

Supporting Information

Design, Synthesis and Characterization of Novel Tetrahydropyran-Based Bacterial Topoisomerase Inhibitors with potent anti-Gram positive activity.

Authors : Jean-Philippe Surivet,* Cornelia Zumbunn, Georg Rueedi, Christian Hubschwerlen, Daniel Bur, Thierry Bruyère, Hans Locher, Daniel Ritz, Wolfgang Keck, Peter Seiler, Christopher Kohl, Jean-Christophe Gauvin, Azely Mirre, Verena Kägi, Marina Dos Santos, Mika Gaertner, Michel Enderlin-Paput, Maria Boehme.

Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, CH-4123 Allschwil, Switzerland

Phone: +41 61 565 65 65. Fax: + 41 61 565 65 00.

E-mail: jean-philippe.surivet@actelion.com.

Content

HPLC-MS: Methods and Equipments	S2
HPLC-HRMS: Methods and Equipments	S2
Preparation of aldehydes 19b-27b	S3
Comparative data of 49e with Ciprofloxacin and Linezolid	S10
Representative Gels	S11
Abbreviations	S12
References	S12

HPLC-MS: Methods and equipment

HPLC : UltiMate 3000 RS pump, UltiMate 3000 column compartment (40 °C), UltiMate 3000 RS DAD, MSQ plus (all Dionex, Switzerland), Sedex 85 ELSD (Sedere, VWR Germany). Column: Zorbax SB-AQ, 5 µm, 120 Å, 4.6 mm × 50 mm (Agilent). Gradient: 5–95% acetonitrile in water containing 0.04% of trifluoroacetic acid, within 1 min. Flow: 4.5 mL/min.

MS: Waters Micromass, ZMD-platform with ESI-probe with Alliance 2790 HT.

HPLC- HRMS Methods and equipment

Analytical pump, Waters Acquity Binary, Solvent Manager. MS: SYNAPT G2MS, source temperature 150 °C, desolvation temperature 400 °C, desolvation gas flow 400 L/h. Cone gas flow: 10 L/h. Extraction cone: 4 RF. lens: 0.1 V. Sampling cone: 30. Capillary: 1.5 kV. High resolution mode. Gain: 1.0. MS function: 0.2 s per scan, 120–1000 amu in full scan, centroid mode. Lock spray: leucine enkephalin 2 ng/mL (556.2771 Da) scan time 0.2 s with interval of 10 s and average of five scans. DAD: Acquity UPLC PDA detector. Column: Acquity UPLC BEH C18 1.7 µm 2.1 mm × 50 mm from Waters, thermostated in the Acquity UPLC column manager at 60 °C. Eluents: water + 0.05% formic acid, B, acetonitrile + 0.05% formic acid. Gradient: 2–98% B over 3.0 min. Flow: 0.6 mL/min. Detection: UV 214 nm and MS; the retention time, *t*_R, is given in min.

PREPARATION OF ALDEHYDES 19b-27b

Preparation of aldehyde 19b

3,5-dibromoquinoline (SI-1). To concentrated H₂SO₄ (130 mL) was added drop wise at 0 °C, over 80 min, 3-bromoquinoline (50 g, 240 mmol) at a rate allowing the internal temperature to be maintained between 0 °C and 10 °C. After the addition was complete, NBS (48 g) was added portion wise and the reaction mixture was stirred at rt overnight. The reaction mixture was poured onto ice (2 l) and the resulting solid was dissolved in DCM (600 mL). The aq layer was further extracted with DCM (600 mL) and the combined extracts were washed with 1M NaOH (300 mL) and concentrated *in vacuo*. The residue was purified by chromatography (DCM-Et₂O) to afford **SI-1** (40 g, 58%). White solid; ¹H NMR (CDCl₃) δ: 8.94 (d, *J* = 2.2 Hz, 1H), 8.73 (d, *J* = 2.2 Hz, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 7.88 (d, *J* = 7.5 Hz, 1H), 7.62 (dd, *J* = 7.5, 8.5 Hz, 1H); ESI-MS (M+H)⁺ *m/z* = 288.1

5-bromo-3-methoxyquinoline (19a). To a mixture of sodium methoxide (14.5 g) in DMPU (350 mL) heated at 125 °C, was added in one portion intermediate **SI-1** (34.5 g, 120 mmol). The reaction was then heated at the same temperature for 1 h. The reaction mixture was then cooled to rt and poured onto ice (300 g). After the ice melt, the solid was filtered off and dried under vacuum. The filtrate was extracted with ether (4 x 150 mL). The combined extracts were washed with brine and dried over Na₂SO₄. After filtration, the solvent was evaporated and the residue purified over SiO₂ (Heptane-EtOAc 4-1) to afford a material that was pooled with the solid. The material was dissolved in DCM and dried over Na₂SO₄. After filtration and evaporation, the solid was further dried under high vacuum to afford **19a** (24.5 g, 85%). Beige solid; ¹H NMR (CDCl₃) δ: 8.68 (d, *J* = 2.8 Hz, 1H), 8.03 (d, *J* = 8.3 Hz, 1H), 7.80 (d, *J* = 7.5 Hz, 1H), 7.72 (d, *J* = 2.8 Hz, 1H), 7.42 (dd, *J* = 7.5, 8.3 Hz, 1H), 4.02 (s, 3H); ESI-MS (M+H)⁺ *m/z* = 239.7.

