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Synthesis and evaluation of 1,3,4-oxadiazole derivatives for development as broad-spectrum antibiotics

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Running title: *Oxadiazole derivatives as broad-spectrum antibiotics*

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ABSTRACT:

The reality and intensity of antibiotic resistance in pathogenic bacteria calls for the rapid development of new antimicrobial drugs. In bacteria, *trans*-translation is the primary quality control mechanism for rescuing ribosomes arrested during translation. Because *trans*-translation is absent in eukaryotes but necessary to avoid ribosomal stalling and therefore essential for bacterial survival, it is a promising target either for novel antibiotics or for improving the activities of the protein synthesis inhibitors already in use. Oxadiazole derivatives display strong bactericidal activity against a large number of bacteria, but their effects on *trans*-translation were recently questioned. In this work, a series of new 1,3,4-oxadiazole derivatives and analogs were synthesized and assessed for their efficiency as antimicrobial agents against a wide range of gram-positive and gram-negative pathogenic strains. Despite the strong antimicrobial activity observed in these molecules, it turns out that they do not target *trans*-translation *in vivo*, but they definitely act on various other cellular pathways.

INTRODUCTION

1,3,4-oxadiazoles are five-membered ring heterocyclic compounds that have a very wide range of biological activities, making them important construction motifs for the development of new drugs (1). Among the various pharmacological activities attributed to 1,3,4-oxadiazole heterocycles, their strong antimicrobial activity is of particular interest (2, 3, 4). Recently, after high-throughput screening, several 1,3,4-oxadiazole compounds active against a wide array of bacteria were identified as inhibitors of *trans*-translation, the process that delivers stalled ribosomes during bacterial protein synthesis (5, 6). In bacteria, *trans*-translation is performed by transfer-messenger RNA (tmRNA) and its partner SmpB, small protein B (7). Two alternative ribosome rescue factors A and B (ArfA and ArfB) can take over when *trans*-

translation is missing or overwhelmed. Ribosomal stalling is a serious issue for cells and without tmRNA, bacteria that are devoid of *arfA* (including *Shigella flexneri*, *Helicobacter pylori*, *Francisella tularensis*, *Mycobacterium tuberculosis*, and *Legionella pneumophila*) cannot survive (8, 9, 10, 11). In addition, even when pathogenic bacteria do manage to survive in the absence of *trans*-translation, they generally lose their virulence (12, 13, 14, 15). Therefore, *trans*-translation inhibitors may act as antibiotics, and have minimal side effects on ribosomes of eukaryotic hosts (16, 17). **KKL-35** is one of the most active oxadiazole compounds, showing strong bactericidal activity against various non-related bacteria, and it is thus a promising molecule for future development (5). However, so far neither its molecular targets nor its exact mechanism of action have been solved. In fact, we and others have recently demonstrated that *trans*-translation is not the only target of **KKL-35** *in vivo* (11, 18). In order to improve the potential anti-*trans*-translation activity of oxadiazole compounds, we designed and synthesized a new series of derivatives, and studied their antimicrobial activities. Despite their strong antimicrobial effects, we were able to show that 1,3,4-oxadiazoles compounds target other pathways *in vivo*.

RESULTS AND DISCUSSION

Design of KKL-35 analogs

Since the oxadiazole **KKL-35** shows very strong antibacterial activity (5), we started with this molecule, designing different derivatives of its structure by varying either its heterocyclic core or the lateral aromatic groups (Scheme 1). First, we focused on the role played by the amide functional group. For this purpose, we prepared the compound **LHH-55**, which corresponds to the **KKL-35** retroamide (Scheme 2). The heterocyclic core was generated *via* one-pot, two-step synthesis starting with commercially available 4-fluorobenzyl hydrazine, first adding ethyl oxalyl chloride, then tosyl chloride. The isolated ethyl ester **LHH-39** was then converted

