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Derivatives of 8-hydroxyquinoline—antibacterial agents that target intra- and extracellular Gram-negative pathogens

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ABSTRACT

Small molecule screening identified 5-nitro-7-((4-phenylpiperazine-1-yl-)methyl)quinolin-8-ol **INP1750** as a putative inhibitor of type III secretion (T3S) in the Gram-negative pathogen *Yersinia pseudotuberculosis*. In this study we report structure–activity relationships for inhibition of T3S and show that the most potent compounds target both the extracellular bacterium *Y. pseudotuberculosis* and the intracellular pathogen *Chlamydia trachomatis* in cell-based infection models.

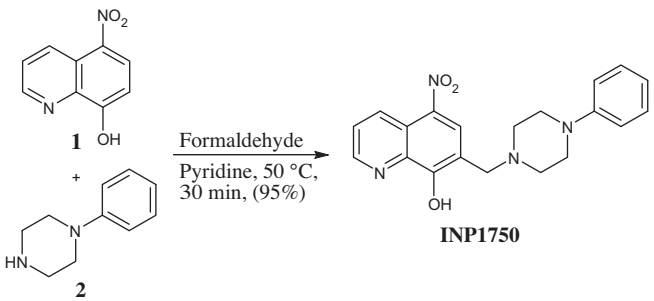
Resistance to antibiotics is one of the major challenges facing treatment of bacterial infections, highlighting the need for novel antibacterial compounds that are effective towards resistant strains. The compounds should have novel modes of action and structural scaffolds different from those of current antibiotics. Such compounds should show antibacterial activity against resistant strains and a responsible use could possibly reduce the frequency of resistance to the new drugs.

Many clinically relevant Gram-negative bacteria utilize T3S to inject toxins into the cytosol of eukaryotic cells and thereby create an environment that allows the bacteria to grow and establish an infection.[1] We have previously used a bacterial reporter-gene assay in *Yersinia pseudotuberculosis* to screen a 9400 compound library for putative inhibitors of bacterial type III secretion (T3S).[2] Three classes of compounds have been pursued[3–9] and inhibitors active against multiple species including *Yersinia*, *Salmonella*, *Chlamydia*, *Escherichia coli*, and *Shigella* were identified.[3,10–16] Compounds have been tested in vivo and the results indicate that T3S inhibitors have a potential for therapy or prevention.[14,17,18]

The T3S reporter-gene assay has also been used in a large screen to identify the known natural product spermatinamine and four new derivatives, pseudoceramine A–D, from a marine sponge *Pseudoceratina* sp. as general antibiotics.[19]

Based on the experience from the first screening campaign and post-screening activities we decided to screen an additional collection consisting of 17,500 synthetic small organic molecules with the hope to find additional T3S inhibitors belonging to new chemotypes. The 8-hydroxyquinoline derivative **INP1750** (Scheme 1, Table 1) was identified as a single hit.[20] **INP1750** and analogs can conveniently be assembled in a one-step synthesis according to classic Mannich chemistry (Scheme 1).[21]

We decided to establish structure–activity relationships by synthesis of compounds using commercially available 8-hydroxy-



Scheme 1. Synthesis of **INP1750**.