3-methoxyquinoline-5-carbaldehyde (19b). To a solution of **19a** (10 g, 42 mmol) in THF (250 mL) cooled to -78 °C, was added *n*-butyl lithium (2.35*N* in hexanes, 22 mL). After 15 min, a solution of DMF (10 mL) in Et₂O (20 mL) was quickly added. The solution was stirred 15 min and EtOH (5 mL), followed by 1M NaHSO₄ (40 mL), was added. After warming to rt, the organic layer was diluted with EA (100 mL). The two layers were separated and the aq layer was extracted once with EtOAc (100 mL). The combined organic layers were washed with brine and concentrated to dryness. The residue was purified by chromatography (EtOAc-Heptane) to afford **19b** (4.75 g, 60%). Yellowish solid; ¹H NMR δ (CDCl₃): 10.32 (s, 1H), 9.02 (d, *J* = 2.9 Hz, 1H), 8.75 (d, *J* = 2.9 Hz, 1H), 8.31 (d, *J* = 8.3 Hz, 1H), 8.02 (d, *J* = 7.1 Hz, 1H), 7.72 (dd, *J* = 7.1, 8.3 Hz, 1H), 4.02 (s, 3H); ESI-MS (M+H)⁺ *m/z* = 187.9.

Preparation of aldehyde 20b

3-fluoro-6-methoxyquinoline-4-carbaldehyde (20b). To a solution of DIPA (15.5 mL) in THF (300 mL), cooled to -78 °C, was added *n*-butyl lithium (2.35*N* in hexanes, 44 mL). The reaction mixture was stirred 5 min at this temperature before warming to 0 °C. The reaction mixture was stirred 15 min before cooling to -78 °C. **20a**⁴ (15 g, 84.7 mmol) in THF (50 mL + 10 mL rinse) was added and the mixture was stirred 3 h at -78 °C. DMF (3 mL, 1.2 eq.) was added quickly (within one min). After 45 min, the reaction mixture was quenched by adding 1-propanol (8 mL). The mixture was warmed to rt and extracted twice with EtOAc. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered and concentrated to dryness. The residue was triturated in Heptane to give **20b** (9.0 g, 52% yield). Orange solid; m.p.= 105.2 °C; ¹H NMR δ (CDCl₃): 10.80 (s, 1H), 8.79 (d, *J* = 1.8 Hz, 1H), 8.48 (d, *J* = 2.8 Hz, 1H), 8.01 (d, *J* = 9.2 Hz, 1H), 7.37 (dd, *J* = 2.8, 9.3 Hz, 1H), 3.98 (s, 3H). ¹³C NMR δ (CDCl₃): 188.9 (d, *J* = 12 Hz), 160.1 (d, *J* = 270 Hz), 158.8 (s), 142.6 (d, *J* = 3 Hz), 138.5 (d, *J* = 28 Hz), 131.4 (s), 125.5 (s), 122.0 (d, *J* = 3 Hz), 119.5 (d, *J* = 2 Hz), 102.9 (d, *J* = 5 Hz), 55.8 (s); HR ESI-MS (M+H)⁺ *m/z* = 206.0619 (calc. for C₁₁H₉NO₂F: 206.0617); ¹R = 1.11.

Preparation of aldehyde 21b

2-chloro-6-fluoro-3-methoxyquinoxaline-5-carbaldehyde (21c). To a solution of DIPA (22.3 mL) in THF (150 mL) was added at -20 °C, *n*-butyl lithium (2.3*M*, 63.7 mL, 159 mmol). The mixture was stirred at -20 °C for 10 min. After cooling to -78 °C, a solution of **21a**¹ (27.1 g, 127.4 mmol, in THF (100 mL + 10 mL rinse) was added over 40 min. The reddish mixture was stirred at -78 °C for 40 min. DMF (15 mL) was added keeping the internal temperature below -70 °C. The reaction proceeded 20 min. Acetic acid (15 mL) was added. Once the mixture had warmed up to rt, 3*M* HCl (180 mL) and EtOAc (500 mL) were added. The mixture was further diluted with EtOAc (400 mL) until two clear layers were obtained. The two layers were separated and the aq layer was extracted with EA (3 x 300 mL). The organic layer was washed twice with a saturated NaHCO₃ solution (200 mL) and brine (200 mL), dried over MgSO₄, filtered and concentrated to dryness. The crude product was triturated with an Et₂O-EtOAc mixture (1-1, 250 mL), washed with ether (2 x 100 mL), and dried to a constant weight to afford **21c** (23 g, 76% yield). Beige solid; ¹H NMR δ (DMSO-*d*₆): 10.96 (dd, *J* = 0.9, 1.5 Hz, 1H), 8.29 (dd, *J* = 5.6, 9.4 Hz, 1H), 7.67 (ddd, *J* = 0.6, 9.1, 10.5 Hz, 1H), 4.17 (s, 3H); ESI-MS (M+H)⁺ *m/z* = 266.0.

