperazine-4H and cyclopropyl-H), 3.87-3.89 (1H, m, cyclopropyl-H), 5.25 (2H, s, CH_2), 6.80 (1H, d, Ar-H), 6.84-6.90 (1H, m, Ar-H), 7.17-7.28 (1H, m, Ar-H), 7.29-7.48 (7H, m, Ar-H), 7.58 (1H, d, Ar-H), 7.88 (1H, d, Ar-H), 8.75 (1H, s, Ar-H).

6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-7-(4-((3-(3-methoxyphenyl)-4-phenyl-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: 61%. M.p.: 250-251 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.77-2.04 (2H, m, cyclopropyl-H, CH_2), 3.10 (4H, s, piperazine-4H), 3.34-3.35 (1H, m, cyclopropyl-H), 3.42 (4H, s, piperazine-4H), 3.60 (3H, s, OCH_3), 3.89-3.90 (1H, m, cyclopropyl-H), 5.32 (2H, s, CH_2), 6.83 (1H, s, Ar-H), 7.02 (2H, s, Ar-H), 7.30-7.33 (1H, m, Ar-H), 7.44-7.45 (2H, m, Ar-H), 7.54 (4H, m, Ar-H), 7.93 (1H, d, Ar-H), 8.77 (1H, s, Ar-H).

6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-7-(4-((3-(2-methoxyphenyl)-4-phenyl-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: 67%. M.p.: 257-259 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.79-1.98 (2H, m, cyclopropyl-H, CH_2), 3.07 (4H, s, piperazine-4H), 3.34-3.42 (5H, m, piperazine-4H and cyclopropyl-H), 3.60 (3H, s, OCH_3), 3.89-3.90 (1H, m, cyclopropyl-H), 5.32 (2H, s, CH_2), 6.82 (1H, s, Ar-H), 7.02 (1H, s, Ar-H), 7.30-7.33 (1H, m, Ar-H), 7.43-7.53 (6H, m, Ar-H), 7.93 (1H, d, Ar-H), 8.77 (1H, s, Ar-H).

7-(4-((3-(3,4-dimethoxyphenyl)-4-phenyl-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 ***(6m)***

Yield: 57%. M.p.: 235-237 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.78-1.93 (2H, m, cyclopropyl-H, CH_2), 3.10 (4H, s, piperazine-4H), 3.38-3.42 (5H, m, piperazine-4H and cyclopropyl-H), 3.50 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.87-3.88 (1H, m, cyclopropyl-H), 5.31 (2H, s, CH_2), 6.79 (1H, s, Ar-H), 6.97-7.02 (2H, m, Ar-H), 7.43-7.44 (2H, m, Ar-H), 7.52-7.55 (4H, m, Ar-H), 7.91 (1H, d, Ar-H), 8.73 (1H, s, Ar-H).

7-(4-((3-(3,5-dimethoxyphenyl)-4-phenyl-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 ***(6n)***

Yield: 56%. M.p.: 232-234 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.75-1.78 (2H, m, cyclopropyl-H, CH_2), 3.10 (4H, s, piperazine-4H), 3.51-3.52 (1H, m, cyclopropyl-H), 3.60-3.65 (10H, m, piperazine-4H and 2×OCH_3), 3.77-3.79 (1H, m, cyclopropyl-H), 5.31 (2H, s, CH_2), 6.49 (2H, s, Ar-H), 6.58 (1H, s, Ar-H), 7.42-7.45 (3H, m, Ar-H), 7.54-7.56 (3H, m, Ar-H), 7.85 (1H, d, Ar-H), 8.66 (1H, s, Ar-H).

6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-7-(4-((4-phenyl-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 ***(6o)***

Yield: 57%. M.p.: 213-214 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.78-1.94 (2H, m, cyclopropyl-H, CH_2), 3.10 (4H, s, piperazine-4H), 3.51-3.58 (7H, m, cyclopropyl-H and 2×OCH_3), 3.68 (3H, s, OCH_3), 3.88-3.89 (1H, m, cyclopropyl-H), 5.32 (2H, s, CH_2), 6.67 (2H, d, Ar-H), 7.46 (2H, d, Ar-H), 7.51-7.52 (4H, m, Ar-H), 7.91 (1H, d, Ar-H), 8.74 (1H, s, Ar-H).

Conclusions

In summary, a series of novel 1-[(1R, 2S)-2-fluorocyclopropyl]CPFX -1,2,4-triazole-5(4*H*)-thione hybrids were designed, synthesized, and evaluated for their *in vitro* antibacterial activity against representative pathogens. The results showed that all of the hybrids were more potent than the parent 1-[(1R, 2S)-2-fluorocyclopropyl]CPFX **1**, and were comparable to CPFX and LVFX 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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Author Contribution Statement

S. Zhang, M. L. Liu and H. Y. Guo designed the study, analyzed the data, and revised the manuscript. Y. Gao, L. X. Na, Z. Xu, A. P. Wang and K. Lv conducted all experiments, collected the data and drafted the manuscript

Conflicts of Interest:

The authors declare no conflict of interest.

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Graphical Illustration

Design, Synthesis and Antibacterial Evaluation of 1-[(1R, 2S)-2-Fluorocyclopropyl]ciprofloxacin-1,2,4-triazole-5(4*H*)-thione Hybrids

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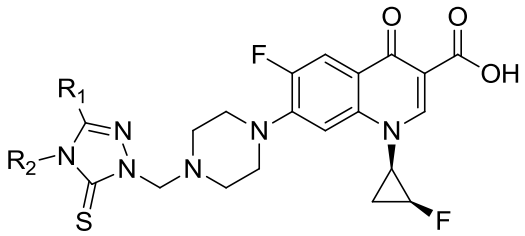

Hybrids 6a-o

A series of 1-[(1R, 2S)-2-fluorocyclopropyl]ciprofloxacin-1,2,4-triazole-5(4*H*)-thione hybrids **6a-o** were designed, synthesized and screened for their *in vitro* antibacterial activities against Gram-positive and Gram-negative bacteria. All hybrids **6a-o** have great potency against the tested strains, and were comparable to ciprofloxacin and levofloxacin against the majority of the tested pathogens, worth to be further investigated.