into the target compound by adding 4-chloroaniline with tBuOK in THF. After this, three other compounds were synthesized by varying just the heterocyclic core. This was done by replacing the oxygen atom with a sulphur atom (**LHH-19**), by removing one of the nitrogen atoms from the heterocyclic ring (**CT1-69**), or by substituting the oxadiazole moiety with a pyridine (**LHH-84**). The 1,3,4-thiadiazole **LHH-19** was obtained after three steps starting from 4-fluoro-benzaldehyde, with the key step being the formation of the heterocycle from the intermediate **LHH-12** compound. Several reaction conditions were tested in order to obtain the compound. The best results were observed when the 1,3,4-thiadiazol-2-amine derivative **LHH-30** was placed in the presence of acyl chloride, using pyridine as solvent. The low yield was mainly due to the difficulty of its isolation (Scheme 3). The two other new compounds were obtained in a standard way. For the first, the formation of 2-oxazolamine **CT1-56** produced the derivative **CT1-69** after an amide-coupling reaction with TBTU. For the second, a Suzuki-Miyaura cross-coupling reaction and amidation starting from 2-amino-6-chloropyridine led to **LHH-84**. In both cases, despite various attempts to optimize the reaction conditions, the last steps produced low yields, again due to the difficulties encountered in isolating the final products (Scheme 4). The antimicrobial activity of these compounds was then evaluated against a panel of 24 bacterial strains (Table S1). Only **CT1-69** acts as a broad-spectrum antibiotic (Table 1), emphasizing the importance of the compound's structure. This is seen for instance with **LHH-55**, where a simple inversion of the **KKL-35** amide functional group results in an absence of antibiotic activity. The results are the same when the heterocycle is changed, such as with the pyridine substitution in **LHH-84**, or when it is slightly modified, as in **LHH-19**, where the oxygen atom was replaced by sulfur.

At the same time, we replaced the fluorine atom in **KKL-35** with a trifluoromethyl group. Thus, **LHH-32** and **CT1-98** were prepared through the synthesis of **LHH-23** and **CT1-96**, respectively. Only **LHH-32** turned out to have remarkable antibacterial activity against a

small panel of pathogenic bacteria (Table 1). Finally we did some variations on the eastern part of **KKL-35** by modifying the chlorophenyl eastern part while keeping the trifluoromethyl substituent on the western benzene ring. The displacement of the chlorine atom from para to the meta position led to **CT1-115** whereas the replacement of the benzene ring by a pyridine provided **CT1-83** (Scheme 6). These 2 new derivatives exhibited interesting broad spectrum antibiotic activities (Table 1).

Antimicrobial activity of the synthetic oxadiazole compounds

We tested the antimicrobial activity of the newly synthesized 1,3,4-oxadiazole compounds against a selection of 24 bacterial species that included international reference strains and clinical isolates (Table S1). Antimicrobial susceptibility testing was first performed by agar disk diffusion, loading the cellulose disks with either 50 µg of a compound or 10 µL DMSO as a control. **KKL-35** was used as a reference for comparison with the newly synthesized molecules (Table 1). Whatever the strain, no growth inhibition is observed around the control disks, demonstrating that the solvent used to dissolve the compounds does not affect the analysis (data not shown). Of the 12 molecules tested, 8 of them (**LHH-55**, **LHH-39**, **LHH-19**, **LHH-77**, **LHH-84**, **CT1-56**, **CT1-96**, and **CT1-98**) did not appear to exhibit any antibacterial activity, as growth of all strains was observed up to the edges of the loaded disks (6mm inhibition diameter, corresponding to the disk diameters, data not shown). The other four molecules (**CT1-69**, **LHH-32**, **CT1-83**, and **CT1-115**) did display antimicrobial activity. Interestingly, three of them (**CT1-69**, **CT1-83**, and **CT1-115**) exhibited a wide spectrum of action and strong antimicrobial activity (diameters over 12 mm). In fact, they seemed to be active against almost all of the gram-positive strains tested, with the exception of *C. perfringens* and *S. pyogenes*. However, the only gram-negative strain affected was *B. fragilis* ATCC 25285 (by **CT1-83**). Interestingly, **CT1-69**, **CT1-83** and **CT1-115** also affected the

growth of the mycobacteria *M. fortuitum*, and **CT1-83** had an effect against *M. abscessus* (Table 1).