6-fluoro-3-methoxyquinoxaline-5-carbaldehyde (21b). Compound **21c** (23 g, 95.6 mmol) was suspended in THF (750 mL) and Et₃N (26.6 mL) was added. The resulting clear solution was treated with 10% palladium on carbon (5 g) and stirred under hydrogen atmosphere for 75 min. The reaction mixture was filtered through Celite and the catalyst was washed with water (100 mL). The two layers were extracted with EtOAc (2 x 200 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated to dryness. The residue was triturated in Et₂O and the solids were filtered off and dried to a constant weight to afford **21b** (16.6 g, 84% yield). Beige solid; ¹H NMR δ

(DMSO-*d*6): 11.15 (dd, $J = 0.6, 1.5$ Hz, 1H), 8.24 (dd, $J = 5.6, 9.4$ Hz, 1H), 7.38 (ddd, $J = 0.6, 9.4, 10$ Hz, 1H), 4.14 (s, 3H); ^{13}C NMR δ (CDCl₃): 188.8, 162.6 (d, $J = 270$ Hz), 153.3, 141.9 (d, $J = 7$ Hz), 139.7 (d, $J = 3$ Hz), 136.7 (d, $J = 12$ Hz), 135.5 (d, $J = 2$ Hz), 111.9 (d, $J = 25$ Hz), 54.4; ESI-MS (M+H)⁺ $m/z = 189.2$.

Compound **22b** was prepared from **22a** as described⁷.

Preparation of aldehyde **23b**

(*E*)-2-methoxy-8-styryl-1,5-naphthyridine (23c). Compound **23a**² (55 g, 230 mmol), *trans*-phenylvinyl boronic acid (34 g, 230 mmol) and K₂CO₃ (38 g, 272 mmol) were introduced in a two-neck flask. The atmosphere was flushed with nitrogen. 1,4-Dioxane (850 mL) and water (21 mL) were added. The mixture was stirred at rt for 5 min and Pd(PPh₃)₄ (12 g, 5 mmol) was added. The mixture was heated at reflux for 5 h. After cooling, the reaction mixture was diluted with EtOAc (400 mL) and water (50 mL). The aq layer was extracted with EtOAc (300 mL). The combined extracts were concentrated to dryness. The residue was purified by chromatography (Heptane-EtOAc) to afford **23c** (58 g, 96%). Colourless solid; ^1H NMR δ (DMSO-*d*6): 8.77 (d, $J = 4.7$ Hz, 1H), 8.28 (d, $J = 9.0$ Hz, 1H), 8.19 (d, $J = 16.7$ Hz, 1H), 8.01 (d, $J = 4.7$ Hz, 1H), 7.91 (d, $J = 16.7$ Hz, 1H), 7.74 (m, 2H), 7.34-7.40 (m, 3H), 7.30 (d, $J = 9.0$ Hz, 1H), 4.12 (s, 3H); ESI-MS (M+H)⁺ $m/z = 263.2$.

1-(6-methoxy-1,5-naphthyridin-4-yl)-2-phenylethane-1,2-diol (23d). To a mixture of **23c** (58 g, 4.8 mmol) in DCM (600 mL) and water (75 mL) were added 4-methylmorpholine N-oxide (53.4 g, 442 mmol) and K₂OsO₄·2H₂O (0.41 g, 1.1 mmol). The reaction mixture was stirred overnight at rt. 10% aq Na₂S₂O₃ (100 mL) was added and after stirring 30 min., the volatiles were removed *in vacuo*. The solid was filtered off, washed with water and dried to a constant weight, affording **23d** (40.5 g, 62% yield.). Yellowish solid; ESI-MS (M+H)⁺ $m/z = 297.1$ (M+H)⁺.

6-methoxy-1,5-naphthyridine-4-carbaldehyde (23b). To a solution of intermediate **23d** (40.5 g) and THF (200 mL) was added a solution of NaIO₄ (49.5 g, 232 mmol) in water (150 mL). The reaction mixture was stirred at rt for 30 min. The reaction mixture was diluted with water (100 mL). The volatiles were removed *in vacuo* and the solids were filtered and thoroughly washed with water and Heptane. After drying, **23b** (24 g, 83%) was recovered. Grey solid; ^1H NMR δ (DMSO-*d*6): 11.25 (s, 1H), 9.02 (d, $J = 4.4$ Hz, 1H), 8.42 (d, $J = 9.1$ Hz, 1H), 7.92 (d, $J = 4.4$ Hz, 1H), 7.40 (d, $J = 9.1$ Hz, 1H), 4.11 (s, 3H); ESI-MS (M+H)⁺ $m/z = 189.1$.

