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Discovery of antibiotics and their targets in multidrug-resistant bacteria

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Chapter 2

Phenotypic antibacterial screen of an in-house compound library

Introduction

There is a great need for new antibiotic treatments to counteract antimicrobial resistance (AMR). The WHO has listed AMR to be one of the biggest health threats facing humanity, with the biggest contributors to the problems being multidrug resistant bacteria, also known as 'superbugs'.[1–3] If left unchecked, the death toll caused by AMR is expected to overtake that of cancer in 2050.[4] Recently, the WHO published a priority list of especially dangerous pathogens. The largest part of their critical-priority pathogens consists of drug-resistant *E. coli* and the "ESKAPE" pathogens. ESKAPE is an acronym for the bacteria *Enterococcus faecium*, *Staphylococcus aureus* (Gram-positive, high-priority), *Klebsiella pneumoniae*, *Acinetobacter*

baumannii, *Pseudomonas aeruginosa*, and *Enterobacter spp.* (Gram-negative, critical-priority). These pathogens are responsible for the majority of nosocomial infections and they often carry high levels of resistance against existing classes of antibiotics.[5,6] Recently, resistance has been detected against antibiotics of last resort.[7–9] To prevent these infections from becoming untreatable, new antibiotics are urgently needed.

To discover new drugs, target-based screening is commonly applied to identify small molecules as chemical starting points (hits), but this strategy is less successful in antibiotic research.[10] This is due to the numerous barriers that bacteria have in comparison to human cells, such as the peptidoglycan layers that constitute the bacterial cell wall as well as efflux mechanisms.[11–13] These difficulties are amplified in Gram-negative bacteria, which have an additional outer membrane layer with orthogonal permeability requirements to the inner membrane.[14–16] As we currently lack generally applicable strategies to optimize hits to circumvent these barriers, phenotypic screening approaches remain of great value in the field of antibiotic research.[17] This entails searching for a hit that already overcomes these barriers, and later finding out the specific working mechanisms.

The classical antibiotic research platform involved mining natural products for antibacterial activity, but in recent decades this has led to reduced output.[18] An alternative approach is to screen synthetic compound libraries for antibacterial activity. These libraries are generally derived from traditional drug discovery efforts and, therefore, are biased towards the tenets of drug-likeness. Recent successes, however, have inspired curiosity to explore existing sets of compounds.[19,20]

In this chapter, an in-house compound library was compiled and screened for antibacterial activity against methicillin-resistant *S. aureus* (MRSA), a model for Gram-positive bacteria, and *E. coli*, a model for Gram-negative bacteria. This resulted in various hits against both organisms that were subsequently validated through resynthesis and structure confirmation.

Results

Library set-up and screening. To construct a unique chemical library, a selection was made of diverse compounds previously developed as part of other projects within the Molecular Physiology group. These compounds were neither designed or previously tested for antibacterial purposes, and therefore provided potential unique antibacterial chemotypes. 352 compounds were tested for antimicrobial activity at a single 100 μM concentration (Figure 2.1) against both MRSA, the Gram-positive model, and *E. coli*, the Gram-negative model. The screen identified 20 hits that were able to inhibit the growth of MRSA, and 12 hits that inhibited growth of *E. coli*. Subsequently, these hits were further explored using a minimum inhibitory concentration (MIC) assay. In MIC assays, bacteria are treated with a concentration range of compounds. The lowest concentration at which a visible inhibitory effect is seen is defined as the MIC. In both MRSA and *E. coli* a single hit was found with a MIC of 6.25 μM. The MRSA hit is termed hit **1**, and the *E. coli* hit is termed hit **2**.