Effects of the changes to the parent KKL-35 molecule on antimicrobial activity

The retroinversion of the **KKL-35** amide functional group led to a total loss of antimicrobial activity in the resulting **LHH-55** compound. The amide functional group is therefore indispensable to the antimicrobial activity of the oxadiazole derivatives, and it was conserved in the following modified molecules. Variations to the heterocyclic core led to a loss of activity when replacing the oxygen atom with a sulfur atom (**LHH-19**), or when substituting the oxadiazole moiety by a pyridine (**LHH-84**). On the other hand, removing one of the nitrogen atoms from the heterocyclic ring (**CT1-69**) improved activity against all of the bacteria already sensitive to **KKL-35**. Moreover, **CT1-69** also exhibited antimicrobial activity against Enterococci, which are resistant to **KKL-35** (Table 1). The third type of modification done on the aryl group of the **KKL-35** parent was certainly the most interesting one. Indeed, antibacterial activities greatly improve when the fluorine atom is replaced by a trifluoromethyl group.. Finally, we did further biological studies on three of the most promising compounds (**CT1-69**, **CT1-83**, and **CT1-115**), chosen because their MIC and IC₅₀ values are significantly lower than those of **KKL-35**.

Determination of minimum inhibitory concentrations

We determined the MICs of **CT1-69**, **CT1-83**, and **CT1-115** molecules for 10 strains, including 1 gram-negative, 7 gram-positive, and 2 *Mycobacterium* strains. Table 2 shows the MIC values (or ranges of values) obtained after three independent experiments. When the bacterial growth inhibition diameters obtained by the agar diffusion method were less than 9 mm, we did not determine the corresponding MICs. **KKL-35** was again used as control, since the main goal was to identify molecule(s) having a MIC at least twice as low as its, a sign of better antimicrobial activity. In this context, **CT1-69** did not seem more efficient than the reference molecules. On the other hand, **CT1-83** displayed lower MICs for both Enterococci and *M. fortuitum*. In addition, **CT1-115** seemed equal to **KKL-35** against *B. fragilis* ATCC 25285, *C. perfringens*, *L. monocytogenes*, *M. abscessus*, *M. fortuitum*, *S. aureus* ATCC 29213, and *S. pyogenes*, and is much stronger against *E. faecalis* ATCC 29212, *E. faecium* AUS0004, and *S. epidermidis*, while having very low (0.0625-1 mg/L) MICs (Table 2).

Synergistic effects with other antibiotics

We observed a synergistic effect between an antibiotic and a test compound as soon as the diameter of growth inhibition measured for the association increased by at least 5 mm over the highest value obtained in individual tests of either of the two molecules. The results are presented in Table 3 for the 6 strains *E. faecalis* ATCC 29212, *E. faecium* AUS0004, *B. fragilis* ATCC 25285, *C. perfringens*, *L. monocytogenes* and *S. pyogenes*. Depending on the strain, we combined the **CT1-69**, **CT1-83**, and **CT1-115** molecules with the antibiotics to which the strain had previously been shown to be either “resistant” or “intermediate” according to EUCAST guidelines. No effects were detected using the strains *B. fragilis* ATCC 25285, *C. perfringens*, or *S. pyogenes* (data not shown). On the other hand, for *E. faecalis* ATCC 29212, significant synergistic effects were observed with the associations

streptomycin/**CT1-115**, and gentamicin/**CT1-115** (Table 3). Moreover, the combination of gentamicin/**CT1-83** showed greater growth inhibitions of *E. faecium* AUS0004 than the compound alone (Table 3). Lastly, **CT1-115** associated with moxalactam also displayed a significant synergistic effect on the growth inhibition of *L. monocytogenes* (Table 3). Note that for this last strain, the diameter of inhibition (15 mm) observed around the disk loaded with ofloxacin and **CT1-69** was lower than that measured with just ofloxacin (21 mm), suggesting that **CT1-69** may inhibit ofloxacin in *L. monocytogenes*.