Preparation of aldehyde **24b**

(*E*)-7-fluoro-2-methoxy-8-styryl-1,5-naphthyridine (24c). The compound **24c** was prepared in 94% yield from **24a**³ using the procedure described for the preparation of **23c**. White solid; ^1H NMR δ (DMSO-*d*6): 8.79 (d, $J = 2.3$ Hz, 1 H), 8.25 (d, $J = 9.0$ Hz, 1 H), 8.07 (d, $J = 17.1$ Hz, 1 H),

7.84 (d, $J = 17:1$ Hz, 1 H), 7.69 (d, $J = 7.4$ Hz, 2 H), 7.43-7.48 (m, 2 H), 7.39 (m, 1 H), 7.23 (d, $J = 9.0$ Hz, 1 H), 4.09 (s, 3H); ESI-MS (M+H)⁺ $m/z = 281.0$.

1-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)-2-phenylethane-1,2-diol (24d). The compound **24d** was obtained in 94% yield starting from **24c** and using the procedure described for the preparation of **23d**. White foam; ¹H NMR δ (CDCl₃): 8.42 (d, $J = 0.7$ Hz, 1H), 8.28 (d, $J = 9.1$ Hz, 1H), 7.24-7.15 (m, 4H), 7.08 (m, 2H), 6.70 (br s, 1H), 5.28 (br s, 1H), 5.10 (d, $J = 7.9$ Hz, 1H), 4.11 (s, 3H), 3.85 (br s, 1H); ESI-MS (M+H)⁺ $m/z = 315.1$.

3-fluoro-6-methoxy-1,5-naphthyridine-4-carbaldehyde (24b). The compound **24b** was obtained in 94% yield starting from **24d** and using the procedure described for the preparation of **23b**. Beige solid; m.p. = 139.6 °C; ¹H NMR δ (DMSO-*d*₆): 11.08 (s, 1H), 9.01 (d, $J = 1.3$ Hz, 1H), 8.41 (d, $J = 9.1$ Hz, 1H), 7.37 (d, $J = 9.1$ Hz, 1H), 4.09 (s, 3H). ¹³C NMR δ (CDCl₃): 189.6, 163.7, 155.4 (d, $J = 277$ Hz), 141.0 (d, $J = 3$ Hz), 140.2 (s), 139.9 (d, $J = 3$ Hz), 139.6 (d, $J = 27$ Hz), 121.2 (dd, $J = 6$ Hz), 116.5 (d, $J = 3$ Hz), 54.5; HR ESI-MS (M+H)⁺ $m/z = 207.0570$ (calc. for C₁₀H₈N₂O₂F: 207.0569); ^rR = 1.06.

Preparation of aldehyde 25b

4-bromo-8-fluoro-6-methoxyquinoline (25a). To a solution of 8-fluoro-6-methoxy-quinolin-4-ol⁵ (13.2 g, 68.3 mmol) in DMF (70 mL), heated to 40 °C in a water-bath, was added PBr₃ (7 mL, 75 mmol). The mixture was stirred at this temperature for 1 h. The reaction mixture was diluted with water (0.5 l) and saturated NaHCO₃ solution was added until pH 8 was reached. The solids were filtered off, taken up in EtOAc and evaporated with silica gel (40 g). The material was eluted (Heptane-EtOAc 3-1) to afford **25a** (7.0 g, 40% yield). Yellow solid. ¹H NMR δ (DMSO-*d*₆): 8.60 (d, $J = 4.6$ Hz, 1H), 8.00 (d, $J = 4.6$ Hz, 1H), 7.48 (dd, $J = 2.6, 11.9$ Hz, 1H), 7.24 (dd, $J = 1.1, 2.6$ Hz, 1H), 3.96 (s, 3H).

(E)-8-fluoro-6-methoxy-4-styrylquinoline (25c). Compound **25c** (5.4 g, 99% yield) was obtained from **25a** (5 g, 19.52 mmol) and *trans*-phenylvinyl boronic acid (3.17 g, 1.1 eq), using the procedure described for the preparation of **23c**. Yellowish solid; ESI-MS (M+H)⁺ $m/z = 279.9$.

1-(8-fluoro-6-methoxyquinolin-4-yl)-2-phenylethane-1,2-diol (25d). Compound **25d** (5.4 g, 89% yield) was obtained from **25c** (5.4 g, 19.4 mmol) and using the procedure described for the preparation of **25d**. Beige solid; ¹H NMR (CDCl₃) δ : 8.67 (d, $J = 4.5$ Hz, 1H), 7.56 (d, $J = 4.5$ Hz, 1H), 7.23-7.14 (m, 5H), 6.96 (d, $J = 2.5, 11.5$ Hz, 1H), 6.68 (d, $J = 2.5$ Hz, 1H), 5.34 (d, $J = 6.3$ Hz, 1H), 4.97 (d, $J = 6.3$ Hz, 1H), 4.83 (br s, 1H), 3.78 (s, 3H), 3.51 (br s, 1H).

8-fluoro-6-methoxyquinoline-4-carbaldehyde (25b). Compound **25b** (2.67 g, 76% yield) was obtained from **25d** (5.3 g, 17 mmol) and using the procedure described for the preparation of **23b**.

White solid; ^1H NMR δ (DMSO-*d*6): 10.51 (s, 1H), 9.09 (d, J = 4.2 Hz, 1H), 8.21 (dd, J = 1.1, 2.6 Hz, 1H), 8.10 (d, J = 4.2 Hz, 1H), 7.47 (dd, J = 2.6, 12.0 Hz, 1H), 3.96 (s, 3H); ESI-MS (M+H) $^+$ m/z = 205.8.