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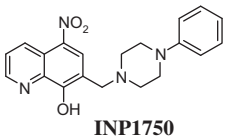
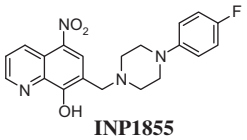
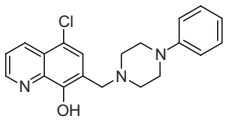
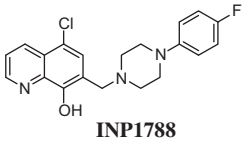
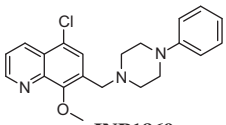
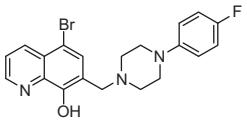
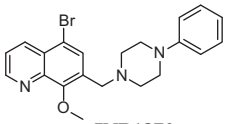
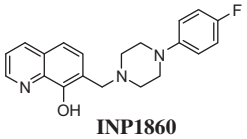
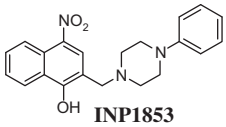
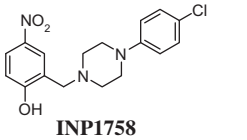
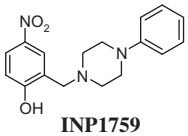
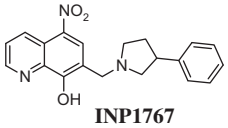
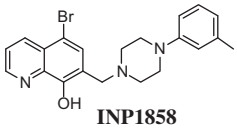
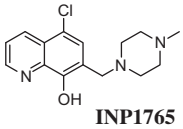
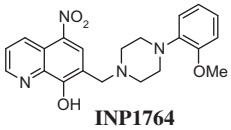
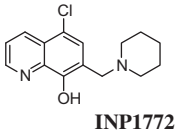
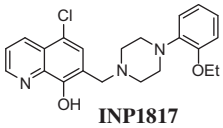
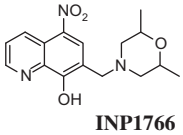
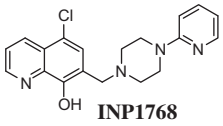
Table 1
Effect of the synthesized compounds on the reporter-gene signal in *Y. pseudotuberculosis*

Substance	Reporter gene inhibition[a]				Substance	Reporter gene inhibition[a]			
	10 μM	20 μM	50 μM	100 μM		10 μM	20 μM	50 μM	100 μM
 **INP1750**	66 49[b]	88 73[b]	94 77[b]	97 79[b]	 **INP1855**	17[b] 38[b,c]	56[b] 66[b,c]	88[b] 81[b,c]	94[b] 83[b,c]
 **INP1813**	NA NA[c]	NA NA[c]	14 22[c]	9 74[c]	 **INP1788**	NA	NA	93	97
 **INP1869**	NA	NA	NA	NA	 **INP1769**	NA	NA	78	89
 **INP1870**	NA[c]	NA[c]	NA[c]	NA[c]	 **INP1860**	NA[c]	NA[c]	58[c]	95[c]
 **INP1853**	NA[c]	NA[c]	NA[c]	NA[c]	 **INP1758**	NA	NA	NA	NA
 **INP1759**	NA	NA	NA	NA	 **INP1767**	54 47[b]	79 64[b]	95 68[b]	98 69[b]
 **INP1858**	NA	NA	41	81	 **INP1765**	NA	NA	NA	48
 **INP1764**	NA	25	77	85	 **INP1772**	NA	NA	8	12
 **INP1817**	NA	NA	37	89	 **INP1766**	NA	NA	76	93
 **INP1768**	NA	NA	80	98					

NA = no activity.
[a] Percent inhibition.
[b] Percent inhibition of phosphatase activity from secreted YopH.
[c] Experiment performed on the HCl salt.

quinolines, phenols, and cyclic secondary amines. In total 19 compounds[22] were screened in the original T3S-linked reporter-gene assay at four concentrations (Table 1).[2,6] The three most potent compounds, **INP1750**, **INP1767**, and **INP1855**[23] were then tested for inhibition of bacterial growth and inhibition of phosphatase activity originating from the secreted tyrosine phosphatase YopH as described previously.[6,7] The compounds showed a clear dose-response effect in both the reporter-gene assay and the YopH assay (Table 1)

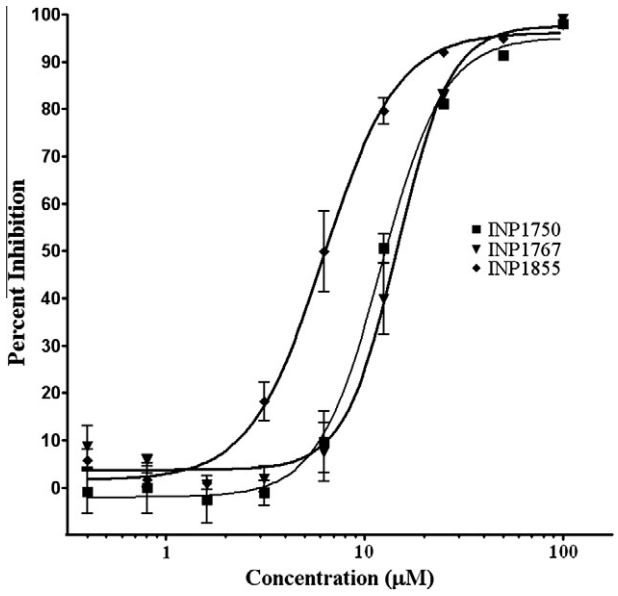


Figure 1. Dose-response curves for **INP1750**, **1767**, and **1855** in the T3S reporter-gene assay.