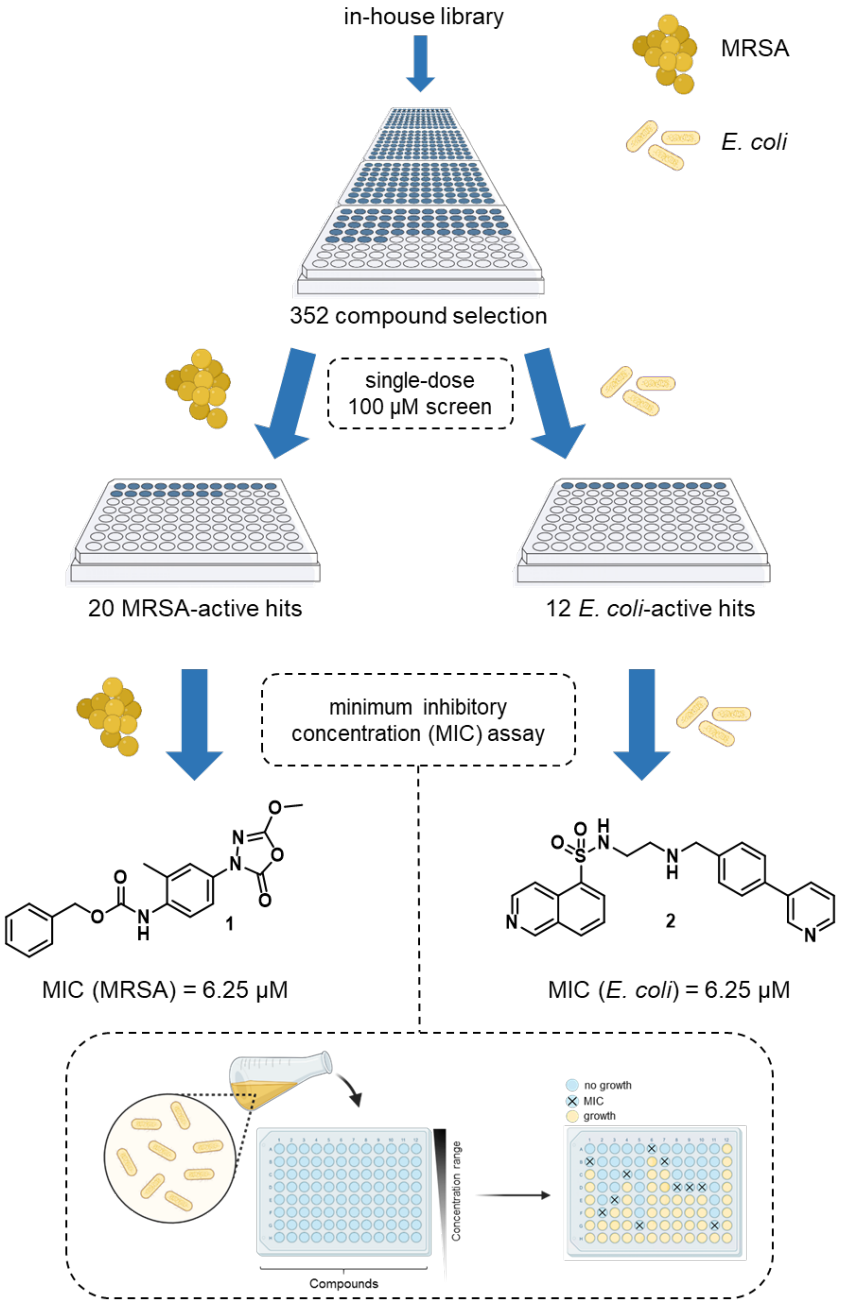
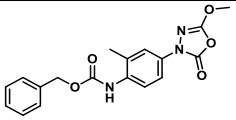
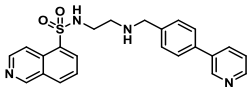
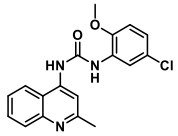
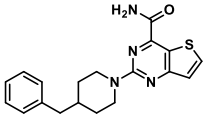
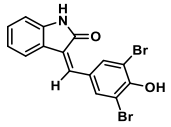
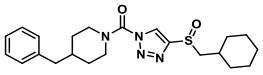
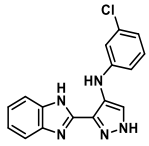
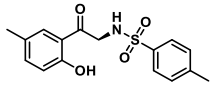


Figure 2.1 | Flowchart detailing the compound library screen. First an in-house library is constructed, which is screened for antibacterial activity against MRSA, and *E. coli*. The MIC of the resulting hits is then determined, yielding hit **1** for MRSA, and hit **2** for *E. coli*.

Table 2.1 | List of hits found with a MIC of 25 μM or lower.