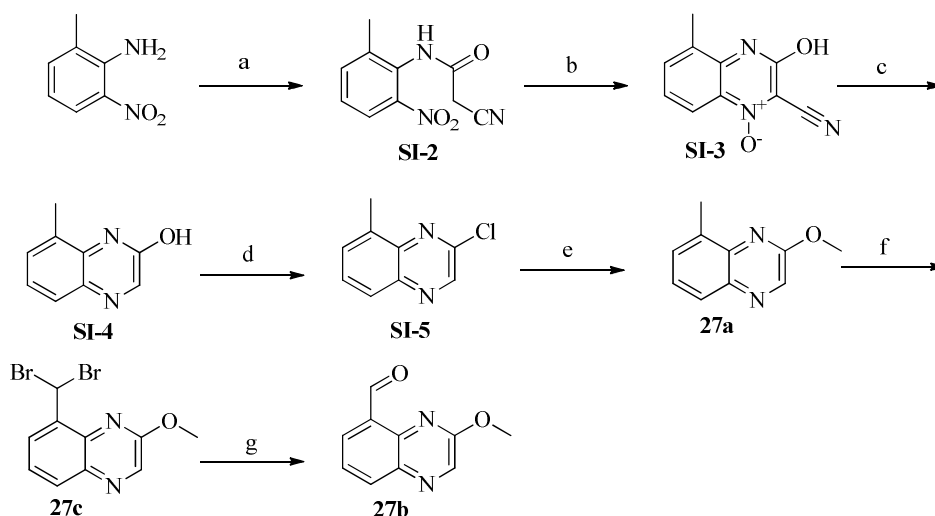
Preparation of aldehyde 26b

(*E*)-2-methoxy-8-styrylquinoline (26c). Compound **26c** (5.98 g, 75% yield) was from 2-methoxyquinolin-8-yl trifluoromethanesulfonate **26⁶** (9.37 g, 30.5 mmol) and *trans*-phenylvinyl boronic acid (4.89 g, 1.05 eq), using the procedure described for the preparation of **23c**. Yellowish solid; ESI-MS (M+H) $^+$ m/z = 262.1.

1-(2-methoxyquinolin-8-yl)-2-phenylethane-1,2-diol (26d). Compound **26d** (2.24 g, 33% yield) was obtained from **26c** (5.98 g, 19.4 mmol) and using the procedure described for the preparation of **23d**. White solid; m.p. = 84.1 °C; ^1H NMR δ (DMSO-*d*6): 8.20 (d, J = 8.9 Hz, 1 H), 7.84 (m, 1 H), 7.76 (dd, J = 1.3, 8.0 Hz, 1 H), 7.44 (t, J = 7.6 Hz, 1 H), 7.31-7.35 (m, 2 H), 7.17-7.22 (m, 2 H), 7.12 (m, 1 H), 6.97 (d, J = 8.8 Hz, 1 H), 5.76 (dd, J = 4.1, 6.5 Hz, 1 H), 5.27 (d, J = 6.5 Hz, 1 H), 5.15 (d, J = 6.0 Hz, 1 H), 4.92 (dd, J = 4.1, 6.0 Hz, 1 H), 4.03 (s, 3H); ^{13}C NMR (CDCl₃) δ : 161.2, 144.1, 140.6, 140.2, 133.2, 130.3, 127.8 (2C), 127.6, 127.3, 127.0 (2C), 125.2, 123.7, 113.0, 81.8, 78.0, 53.9; HR ESI-MS (M+H) $^+$ m/z = 296.1289 (calc. for C₁₈H₁₈NO₃: 296.1286); ^tR = 1.15.

8-fluoro-6-methoxyquinoline-4-carbaldehyde (26b). Compound **26b** (1.23 g, 88% yield) was obtained from **26d** (2.2 g, 7.4 mmol) and using the procedure described for the preparation of **23b**. White solid; m.p. = 90.7 °C; ^1H NMR δ (DMSO-*d*6): 11.26 (d, J = 0.6 Hz, 1 H), 8.40 (d, J = 9.0 Hz, 1 H), 8.26 (dd, J = 1.5, 8.0 Hz, 1 H), 8.14 (dd, J = 1.6, 7.4 Hz, 1 H), 7.58-7.63 (m, 1 H), 7.18 (d, J = 8.9 Hz, 1 H), 4.09 (s, 3 H); HR ESI-MS (M+H) $^+$ m/z = 188.0714 (calc. for C₁₁H₁₀NO₂: 188.0711); ^tR = 1.22.

Preparation of aldehyde 27b^a



^aReagents : (a) cyanoacetic acid, PCl₅, benzene, 70 °C; (b) 1M NaOH, pyridine, rt; (c) NaHSO₃, aq EtOH, 60 °C; (d) POCl₃, 110 °C; (e) MeONa, DMF, 45 °C; (f) NBS, AIBN, CCl₄, rt; (g) AgNO₃, aq EtOH, rt.