Table 2
EC_{50} values for the reporter-gene signal in *Y. pseudotubercolosis*, MIC for *C. trachomatis* infection of HeLa cells, and HeLa cell viability for **INP1750**, **INP1767**, and **INP1855**

Substance	EC_{50}[a]	C. trachomatis MIC	Cell viability[b]
INP1750	12.4	25	66.3 ± 5.4
INP1767	14.6	12.5	92.1 ± 2.4
INP1855	6.24	3.13	90.2 ± 4.0

[a] Calculated from Figure 2 using GraphPad prism.
[b] Percent cell viability (±SD) at 2× MIC.

with EC_{50} values between 6 and 12 μM (Fig. 1, Table 2). **INP1750** and **INP1855** did not affect bacterial growth at 100 μM and **INP1767** showed no effect at 50 μM for periods up to 6 h (data not shown).[6] The effect of the compounds in the bacterial reporter-gene assay was unaffected in presence of detergent indicating that compound aggregation is not an underlying reason for the observed activity (data not shown).[24] This compound class clearly constitutes putative T3S inhibitors that either target the secretion machinery directly or act on regulatory pathways.[25]

From the data in Table 1 some conclusions regarding structure–activity relationships can be drawn. It is clear that the fused pyridine ring is critical since **INP1759** and **INP1758**, that lack the ring completely, and the naphthalene based **INP1853** are void of activity. The aromatic hydroxyl group appears to be preferred as the corresponding methyl ethers **INP1869** and **INP1870** are inactive. The nitro group in **INP1750** can be exchanged for a bromo or chloro substituent but when comparing **INP1750** with **INP1813** and **INP1855** with **INP1788** it is obvious that the nitro group is superior. **INP1860** that lacks a substituent in this position still shows moderate activity at higher concentrations but it was found that this effect most likely results from general growth inhibition (data not shown). Modification of the *N*-phenyl piperazine moiety in **INP1750** can be executed resulting in retained or improved activity as shown by **INP1767** and **INP1855**. **INP1767** was tested in racemic form and it remains to be established if the individual stereoisomers display different biological profiles.

The three most promising compounds **INP1750**, **INP1767**, and **INP1855** were further evaluated in a macrophage infection model as described previously.[6,26] The macrophage cell line J774 was infected with wild-type *Y. pseudotuberculosis*. The T3S system translocates toxins into the macrophage cytosol resulting in cell death or reduced viability. The status of the J774 cells was monitored using CalceinAM, which is converted to a green fluorescent molecule in healthy cells. The bacteria cause a reduction of cell viability and this effect was reversed by all three compounds (Fig. 2a). The compounds show little effect when the macrophages are infected with a non-virulent T3S-deficient mutant (Fig. 2b) and display modest toxicity as observed for treated uninfected macrophages (Fig. 2c).

We have previously shown that compounds active in *Y. pseudotuberculosis* are active against multiple bacterial species including *Chlamydia*. As a next step we evaluated **INP1750**, **INP1767**, and **INP1855** against *Chlamydia trachomatis* in an ex vivo infection model as described previously.[6,26] All three compounds proved to be active also against *C. trachomatis* with minimum inhibitory concentrations (MICs) of 3–25 μM (Table 2).

Cell viability was scored with rezazurin[27,28] at concentrations twice the MIC values. **INP1750** was somewhat toxic as also noted in the *Yersinia* infection model (cf. Fig. 2) while the most potent compounds **INP1767** and **INP1855** result in cell viability >90% (Table 2). The effect of **INP1855** on growth of *C. trachomatis* was studied by fluorescence microscopy after immunostaining (Fig. 3). In absence of inhibitor large *Chlamydia* inclusions are visible (Fig. 3, left panel). At 1.56 μM of **INP1855** the size of the inclusions are slightly reduced and at the MIC, 3.13 μM, no inclusions are visible and HeLa cell morphology is similar to the DMSO treated control (Fig. 3, middle and right panels).