Hit	Structure	MIC MRSA	MIC *E. coli*	MW (Da)	cLogP	PSA (Å^2)	HBA	HBD	RB
1		6.25	-	355	1.16	89.4	8	1	6
2		-	6.25	419	2.57	82.9	6	2	7
3		12.5	-	342	5.17	62.7	5	2	3
4		12.5	-	352	4.24	71.0	5	1	4
5		25	-	395	4.13	49.3	3	2	1
6		25	-	415	4.73	65.3	6	0	6
7		25	-	310	5.22	60.8	5	3	3
8		50	25	319	3.52	83.5	5	2	4

MW: molecular weight; cLogP: partition coefficient (calculated with ChemDraw); PSA: polar surface area (calculated with ChemDraw); HBA: ; HBD: ; RB: rotatable bonds

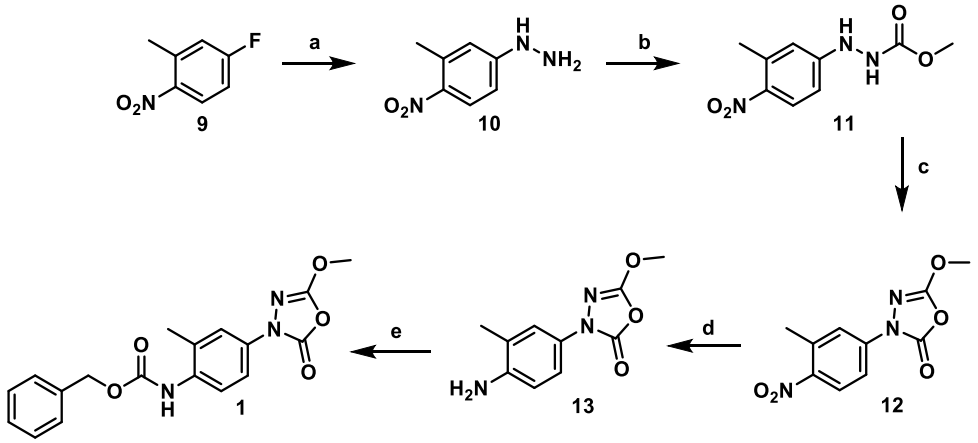
Table 2.1 summarizes the most potent hits found. In MRSA, besides hit **1**, two other hits were found with an MIC of 12.5 μM, and three hits with an MIC of 25 μM. The *E. coli* screen yielded only hit **8** as other hit besides hit **2**, with a MIC of 25 μM. Both hits **1** and **2** had favorable physicochemical properties (cLogP < 5, PSA < 140 Å^2, HBA < 10, HBD < 5, RB < 10).[21]

For the MRSA screen, hit **1** was of specific interest. Not only because it is the most potent MRSA hit, but also due to its potential covalent binding mode-of-action via the oxadiazolone moiety. Hit **1** (CAY10499), was previously reported to be an inhibitor of monoacylglycerollipase (MAGL) and hormone-sensitive lipase (HSL).[22,23] It has been shown that the oxadiazolone moiety of CAY10499 covalently binds to the catalytic serine present in lipases.[24,25] A covalent binding mechanism to reactive amino acids in catalytic sites provides

opportunities for unbiased target identification through activity-based protein profiling (See Chapter 4).[26]

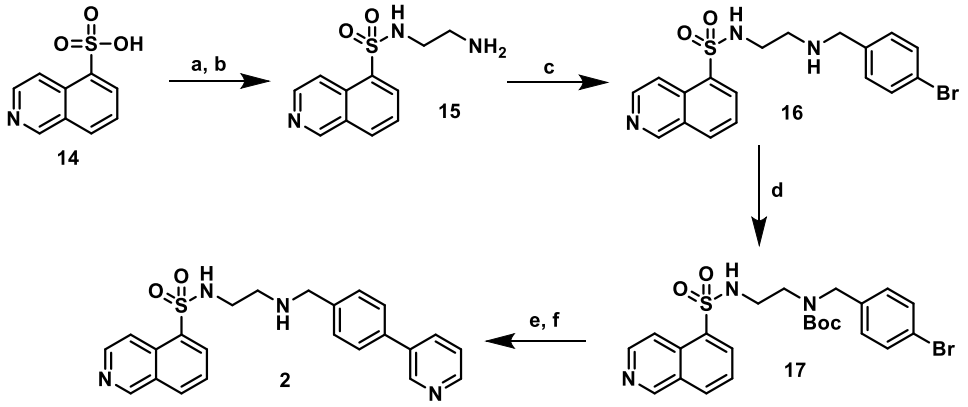
Against *E. coli*, hit **2** was the most potent compound, with hit **8** being four-fold less potent, and the other hits not showing activity at lower concentrations than 100 μM. Hit **2** was developed as part of a structure-activity relation (SAR) study where derivatives of kinase inhibitor H-89 were synthesized to improve inhibition of kinase Flt3, a target kinase in acute lymphoid leukemia.[27] The antibacterial activity of the remaining compounds in this series could maybe provide an insight in the essential pharmacophores of hit **2**.