2-cyano-N-(2-methyl-6-nitrophenyl)acetamide (SI-2). To a solution of 2-methyl-6-nitroaniline (25 g, 164.3 mmol) in benzene (200 mL) were added cyanoacetic acid (14.5 g, 170.46 mmol) and PCl₅ (35 g, 168 mmol). The reaction mixture was heated at 60 °C for 7 h. After cooling to rt, the reaction mixture was filtered and the solid was washed with benzene and water. The solid was dried *in vacuo* to afford **SI-2** (24 g, 66% yield). Yellow solid; ¹H NMR δ (DMSO-*d*₆): 10.2 (s, 1H), 7.78 (d, *J* = 8.3 Hz, 1H), 7.65 (d, *J* = 8.3 Hz, 1H), 7.43 (t, *J* = 8.3 Hz, 1H), 3.95 (s, 2H), 2.30 (s, 3H).

2-cyano-3-hydroxy-5-methylquinoxaline 1-oxide (SI-3). To a solution mixture of **SI-2** (24 g, 109.5 mmol) in 1M aq NaOH (100 mL) was added pyridine (100 mL). The reaction mixture was stirred at rt for 4 h. The pH was adjusted to 6 by addition of 1M HCl. The solid was filtered off and washed with water. The solid was triturated with EtOH and dried to a constant weight, yielding **SI-3** (17.7 g, 80% yield). Yellow solid; ESI-MS (M+H)⁺ *m/z* = 202.1.

8-methylquinoxalin-2-ol (SI-4). To a solution of **SI-3** (17.7 g, 87.9 mmol) in water (300 mL) and EtOH (24 mL) was added sodium dithionite (35.4 g, 203.9 mmol). The reaction mixture was heated at 60 °C for 1 h. The reaction mixture was filtered till warm, and the pH of the filtrate was adjusted to 2 by adding 1M HCl. The pH of the solution was subsequently made basic by adding solid NaOH (10 g). EtOAc (150 mL) was added. The aq layer was extracted twice more with EtOAc (2 x 150 mL). The combined organic extracts were dried over Na₂SO₄, filtered and concentrated to dryness. The residue was dried to a constant weight to afford **SI-4** (11.1 g, 78% yield). Yellow solid; ¹H NMR (DMSO-*d*₆) δ: 11.75 (br s, 1H), 8.17 (s, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.21 (t, *J* = 8.4 Hz, 1H), 2.42 (s, 3H); ESI-MS (M+H)⁺ *m/z* = 161.1.

2-chloro-8-methylquinoxaline (SI-5). A solution of intermediate **SI-4** (11.1 g, 69.5 mmol) in POCl₃ (80 mL) was heated at 110 °C during 2 h. After cooling to rt, the reaction mixture was poured onto ice (200 g). The aq layer was extracted with EtOAc (2 x 200 mL). The combined extracts were washed with brine (100 mL), dried over Na₂SO₄, filtered and concentrated to dryness. The residue was purified by chromatography (Heptane- EtOAc 1-1) to afford **SI-5** (12.5 g, 99% yield). Reddish solid; ¹H NMR (DMSO-*d*₆) δ: 8.99 (s, 1H), 7.97 (m, 1H), 7.80 (m, 2H), 2.68 (s, 3H); ESI-MS (M+H)⁺ m/z = 179.2.

2-methoxy-8-methylquinoxaline (27a). To a solution of **SI-5** (12.5 g, 69.5 mmol) in DMF (80 mL) was added sodium methoxide (9 g, 166 mmol). The reaction mixture was heated at 45 °C for 4 h. After cooling to rt, the reaction mixture was partitioned between water (10 mL) and EtOAc (200 mL). The organic layer was washed once with water (100 mL), dried over Na₂SO₄, filtered and concentrated to dryness. The residue was purified by chromatography (Heptane- EtOAc 1-4) to afford **27a** (10.2 g, 84% yield). Yellow solid; ¹H NMR δ (CDCl₃): 8.48 (s, 1H), 7.88 (d, *J* = 7.9 Hz, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.47 (t, *J* = 7.9 Hz, 1H), 4.12 (s, 3H), 2.69 (s, 3H); ESI-MS (M+H)⁺ m/z = 175.4.

8-(dibromomethyl)-2-methoxyquinoxaline (27c). To a solution of compound **27a** (10.2 g) in CCl₄ (560 mL) were added AIBN (0.96 g) and NBS (25.9 g, 145.5 mmol). The reaction mixture was heated at 80 °C for 3 h. After cooling to rt, the reaction mixture was washed with water (200 mL) and the organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was triturated with MeOH to give, after drying under high vacuum, **27c** (14.4 g, 43.3 mmol). Beige solid; ¹H NMR (DMSO-*d*₆) δ: 8.69 (s, 1H), 8.25 (dd, *J* = 1.3, 7.5 Hz, 1H), 8.07 (dd, *J* = 1.3, 8.3 Hz, 1H), 8.02 (s, 1H), 7.74 (dd, *J* = 7.5, 8.3 Hz, 1H), 4.14 (s, 3H); ESI-MS (M+H)⁺ m/z = 332.8.