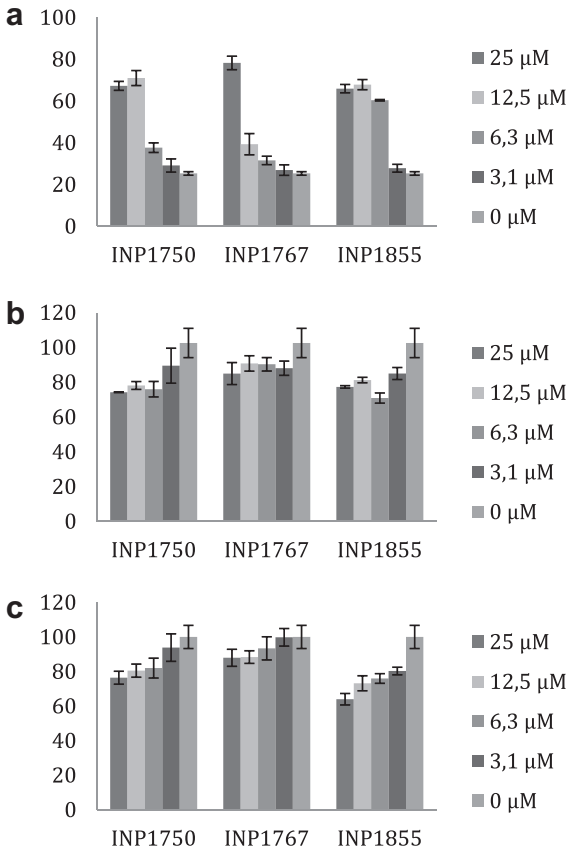


Figure 2. Effect of **INP1750**, **INP1767**, and **INP1855** on T3S mediated virulence in a macrophage infection model.[6,26] Macrophage health was measured according to the CalceinAM method. 100% on the *y*-axis corresponds to uninfected cells treated with DMSO alone. (a) Wild-type *Y. pseudotuberculosis* (pIB102), (b) T3S deficient mutant (pIB604), (c) uninfected control.

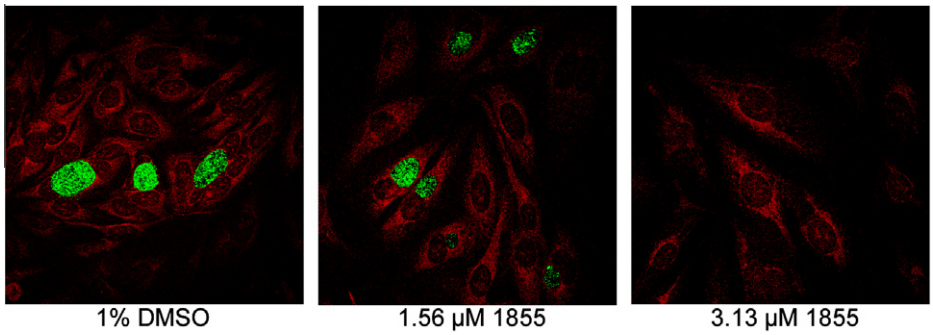


Figure 3. Effect of **INP1855** on growth of *C. trachomatis* in HeLa cells.[6,26] The HeLa cells are stained red and the *C. trachomatis* inclusions are stained green (pathfinder chlamydia, bio-rad).

In conclusion we have employed whole cell screening to identify putative inhibitors of T3S in Gram-negative bacteria. The identified inhibitors are based on a different chemical scaffold than previously reported small molecule T3S inhibitors.[29–35] Subsequent investigation of SARs resulted in inhibitors that show low micromolar activity against both the extracellular pathogen *Y. pseudotuberculosis* and the intracellular pathogen *C. trachomatis*.