Resynthesis of hit 1. To validate the antibacterial activity, the hits were resynthesized and tested. Hit **1** benzyl (4-(5-methoxy-2-oxo-1,3,4-oxadiazol-3(2*H*)-yl)-2-methylphenyl)carbamate was made in a five-step procedure, starting with the creation of the oxadiazolone moiety (Scheme 2.1). The nitro group was reduced to an anilline, which was reacted with benzyl chloroformate to form carbamate-containing hit **1**. Subsequent MIC assay validated the found MIC value of 6.25 μM.



Scheme 2.1 | Synthesis of hit **1**. Reagents and conditions: a) NH_2-NH_2, EtOH, 80°C, 80%; b) CH_3OCOCl, pyridine, NMP, 0°C, 90%; c) triphosgene, pyridine, DCM, 0°C, 87%; d) H_2, Pd/C, MeOH/DCM, RT, 99%; e) BnOCOCl, pyridine, DCM, 0°C, 92%.

Resynthesis of hit 2. Hit **2** *N*-(2-((4-(pyridin-3-yl)benzyl)amino)ethyl)isoquinoline-5-sulfonamide was resynthesized in a six-step procedure (Scheme 2.2). Isoquinolinesulfonic acid could be reacted with ethylenediamine through first transforming it to the sulfonyl chloride. A reductive amination of 4-bromobenzaldehyde resulted in benzylation of the primary amine, which was Boc-protected. A Suzuki coupling, followed by Boc-deprotection yielded hit **2**, of which the activity was validated (MIC = 6.25 μM).



Scheme 2.2 | Synthesis of hit **2**. Reagents and conditions: a) $SOCl_2$, DMF, 60°C; b) ethylene diamine, DCM, 0°C to RT, 93% (over two steps); c) R_2-bromobenzaldehyde, $NaBH(OAc)_3$, AcOH, THF, RT, 79%; d) Boc_2O, $NaHCO_3$, THF, 0°C to RT, 86%; e) $R_3(BOH)_2$, $Pd(PPh_3)_4$, K_2CO_3, H_2O, dioxane, 90°C; f) TFA, DCM, 0°C, 62% (over two steps).

Conclusion

A phenotypic antibacterial screen of a diverse compound library resulted in two validated hits, one with activity against MRSA and the other against *E. coli*. Resynthesis and testing of the hit compounds, confirmed their structure and activity. The structure-activity relationships of compound **1** and **2** are further described in Chapters 3 and 5, respectively, followed by their target identification in Chapters 4 and 6.

Acknowledgments

The following people are kindly acknowledged for their contribution to this chapter: Anthe Janssen for assisting in setting up the compound library, Ioli Kotsogianni for assisting with the MIC assays, Bing Liu for assisting with the resynthesis of hit **2**, Berend Gagestein for the initial synthesis of hit **2**.

Methods

Reagents & materials. Buffers and salts were of ACS reagent grade or higher and were purchased commercially, from Carl Roth GmbH (Karlsruhe, Germany) and Sigma-Aldrich (Darmstadt, Germany), biological materials and growth media were purchased from Sigma-Aldrich, Scharlab S.L. (Barcelona, Spain) and Fisher Scientific (Landsmeer, Netherlands). Antibiotics (TRC, Combi-Blocks, Sigma-Aldrich) were dissolved in ultrapure H_2O or DMSO, stock solutions were stored in -20°C, apart from meropenem which was used fresh. All test compounds were used from 10 mM DMSO stock solutions made from freeze dried powder and stored at -20°C.

Bacterial strains. *S. aureus* USA300 (ATCC BAA1717) belongs to the American Type Culture Collection (ATCC). *E. coli* W3110 belongs to the bacterial strain collection of prof. Nathaniel I. Martin.