Cytotoxicity

The cytotoxicity of the lead compounds was assessed using eight mammalian cell lines: Huh7, Caco-2, MDA-MB-231, HCT116, PC-3, MCF-7, NCI-H727, and a fibroblast cell line (Table 4). Cell viability was determined by MTT assay. Overall, the IC₅₀ values are in the same range (14-25 μ M) as for **KKL-35**, except in the case of **CT1-83**, which is not cytotoxic below 25 μ M.

***E. coli* resistance to 1,3,4-oxadiazole compounds is linked to efflux**

Considering the lack of antimicrobial effects on *Enterobacteriaceae*, our intention was to study how *E. coli* resists 1,3,4-oxadiazole derivatives. We began by doing disk diffusion assays, and by calculating the MICs in *E. coli* BW25113 for the three compounds previously identified as being the most promising, **CT1-69**, **CT1-83**, and **CT1-115**. However, no inhibition zone was seen in the wild-type strain, as already noticed with **KKL-35** (5, 18), and the MIC was higher than 128 μ M for all of the tested compounds (Table 5). Experiments were then conducted using a strain deleted for TolC. Indeed, in *E. coli* the antimicrobial drug efflux pathway is composed of the outer membrane porin protein TolC combined with cytoplasmic membrane pumps belonging to five major superfamilies. We and others have in fact

documented growth inhibition in the presence of **KKL-35** when the AcrAB pump was inactivated, indicating that **KKL-35** is ejected by the AcrAB-TolC multidrug efflux pump in *E. coli*. TolC inactivation in the presence of 1,3,4-oxadiazoles results in restricted growth, showing an inhibition halo of 19 mm in disk inhibition assays and a MIC of 0.25 μM for **CT1-115**, the most potent derivative, as compared to **KKL-35**'s inhibition halo of 22 mm and a MIC of 2 μM . These data confirm that the 1,3,4-oxadiazole compounds described here are ejected by the same pathway as **KKL-35**. Since the TolC system exists only in gram-negative bacteria, these results also explain why the compounds are mainly active in gram-positive bacterial strains or in strains lacking efficient efflux (Table 1).

Correlation between the antimicrobial activity of oxadiazole compounds and the in vivo inhibition of trans-translation

Since the links between the antimicrobial activity of **KKL-35** and its *in vivo* activity on *trans*-translation were recently called into question (11, 18), we performed the same inhibitory assays on various strains mutated for rescuing systems which are involved in protein synthesis quality control. Indeed, since ribosome rescue is essential, the double deletion of *ssrA* and *arfA* is lethal in *E. coli* (8). Assuming that a compound inhibited *trans*-translation, we expected that it would display a stronger inhibition of bacterial growth in the absence of ArfA. Since ArfB plays also a partial role in cell viability in the absence of SsrA and ArfA (19), we constructed mutant cells deficient in (*arfA*, *arfB*) in a ΔtolC background. However, we observed similar inhibition diameters around the disks for strains deficient in *tolC* or (*tolC*, *arfA*, *arfB*) (Table 6). We thus performed the same experiments in the absence of the putative tmRNA, but also ClpP targets. Indeed, when Clp is absent or inhibited, *trans*-translated proteins will not degrade and the process of *trans*-translation is incomplete. If a molecule targets *trans*-translation, in the absence of that process, the molecule should no

longer be effective. Yet the molecules studied inhibit growth in the same way as in strains deficient for (*tolC*, *ssrA*) and (*tolC*, *clpP*), suggesting that they affect other targets present in those mutants (Table 6).