Acknowledgments

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20. The screening was performed at Umeå Small Molecule Screening Facility that currently is incorporated as the screening platform in Laboratories for Chemical Biology Umeå. The screening was performed essentially as described in Ref. 1 with the differences that the compound DMSO solutions were transferred using a 96-channel pipetting instrument and the luminescence signal was recorded with a conventional filter-based plate reader.
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22. ***INP1758***, ***1759***, ***1764***, ***1765***, ***1766***, ***1768***, ***1769***, ***1772***, and ***1788*** were purchased from ChemBridge. All other compounds were synthesized in house or by Syngene International Ltd. Typical experimental procedure: All starting materials and reagents were commercially available and used as received. Formaldehyde (75 mmol) and the cyclic secondary amine (20 mmol) were reacted under cooling on ice and the resulting hard white precipitation was added portionwise to the 8-hydroquinoline derivative (20 mmol) dissolved in 60 mL pyridine under stirring at 50 °C. The added precipitate reacted readily and after a couple of minutes a yellowish precipitation was formed. Aftert 30–40 min the mixture was filtered through a Büchner funnel. The precipitate was washed with 150 mL ethanol, dissolved in 1 M HCl under heating and crystallized by addition of 1 M NaOH under gentle stirring until pH 5–6. The crystals were washed with water and the isolated product was >95% pure according to LC–MS and ^{1}H NMR spectroscopy.
23. *5-Nitro-7-((4-phenylpiperazin-1-yl)methyl)quinolin-8-ol* (***INP1750***): ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.19 (dd, J = 1.4, 8.7 Hz, 1H), 8.72 (dd, J = 1.2, 4.1 Hz, 1H), 8.6 (s, 1H), 7.64 (dd, J = 4.1, 8.7 Hz, 1H), 7.24–7.20 (m, 2H), 6.95–6.93 (m, 2H), 6.82–6.78 (m, 1H), 4.1 (s, 2H), 3.72–3.35 (m, 4H), 3.12–3.10 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 150.2, 147.1, 139.8, 132.5, 132.2, 129.0, 127.4, 124.6, 124.3, 119.3, 116.0, 115.8, 115.7, 55.7, 51.7, 46.7. LC-MS $[M-H^+]^-$ Calcd for $[C_{20}H_{20}N_4O_3]$: m/z 363.15. Found 363.17. *5-nitro-7-((3-phenylpyrrolidin-1-yl)methyl)quinolin-8-ol* (***INP1767***): ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.31–9.29 (m, 1H), 8.65 (s, 1H), 8.58 (br s, 1H), 7.54 (br s, 1H), 7.33 (s, 4H), 7.24 (br s, 1H), 4.38–4.35 (m, 2H), 3.83–3.79 (m, 1H), 3.66–3.52 (m, 3H), 3.36–3.31 (m, 1H), 2.39 (m, 1H), 2.13–2.10 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 146.3, 140.8, 134.6, 132.6, 129.2, 129.1, 127.8, 127.6, 127.5, 127.1, 125.1, 122.7, 115.5. LC-MS $[M-H^+]^-$ Calcd for $[C_{20}H_{19}N_3O_3]$: m/z 348.14. Found 348.19. *7-((4-(4-fluorophenyl)piperazin-1-yl)methyl)-5-nitroquinolin-8-ol* (***INP1855***): ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.53 (br s, 1H), 9.31 (dd, 1.4, 8.9 Hz, 1H), 9.04 (dd, 1.4, 4.3 Hz, 1H), 9.01 (s, 1H), 8.00 (dd, 4.4, 8.9 Hz, 1H), 7.11–7.06 (m, 2H), 7.03–7.00 (m, 2H), 4.58 (s, 2H), 3.70 (br s, 2H), 3.52 (br s, 2H), 3.30 (br s, 2H), 3.21 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.2, 156.8 (d, 237.3 Hz), 147.7, 146.2, 136.3, 134.7, 133.5, 132.3, 126.1, 123.6, 118.0 (d, 7.8 Hz), 115.6 (d, 22.4), 111.3, 52.5, 50.3, 46.2. LC-MS $[M-H^+]^-$ Calcd for $[C_{20}H_{19}FN_4O_3]$: m/z 381.14. Found 381.24.
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