Library screen. From glycerol stocks, USA300, as the Gram-positive representative strain, and W3110, as the Gram-negative representative strain, were cultured on blood agar plates (PB5039A, Thermo Scientific) by overnight (18 ± 2 h) aerobic incubation at 37°C. A single colony was transferred to tryptic soy broth (TSB, 02-200-500, Scharlab). The cultures were grown to exponential phase (OD_{600} = 0.5) at 37°C. The bacterial suspensions were diluted 200-fold in cation adjusted Mueller-Hinton broth (CAMHB) and 99 µL were added in a library of test compounds (1 µL DMSO stock solution, per well in technical duplicates) in polypropylene 96-well microtiter plates to reach a volume of 100 µL and a final concentration of 100 µM for each test compound and a maximum of 1% DMSO. The plates were sealed with breathable membranes and incubated at 37°C for 18 ± 2 h with constant shaking (600 rpm). Screening hits were selected from the wells where no visible bacterial growth was observed, as compared to the inoculum controls, containing 1% DMSO.

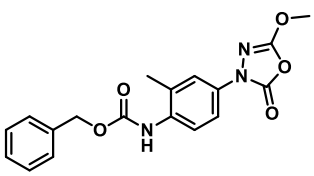
Minimum inhibitory concentration (MIC). From glycerol stocks, bacterial strains were cultured on blood agar plates by overnight incubation at 37°C. A single colony was transferred to TSB. In case of VRSA strains, 6 µg/mL vancomycin was supplemented to the media. The cultures were grown to exponential phase (OD_{600}: 0.5) at 37°C. The bacterial suspensions were diluted 100-fold in CAMHB and 50 µL was added to a 2-fold serial dilution series of test compounds (50 µL per well) in polypropylene 96-well microtiter plates to reach a volume of 100 µL. The plates were sealed with breathable membranes and incubated overnight at 37°C with constant shaking (600 rpm). For *Enterococci species* direct colony suspension was used by immediately suspending multiple colonies from fresh blood agar plates in CAMHB to an OD_{600} of 0.5 and subsequent 100-fold dilution. The MIC was determined as the lowest concentration at which no visible bacterial growth was observed, as compared to the inoculum controls, from the median of a minimum of triplicates.

Synthetic procedures

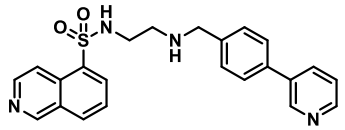
General remarks

All chemicals (Sigma-Aldrich, Fluka, Acros, Merck, Combi-Blocks, Fluorochem, TCI) were used as received. All solvents used for reactions were of analytical grade. THF, Et_2O, DMF, ACN and DCM were dried over activated 4 Å molecular sieves, MeOH over 3 Å molecular sieves. H_2O used in synthesis procedures was of Milli-Q-grade quality. Column chromatography was performed on silica gel (Screening Devices BV, 40-63 µm, 60 Å). The eluent EtOAc was of technical grade and distilled before use. Triethylamine was distilled over KOH, and triethylamine and pyridine were stored over KOH pellets. Starting materials were coevaporated with toluene (3×) before use in water-sensitive reactions.

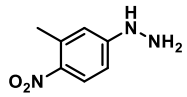
Reactions were monitored by thin layer chromatography (TLC) analysis using Merck aluminium sheets (Silica gel 60, F254). Compounds were visualized by UV-absorption (254 nm) and spraying for general compounds: $KMnO_4$ (20 g/L) and K_2CO_3 (10 g/L) in H_2O, or for amines: ninhydrin (0.75 g/L) and acetic acid (12.5 mL/L) in ethanol, followed by charring at 150°C. ^1H and ^{13}C NMR experiments were recorded on a Bruker AV-300 (300/75 MHz), Bruker AV-400 (400/101 MHz), Bruker DMX-400 (400/101 MHz), Bruker AV- 500 (500/126 MHz) and Bruker AV-600 (600/151 MHz). Chemical shifts are given in ppm (δ) relative to tetramethylsilane, as internal standard. Multiplicity: s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, quint = quintet, non = nonet m = multiplet. Coupling constants (*J*) are given in Hz. LC-MS measurements were performed on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (ESI+) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a standard C18 (Gemini, 4.6 mm D × 50 mm L, 5 µm particle size, Phenomenex) analytical column and buffers A: H_2O, B: ACN, C: 0.1% aq. TFA. High resolution mass spectra were recorded on a LTQ Orbitrap (Thermo Finnigan) mass spectrometer or a Synapt G2-Si high-definition mass spectrometer (Waters) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250°C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphtalate (m/z = 391.28428) as a lock mass. Preparative HPLC was performed on a Waters Acquity Ultra Performance LC with a C18 column (Gemini, 150 × 21.2 mm, Phenomenex) using an ACN in H_2O (+0.2% TFA) gradient. All final compounds were determined to be > 95% pure by LC-UV analysis.