The next step was to use a fluorescence reporter system to check 1,3,4-oxadiazole compound activity in cells. Towards this aim, we recently developed a reliable *in vivo* double-fluorescence reporter system for the simultaneous quantification of both *trans*-translation and the associated proteolysis activities in bacteria. In this system, two different fluorescent proteins are simultaneously used in one strain: red mCherry and green fluorescent protein (GFP). If *trans*-translation is inhibited, bacteria will glow green, and if inhibitors target proteases, the bacteria glow both green and red (18). We chose the Δ *tolC* strain for the experiments, as this has a higher sensitivity to 1,3,4-oxadiazoles than the wild-type strain. The concentrations used were less than or equal to the minimum inhibitor concentrations calculated for each compound in *E. coli*. However, as previously shown for **KKL-35** (18), the fluorescence levels did not change for these molecules as compared to the positive controls (data not shown). All together, these results suggested that the antibacterial effects of oxadiazoles in *E. coli* might not be due to the inhibition of tmRNA or ClpP, challenging the theory that *trans*-translation is the main target for oxadiazole derivatives *in vivo*.

CONCLUSION

The three new oxadiazole compounds **CT1-69**, **CT1-83**, and **CT1-115** have antimicrobial activities that are higher than the **KKL-35** reference molecule against a large panel of gram-positive pathogenic strains. Furthermore, these new compounds show synergistic activity with conventional antibiotics and have low toxicities. Taking into account their negative *trans*-translation *in vivo* assay results in *E. coli*, the overall global activity of the three compounds on a large panel of pathogenic bacteria suggests that oxadiazole derivatives may have one or

several other major cellular targets in addition to *trans*-translation. So far, we have failed to obtain spontaneous, UV, or nitrosoguanidine-induced resistant mutants with wild-type as well as delta tolC strains, which suggests that the 1,3,4-oxadiazole target is either important for survival, or there are multiple targets. However, our cellular assays were essentially performed using *E. coli* cells, and we cannot rule out the idea that the anti-*trans*-translational effects are improved in certain bacteria, as recently suggested for *Francisella tularensis* or *Mycobacterium tuberculosis* (2, 6). Our goal now is to further improve the activity, selectivity, and cellular behavior of 1,3,4-oxadiazoles compounds and find their other cellular targets.

EXPERIMENTAL PROCEDURES

General information

All reagents and solvents were purchased from commercial suppliers or when necessary were purified/dried according to W. L. F. Armarego and C. L. L. Chai (20). ¹H and ¹³C-NMR spectra were recorded on a Bruker DMX 500 or Avance 300 instrument using TMS and CDCl₃, respectively as the internal standard. δ values are given in parts per million (ppm) and coupling constants (*J* values) are given in Hertz (Hz). Signal multiplicity is reported as follows: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quadruplet; qt, quintet; sext, sextuplet; hept, heptuplet; dd, doublet of doublets; dq, doublet of quadruplets; td, triplet of doublets; tq, triplet of quadruplets; dsept, doublet of septuplets; ddd, doublet of doublets of doublets; and m, multiplet. High-resolution mass spectrometry (HRMS) analysis was done using a Waters Q-ToF II, a Micromass ZabSpec TOF, a Bruker micrO-TOF Q II, or an LTQ Orbitrap XL instrument for ESI. X-ray crystallographic data were collected on an APEXII crystal diffractometer. Optical rotations were recorded on a PerkinElmer 341 polarimeter. Melting points were obtained on a hot bench. TLC analysis was performed using precoated

Merck TLC silica gel 60 F₂₅₄ plates. Column chromatography purifications on silica gel were done using Merck silica Gel 60 (70-230 mesh), those on neutral alumina used Merck aluminium oxide 90 active, and the preparative thin-layer chromatography purifications were performed using Merck Silica Gel 60 PF₂₅₄. The petroleum ether (PE) used for purifications was at a low boiling point (40-60 °C).

