**SUPPLEMENTARY MATERIAL**

**Conjugation of LasR quorum-sensing inhibitors with ciprofloxacin increases antibiotic susceptibility of *P. aeruginosa* clinical strains.**

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**Synthesis of chemical compounds**

* 1. **Materials and equipment**

Proton nuclear magnetic resonance (1H NMR), carbon nuclear magnetic resonance (13C NMR) were recorded using VARIAN 400 MHz. Chemical shifts (δ) were quoted in ppm relative to residual solvent and coupling constants (J) were quoted in Hertz (Hz). Multiplicity was reported with the following abbreviations: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet;  dd = doublet of  doublet; dt = doublet of triplet, dq = doublet of quartet. All the NMR spectra were elaborated using Mestre Nova 6.0.2 software and FID data are available on request. Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR Spectrum 100 using as cell zirconium-selenium diamond. Analytical thin layer chromatography (TLC) was performed on silica gel Macherey-Nagel poligram SIL/UV 254 of 0.25 mm, visualization was achieved using UV light (254 nm) and Potassium Permanganate (KMnO4) 2% in water. Flash column chromatography were undertaken on silica gel Merck 60-200 mesh using ISOLERA ONE® (Biotage Sweden) instrument with a linear gradient of the eluting solvents and the Biotage KP-SIL cartridge. HPLC analysis were performed using a Beckmann system gold equipped with Beckmann 166 UV detector, the chromatogram were performed using a linear gradient from 0% solvent B to 100% solvent B in 25 minutes at 0.7mL flow (solvent A: Water/0.1% trifluoracetic acid, Solvent B Acetonitrile (0.1% Trifluoroacetic acid). All the chemicals were purchased from Sigma-Aldrich (Milan Italy) or Fluorochem (UK).

Molecular weights were measured with a mass spectrometer electrospray ESI MICROMASS ZMD 2000 (Micromass UK) and high resolution spectra with an Agilent ESI-Q-TOF LC/MS 6520 System equipped with a nano HPLC Chip Cube® (Agilent Technologies USA).

**1.2 Synthesis of 1-cyclopropyl-7-(4-(3-ethoxy-3-oxopropanoyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid** (**6**)

To a stirred solution of decanoic acid monoethyl ester (380mg, 1.65mmol) in dry DMF (10mL) at 0°C were added WSC (316mg, 1.65mmol) and HOBt (252mg, 1.65mmol), after 30 minutes ciprofloxacin (500mg, 1.5mmol) was added and the reaction was stirred over night at room temperature. The solvent was removed in vacuo and the crude material dissolved in 30mL of ethyl acetate. The organic layer was washed twice with 5% HCl solution, filtered off in a Gooch funnel to obtain a yellow solid in 88% yield.

**1 H-NMR:**

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**13 C-NMR**



**1.3 Synthesis of 7-(4-(2-carboxyacetyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7)**

Compound **6** (633mg, 1.16mmolo) was dissolved in 50mL of ethanol and 5 equivalent of NaOH 2N were added. The reaction was stirred over night at room temperature. The crude mixture was dryness under vacum and the corresponding oil dissolved in 30mL of ethyl acetate. The organic layer was washed twice with 1N hydrochloric acid (15mL each) and with 15mL of brine. The organic layer was dried over anhydrous sodium sulphate and evaporated under vacuum to dryness to obtain compound **7** in quantitative yield.

**1H NMR** (400 MHz, Methanol-*d*4)  7.88 (dd, *J* = 6.3, 3.1 Hz, 1H), 7.49 (dd, *J* = 6.4, 3.1 Hz, 1H), 2.29 (dt, *J* = 13.5, 7.4 Hz, 3H), 2.02 (d, *J* = 3.5 Hz, 1H), 1.59 (t, *J* = 7.3 Hz, 4H), 1.32 (m, 16H).

**13C NMR** (101 MHz, Methanol-*d*4) : 178.63, 176.99, 140.90, 129.38, 128.16, 119.60, 116.59, 112.37, 35.87, 35.73, 31.22, 31.16, 31.11, 31.05, 27.00, 26.93.

**1.4 Synthesis of 7-(4-(3-(cyclopentylamino)-3-oxopropanoyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (ET37)**

To a stirred solution of compound **7** (750mg, 1.45mmol) in 20mL of dry DMF were added WSC (306mg, 1.6mmol) and HOBt (245mg, 1.6mmol), after 10 minutes at 0°C, cyclopentilamine (136mg, 1.6mmol, 158mL) was added and the reaction was stirred 12 h at room temperature, after this time was checked by mass spectrometry analysis. The DMF was removed in vacuo and the crude material dissolved in 25mL of ethyl acetate; the organic layer was washed with 1N HCl (15mL), 5% NaHCO3 (15mL) and brine (20mL). The organic phase was dried over sodium sulphate anhydrous and evaporated under vacuum to dryness. The crude material was purified by flash chromatography (methanol/DCM; 0.5/9.5) to obtain the final compound **ET37** in 71% yield.

**1H NMR** (400 MHz, Chloroform-*d*)  7.88-7.60 (m, 1H), 7.40 (dddd, *J* = 15.0, 8.1, 6.9, 1.4 Hz, 1H), 5.86-5.72 (m, 1H), 4.15 (q, *J* = 7.0 Hz, 1H), 3.91-3.52 (m, 2H), 3.32 (dt, *J* = 27.6, 5.1 Hz, 2H), 2.21-2.04 (m, 4H), 1.94 (s, 4H), 1.72-1.49 (m, 4H), 1.45-1.13 (m, 21H).



**13C NMR** (101 MHz, CDCl3)  177.21, 173.65, 172.42, 167.34, 147.73, 128.60, 126.72, 126.09, 117.52, 111.13, 105.34, 51.55, 51.40, 49.56, 45.60, 41.38, 36.89, 35.54, 33.33, 33.16, 33.11, 29.35, 29.11, 28.99, 25.84, 25.34, 23.83, 23.77, 23.45, 8.41.



**COSY:**

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**HMQC:**

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**HMBC:**

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**19F**

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**HR-MS (ESI): [M+H]+** = 583.3293 (found), 583.3290 (calc.)

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**1.5 Synthesis of (*S*)-1-cyclopropyl-6-fluoro-4-oxo-7-(4-(3-oxo-3-((2-oxotetrahydrofuran-3-yl)amino)propanoyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid. (ET39)**

To a stirred solution of compound **7** (750mg, 1.45mmol) in 20mL of dry DMF were added WSC (306mg, 1.6mmol) and HOBt (245mg, 1.6mmol) and NMO (323mg, 3.2mmol, 318L) after 10 minutes at 0°C, homoserinelactone (162mg, 1.6mmol) was added and the reaction was stirred 12 h at room temperature, after this time was checked by mass spectrometry analysis. The DMF was removed in vacuo and the crude material dissolved in 25mL of ethyl acetate; the organic layer was washed with 1N HCl (15mL), 5% NaHCO3 (15mL) and brine (20mL). The organic phase was dried over sodium sulphate anhydrous and evaporated under vacuum to dryness. The crude material was purified by flash chromatography (methanol/DCM; 0.5/9.5) to obtain the final compound **ET39** in 63% yield.

**HPLC:**

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**HR-MS (ESI): [M+H]+** = 599.2888 (found), 599.2875 (calc.)