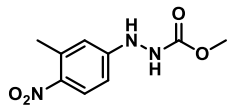
Benzyl (4-(5-methoxy-2-oxo-1,3,4-oxadiazol-3(2*H*)-yl)-2-methylphenyl)carbamate (1). 13 (40 mg, 0.18 mmol) and benzyl chloroformate (28 µL, 0.20 mmol) were dissolved in DCM (0.05 M) along with pyridine (1.1 equiv.) and the mixture was cooled to 0°C, after which acyl chloride (1.1 equiv.) was added. The mixture was stirred until TLC analysis indicated full conversion of the starting material. The mixture was then washed with H_2O (3×), and the organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The title compound was obtained as a white solid (59 mg, 0.17 mmol, 92%) without need for further purification. ^1H NMR (300 MHz, $CDCl_3$) δ 7.90 – 7.76 (m, 1H), 7.62 – 7.54 (m, 2H), 7.44 – 7.30 (m, 5H), 6.52 (bs, 1H), 5.20 (s, 2H), 4.08 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.86, 153.69, 148.35, 136.06, 133.51, 133.47, 128.74, 128.52, 128.48, 119.89, 116.67, 67.32, 57.79, 17.96. HRMS $[C_{18}H_{17}N_3O_5 + H]^+$: 356.12410 calculated, 356.12409 found.



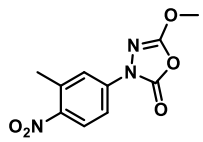
***N*-(2-((4-(Pyridin-3-yl)benzyl)amino)ethyl)isoquinoline-5-sulfonamide (2). 17** (20 mg, 38 µmol) was reacted with 4-pyridinyl boronic acid (1.2 equiv.), $Pd(PPh_3)_4$ (0.015 equiv.), K_2CO_3 (4 equiv.) in 1,4-dioxane and water (1:3, 0.1 M) in a sealed microwave tube. The reaction mixture was degassed with under nitrogen flow for 15 min and then stirred overnight at 90°C. The reaction mixture was then filtered over a silica gel pad with EtOAc and concentrated *in vacuo*. The crude product was purified by column chromatography (1% → 10% MeOH (10% aq. NH_3) in DCM) to give pure Boc-protected product. This was then dissolved in DCM (0.1 M), and TFA (17% v/v) was added at 0°C and the mixture was allowed to steer at RT for 4 h. The reaction was quenched with sat. aq. Na_2CO_3, diluted with water and extracted with DCM (3×). The combined organic layers were dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the crude material by column chromatography (0% → 10% MeOH (10% aq. NH_3) in DCM) afforded the pure product (12 mg, 29 µmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (d, *J* = 1.1 Hz, 1H), 8.79 (dd, *J* = 2.4, 0.9 Hz, 1H), 8.63 (d, *J* = 6.1 Hz, 1H), 8.58 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.50 – 8.42 (m, 2H), 8.17 (dt, *J* = 8.4, 1.1 Hz, 1H), 7.85 (ddd, *J* = 7.9, 2.4, 1.6 Hz, 1H), 7.68 (dd, *J* = 8.2, 7.3 Hz, 1H), 7.45 (d, *J* = 8.6 Hz, 2H), 7.37 (ddd, *J* = 7.9, 4.8, 0.9 Hz, 1H), 7.22 (d, *J* = 8.6 Hz, 2H), 3.59 (s, 2H), 3.04 (t, *J* = 5.7 Hz, 2H), 2.69 (t, *J* = 6.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.37, 148.40, 148.10, 145.14, 139.67, 136.58, 136.30, 134.46, 134.41, 133.55, 133.35, 131.31, 129.08, 128.69, 127.19, 126.01, 123.74, 117.33, 52.78, 47.60, 42.56. HRMS $[C_{23}H_{22}N_4O_2S + H]^+$: 419.15335 calculated, 419.15335 found.