Bacterial strains and growth conditions

Escherichia coli BW25113 deletion strains and derivatives used in this study were previously described (18). All the other bacterial strains are listed in Supplementary Table 1. Identification of the different species was carried out from culture of 20 ± 4 hours and from a 4 days culture for *Mycobacterium*, by MALDI-TOF (Bruker MALDI Biotyper CA System, Germany). Strains belonging to the *Enterobacteriaceae* (8 strains), *Enterococcaceae* (3 strains), *Staphylococcaceae* (3 strains) families and *Kocuria rhizophila*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Bacillus subtilis* species were grown 20 ± 4 h in aerobic conditions, on Trypticase-Soja agar, at 35 ± 2 °C. *Streptococcus pyogenes* was grown 20 ± 4 hours in an aerobic atmosphere + 5% CO₂ on trypticase soy agar + 5% horse blood (Biomérieux, France), at 35 ± 2 °C. The species *Clostridium perfringens* and *Bacteroides fragilis* were cultured 20 ± 4 hours anaerobically (AnoxomatTM, Mart[®] Microbiology B.V., Netherlands), on TSH agar at 35 ± 2 °C. The two *Mycobacterium* strains were cultured for 4 days in a humid aerobic atmosphere TSH on agar at 35 ± 2 °C. After identification, the strains were stored at -80 °C.

Screening of the antimicrobial activity

The antimicrobial activity of the compounds was measured according to the diffusion method in agar medium. Mueller-Hinton (MH) medium was used for bacteria other than slow-

growing bacteria and MHF (5% horse blood added to MH) medium was used for strains of *S. pyogenes*, *L. monocytogenes*, *C. perfringens*, *B. fragilis* ATCC 25285 as well as for *Mycobacterium* strains (*M. abscessus* and *M. fortuitum*). The surface of the agar was seeded from a swab of a standardized 0.5 McFarland inoculum (around 10^8 Colony Forming Units (CFU)/ml). Sterile cellulose discs were disposed on its surface and loaded with 50 μg of each compound (10 μL of a 5 g/L solution into DMSO). For each strain, a control was carried out by depositing 10 μL of DMSO on a cellulose disc. The media were incubated at 35 ± 2 °C aerobically, except for *S. pyogenes* (incubated aerobically + 5% CO_2) and *C. perfringens* and *B. fragilis* ATCC 25285 (incubated anaerobically). The two *Mycobacterium* strains were incubated in humid aerobic conditions for 4 days at 35 ± 2 °C. All other strains are incubated for 20 ± 4 h. Lastly, the diameters of the growth inhibitions around the discs were measured.

Antimicrobial susceptibility testing

MICs of tested molecules as well as antibiotics were determined using the broth microdilution method according to EUCAST (<http://www.eucast.org/>) guidelines. The strains used for these determinations were *C. perfringens*, *E. faecalis* ATCC 29212, *E. faecium* AUS0004, *L. monocytogenes*, *S. aureus* ATCC 29213, *S. epidermidis*, *S. pyogenes*, *B. fragilis* ATCC 25285, *M. abscessus* and *M. fortuitum* and the growth conditions were those as described above.

Effects of the combination molecule-antibiotic

In order to determine whether the activity of antibiotics combined with our molecules was able to be modified, synergistic tests were carried out. To do this, the selected antibiotics were those for which the categorization as "intermediate" or "resistant" were obtained by standard antibiogram (according to the EUCAST guidelines). For this purpose, the agar diffusion

technique was used. A swab of bacterial standard inoculum (0.5 MacFarland suspension), was used to seed the surface of medium. MH medium was used for *E. faecalis* ATCC 29212 and *E. faecium* AUS0004 and MHF for *B. fragilis* ATCC 25285, *C. perfringens*, *L. monocytogenes* and *S. pyogenes*. Standard discs containing the selected antibiotics were put on the surface and then loaded with 50 µg of each anti-microbial compound. The control tests corresponded to the antibiotic discs not supplemented with compounds and to discs only loaded with 50 µg of molecules to be tested. The medium were incubated as mentioned above. A synergistic effect was observed when the diameter of the growth inhibition was larger (at least 5 mm) than the aureoles obtained with the antibiotic or the molecule alone.