3-methoxyquinoxaline-5-carbaldehyde(27b). To a solution of **27c** (10.7 g, 32.2 mmol) in EtOH (330 mL) was added, at rt, a solution of AgNO₃ (15 g) in water (70 mL). The reaction was stirred at rt for 1 h. The reaction mixture was diluted with CH₃CN (200 mL) and the solids were filtered off. The filtrate was concentrated *in vacuo*. The residue was filtered over a silica gel pad (EtOAc) to afford **27b** (6.2 g, 99% yield). Yellow solid; ¹H NMR δ (DMSO-*d*₆): 11.15 (s, 1H), 8.74 (s, 1H), 8.36 (dd, *J* = 1.3, 8.1 Hz, 1H), 8.21 (dd, *J* = 1.3, 7.9 Hz, 1H), 7.80 (dd, *J* = 7.9, 8.1 Hz, 1H), 4.14 (s, 3H); ESI-MS (M+H)⁺ m/z = 189.2.

Comparative antibacterial activities of 49e and reference antibiotics against clinical isolates⁸

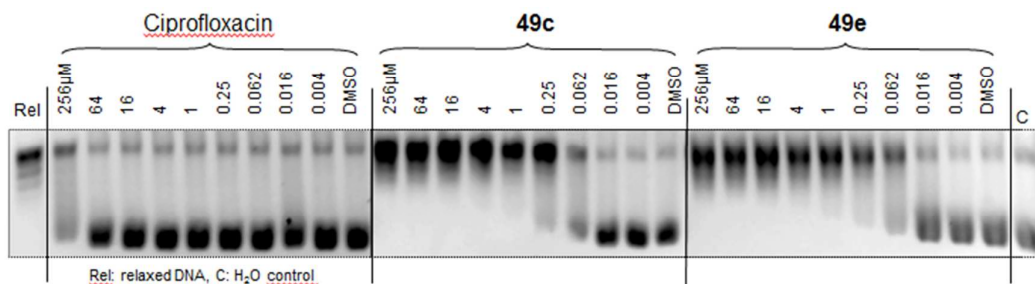
Organism (n)	Cpds ^a	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	Range (mg/L)
<i>S. aureus</i> (35) including MRSA (25)	49e	≤ 0.015	0.03	≤ 0.015-0.06
	CIP	32	>32	0.25->32
	LZD	2	4	0.5->32
	49e	≤ 0.015	0.03	≤ 0.015-0.03
	CIP	>32	>32	0.5->32
	LZD	2	4	0.5-4
<i>S. epidermidis</i> (16)	49e	0.06	0.25	0.03-0.25
	CIP	8	16	0.125->32
	LZD	1	2	1-2
<i>Enterococcus faecalis</i> (20)	49e	0.5	0.5	0.06-1
	CIP	1	>32	0.5->32
	LZD	2	4	0.5-8
<i>Enterococcus faecium</i> VRE (20)	49e	0.25	1	0.03-4
	CIP	16	>32	0.5->32
	LZD	2	4	1->32
<i>S. pneumoniae</i> (24)	49e	0.06	0.125	0.03-0.25
	CIP	2	16	0.125-16
	LZD	2	2	0.5-2
<i>Streptococcus pyogenes</i> (14)	49e	0.03	0.06	0.015-0.125
	CIP	0.5	0.5	0.25-1
	LZD	1	2	0.5-4
<i>Streptococcus agalactiae</i> (13)	49e	0.25	0.5	0.125-0.5
	CIP	1	1	0.5-1
	LZD	2	2	1-4
<i>H. influenzae</i> (11)	49e	0.5	4	0.25-8
	CIP	≤ 0.015	≤ 0.015	≤ 0.015->8
	LZD	16	32	4-32
<i>M. catarrhalis</i> (11)	49e	0.03	0.125	≤ 0.015-125
	CIP	0.03	0.03	≤ 0.015-0.03
	LZD	8	8	2-8

^a. CIP, ciprofloxacin; LZD, linezolid.

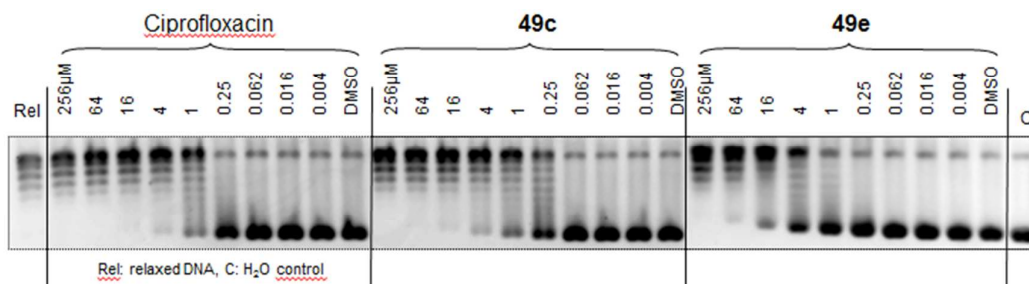
Representative Gels for Supercoiling Inhibitory Activity and Relaxation Inhibitory Activity assays of NBTIs 49c, 49e and Ciprofloxacin.