(3-Methyl-4-nitrophenyl)hydrazine (10). 4-Fluoro-2-methyl-1-nitrobenzene (**9**) (10.0 g, 64.5 mmol) was suspended in ethanol (100 mL), after which hydrazine monohydrate (10.0 mL, 191 mmol) was added dropwise. The mixture was heated to 80°C, and stirred for 16 h. The mixture was then transferred to a beaker and cooled to 0°C, which formed an orange precipitate. The mixture was filtered, and washed on the filter with ice cold EtOH (100 mL). The residue was collected and concentrated *in vacuo* to give the title compound as an orange powder (8.60 g, 51.4 mmol, 80%). ^{1}H NMR (400 MHz, DMSO) δ 8.17 (s, 1H), 7.95 (d, *J* = 9.2 Hz, 1H), 6.67 – 6.61 (m, 2H), 4.41 (s, 2H), 2.50 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 156.21, 137.16, 136.03, 128.02, 112.12, 108.19, 22.46.

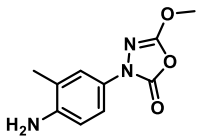


Methyl 2-(3-methyl-4-nitrophenyl)hydrazine-1-carboxylate (11). 10 (203 mg, 1.21 mmol) and NMP (104 µL, 1.09 mmol) were dissolved in pyridine (1.2 mL). The mixture was cooled to 0°C, after which methyl chloroformate (0.141 mL, 1.80 mmol) was added dropwise. The reaction mixture was stirred for 90 min, after which the mixture was resuspended in excessive amounts of EtOAc and 1 M aq. HCl. The organic layer was washed with brine, and subsequently dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification of the crude material by column chromatography (10% → 50% EtOAc in pentane) yielded the title compound as a yellow oil (246 mg, 1.09 mmol, 90%). ^{1}H NMR (400 MHz, DMSO) δ 9.38 (bs, 1H), 8.80 (s, 1H), 7.99 (d, *J* = 9.1 Hz, 1H), 6.60 (dd, *J* = 9.1, 2.6 Hz, 1H), 6.58 – 6.50 (m, 1H), 3.63 (s, 3H), 2.52 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 157.02, 153.78, 138.71, 136.78, 127.81, 113.15, 108.87, 52.17, 21.77.

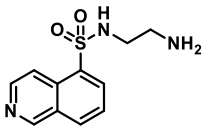


5-Methoxy-3-(3-methyl-4-nitrophenyl)-1,3,4-oxadiazol-2(3*H*)-one (12). 11 (500 mg, 2.22 mmol) and pyridine (2 equiv.) were dissolved in DCM (0.2 M), and cooled to 0°C after which a solution of triphosgene (1 equiv.) in DCM (1 M) was added dropwise. The mixture was stirred until TLC analysis indicated full conversion of the starting material. Then, 5% aq. NH_4OH (equal volume to DCM) was added, and the mixture was stirred for 10 min. The resulting organic layer was washed with 1 M aq. HCl, subsequently dried ($MgSO_4$), filtered and concentrated *in vacuo* to obtain the crude product. Purification of the crude material by column chromatography

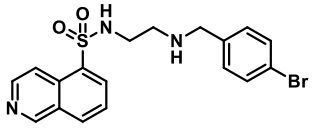
(5% → 10% EtOAc in pentane) yielded the desired product as an off-white solid (484 mg, 1.93 mmol, 87%). ^{1}H NMR (400 MHz, DMSO) δ 8.16 (d, *J* = 8.9 Hz, 1H), 7.74 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.71 (d, *J* = 2.5 Hz, 1H), 4.11 (s, 3H), 2.58 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 155.78, 147.85, 144.79, 139.52, 135.37, 126.64, 119.86, 115.16, 58.43, 20.47.



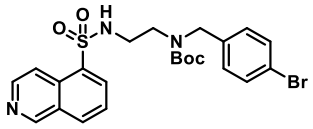
3-(4-Amino-3-methylphenyl)-5-methoxy-1,3,4-oxadiazol-2(3*H*)-one (13). 12 (470 mg, 1.87 mmol) was dissolved in DCM (25 mL) and MeOH (15 mL), and under nitrogen atmosphere Pd/C catalyst (10 wt%, 150 mg, 0.14 mmol) was added. The mixture was stirred, and hydrogen gas was bubbled through the solution. Once TLC analysis showed complete conversion of the starting material, the atmosphere was displaced with nitrogen, followed by filtering over Celite®, and concentration of the filtrate *in vacuo*. This yielded the title compound as an off-white solid (408 mg, 1.84 mmol, 99%) without need for further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (d, *J* = 2.5 Hz, 1H), 7.36 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.67 (d, *J* = 8.5 Hz, 1H), 4.05 (m, 3H), 3.65 (bs, 2H), 2.19 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.65, 148.65, 143.03, 127.28, 122.90, 121.33, 118.08, 114.94, 57.59, 17.51. HRMS $[C_{10}H_{11}N_3O_3 + H]^+$: 222.08732 calculated, 222.08730 found.



***N*-(2-Aminoethyl)isoquinoline-5-sulfonamide (15).** Isoquinoline-5-sulfonic acid (**14**) (10.0 g, 47.8 mmol) was dissolved in $SOCl_2$ (60 mL) and DMF (1.2 mL). The mixture was refluxed at 60°C until TLC analysis showed the complete conversion of the starting material. The $SOCl_2$ was evaporated *in vacuo*, and the reaction mixture was washed with DCM and then filtered. The crude sulfonyl chloride formed was immediately used in the following reaction. Ethylene diamine (15.1 mL, 227 mmol) was added dropwise to a cooled (0°C) and stirred solution of the crude sulfonyl chloride (10.0 g, 37.7 mmol) in DCM (600 mL). The mixture was then stirred at RT for 2 h. The reaction mixture was diluted with sat. aq. Na_2CO_3 (10 mL), washed with brine (50 mL) and extracted with DCM (3×). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated. The residue was then co-evaporated with toluene to remove the remaining ethylene diamine giving **15** (10.3 g, 35.0 mmol, 93%) as a dark yellow solid that was used without further purification.



***N*-(2-((4-Bromobenzyl)amino)ethyl)isoquinoline-5-sulfonamide (16). 15** (0.10 g, 3.96 mmol) and 4-bromobenzaldehyde (0.36 g, 1.93 mmol) were dissolved in THF (20 mL) in the presence of activated 3 Å molecular sieves. Then, sodium triacetoxyborohydride (0.84 g, 3.96 mmol) and glacial acetic acid (110 µL, 1.93 mmol) were added. The reaction mixture was stirred overnight, after which sat. aq. Na_2CO_3 (5 mL) was added to quench the reaction. The mixture was then diluted with brine (5 mL), extracted with Et_2O (10 mL) and DCM (3×). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product purified by column chromatography (1% → 10% MeOH (10% aq. NH_3) in DCM) to give **16** (0.64 g, 1.52 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (d, *J* = 1.0 Hz, 1H), 8.65 (d, *J* = 6.1 Hz, 1H), 8.45 – 8.40 (m, 2H), 8.20 (dt, *J* = 8.2, 1.2 Hz, 1H), 7.69 (dd, *J* = 8.2, 7.4 Hz, 1H), 7.38 – 7.31 (m, 2H), 7.00 – 6.95 (m, 2H), 3.49 (s, 2H), 3.02 – 2.96 (m, 2H), 2.65 – 2.59 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.46, 145.24, 138.59, 134.28, 133.70, 133.44, 131.59, 131.30, 129.67, 129.10, 126.05, 121.01, 117.26, 52.53, 47.42, 42.54.



***tert*-Butyl (4-bromobenzyl)(2-(isoquinoline-5-sulfonamido)ethyl)carbamate (17). 16** (2.26 g, 5.38 mmol) and $NaHCO_3$ (500 mg, 5.92 mmol) were suspended in THF (15 mL) and cooled to 0°C. Boc_2O (1.26 g, 5.92 mmol) was then carefully added to this mixture, followed by 6 h of stirring. The reaction was diluted with sat. aq. Na_2CO_3 (5 mL), followed by dilution with brine

(10 mL) and extraction with DCM (3×). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (20% → 40% EtOAc in pentane) to give title compound **17** (2.42 g, 4.65 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (s, 1H), 8.66 (d, *J* = 6.1 Hz, 1H), 8.45 – 8.32 (m, 2H), 8.21 (d, *J* = 8.2 Hz, 1H), 7.69 (t, *J* = 7.8 Hz, 1H), 7.39 – 7.30 (m, 2H), 6.96 (d, *J* = 8.2 Hz, 2H), 4.27 (s, 2H), 3.39 – 3.20 (m, 2H), 3.11 – 2.89 (m, 2H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.93, 153.37, 145.25, 136.89, 133.64, 133.29, 131.83, 131.32, 129.17, 128.94, 126.00, 121.41, 117.41, 81.25, 51.35, 46.77, 42.63, 28.43.

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