Supercoiling Inhibitory Activity assay

***S. aureus* Gyrase**

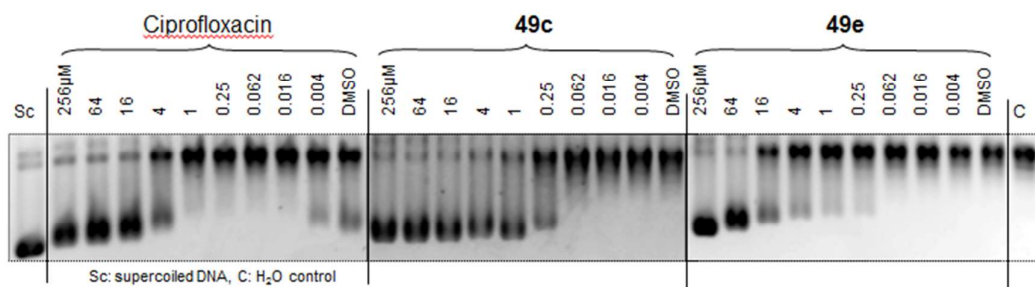


***E. coli* Gyrase**

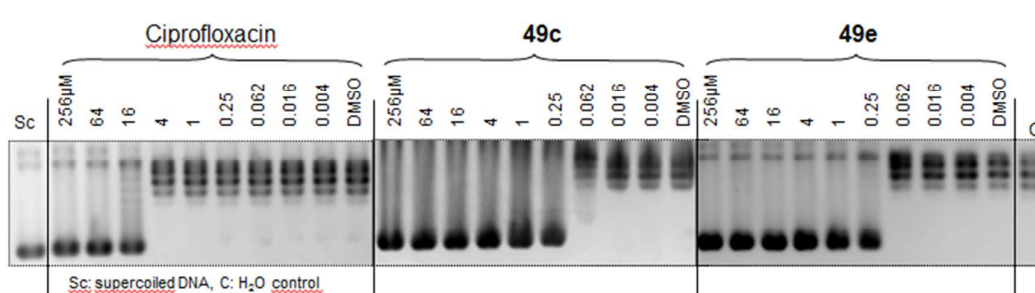


Relaxation Inhibitory Activity assay

***S. aureus* TopoIV**



***E. coli* TopoIV**



ABBREVIATIONS USED

BINAP, 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl; DIPA diisopropylamine; DIAD, diisopropyl aza-1,2-dicarboxylate; DIPEA, diisopropylethylamine; KHMDS, potassium bis(trimethylsilyl)amide; LiHMDS, lithium bis(trimethylsilyl)amide; Et₂O, diethyl ether; EtOAc, ethyl acetate; EtOH, ethanol; Pd(PPh₃)₄, tetrakis(triphenylphosphine)palladium(0); (dba)₃Pd₂.CHCl₃, tris-(dibenzylideneacetone)dipalladium(0) chloroform complex

REFERENCES

1. Sarges, R.; Howard, H. R.; Browne, R. G.; Lebel, L.A.; Seymour, P. A.; Koe, B. K. 4-Amino[1,2,4]triazolo[4,3-*a*]quinoxalines. A novel class of potent adenosine receptor antagonists and potential rapid-onset antidepressants. *J. Med. Chem.* **1990**, *33*, 2240-2254
2. Commercially available; **23a** can be alternatively prepared as described in Allison, B. D.; Gomez, L.; Grice, C. A.; Hack, M. D.; Morrow, B. J.; Motley, S. T.; Santillan, A.; Shaw, K. J.; Schwarz, K. L.; Tang, L. Y.; Venkatesan, H.; Wiener, J. J. M. US2006223810, 2006.
3. Miller, W. H.; Rouse, M. B.; Seefeld, M. A. WO200681178, 2006.
4. Li, B.; Zhang, Z.; Mangano, M. A Facile Two-Step Synthesis of 3-Fluoro-6-methoxyquinoline. *Org. Process Res. Dev.* **2008**, *12*, 1273-1275.
5. Davies, D. T.; Jones, G.E.; Peightfoot, A.; Markwell, R. E.; Pearson, N. D. US2004/38998, 2004.
6. Axten, J. M.; Daines R. A.; Davis, D. T.; Gallagher T. F.; Jones G. E.; Miller W. H.; Pearson N. D.; Pendrak, I. WO20042490, 2004.
7. Mao, J.; Yuan, H.; Wang, Y.; Wan, B.; Pieroni, M.; Huang, Q.; van Breemen, R. B.; Kozikowski, A. P.; Franzblau, S. G. From Serendipity to Rational Antituberculosis Drug Discovery of Mefloquine-Isoxazole Carboxylic Acid Esters. *J. Med. Chem.* **2009**, *52*, 6966-6978.
8. Clinical Laboratory and Standards Institut. *Methods for Dilution Susceptibility Test for Bacteria That Grow Aerobically*; Approved Standard Seventh Edition, document. M7-A7, Clinical Laboratory and Standards Institut, Wayne, PA, USA, 2006.