Toxicity test

Cytotoxicity tests were performed on the ImPACcell robotic platform (BIOSIT, Université de Rennes 1). This featured high-throughput multiparameter image analysis, with both high-content screening and high-content analysis. The platform is equipped with an Olympus microscope and Compix SimplePCI software; a Zeiss Axio Imager M1 microscope with a Zeiss camera and AxioVision software; and imaging systems including an ArrayScan VTI Cellomics reader (Thermo Fisher), Hamilton STARlet and NIMBUS workstations, and a Scienion spotter. For the tests, seven different cell lines were used: human hepatocellular carcinoma (HuH7); colorectal adenocarcinoma (Caco-2); breast adenocarcinoma (MDA-MB231); colorectal carcinoma (HCT116); prostatic adenocarcinoma (PC3); lung carcinoma (NCL-H727); and breast cancer (MCF7). The residual cell percentages reported correspond to viable cells compared to the average viable cells in the DMSO control. Viability of 100% represents no cytotoxicity or inhibition of cell growth, while under 25-30% is considered cytotoxic and 0% represents acute cytotoxicity.

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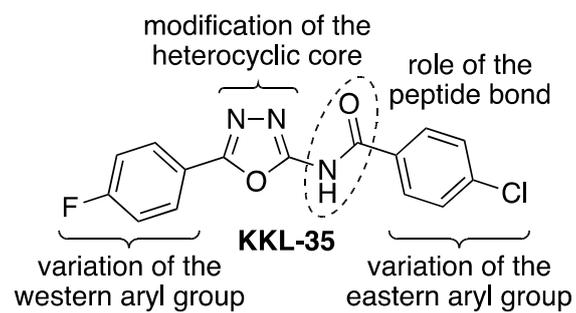
Conflicts of Interest: None.

Author contributions: CT, HHL, PVW and MJ performed chemical experiments; MT, RGVB, FD performed *in vivo trans*-translation experiments; RR, SG, AD, AT, GE, CB and JCG performed microbiology experiments; CB, JCG, PVW, and RG wrote the paper. RG supervised the project.

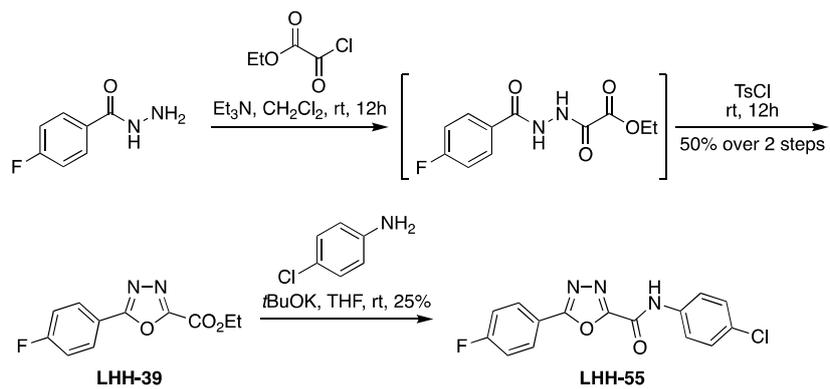
REFERENCES

1. de Oliveira, C.S., Lira B.F., Barbosa-Filho, J.M., Lorenzo, J.G., de Athayde-Filho, P.F. (2012) Synthetic approaches and pharmacological activity of 1,3,4-oxadiazoles: a review of the literature from 2000-2012. *Molecules* **17**, 10192-101231
2. Goralski, T.D., Dewan, K.K., Alumasa, J.N., Avanzato, V., Place, D.E., Markley, R.L., Katkere, B., Rabadi, S.M., Bakshi, C.S., Keiler, K.C., Kirimanjeswara, G.S. (2016) Inhibitors of Ribosome Rescue Arrest Growth of *Francisella tularensis* at All Stages of Intracellular Replication. *Antimicrob. Agents Chemother.* **60**, 3276-3282.
3. Chandrakantha, B., Shetty, P., Nambiyar, V., Isloor N., Isloor, A.M. (2010) Synthesis, characterization and biological activity of some new 1,3,4-oxadiazole bearing 2-flouro-4-methoxy phenyl moiety. *Eur. J. Med. Chem.* **45**, 1206-1210.
4. Patel, N.B. and Patel, J.C. (2010) Synthesis and antimicrobial activity of 3-(1,3,4-oxadiazol-2-yl)quinazolin-4(3H)-ones. *Sci. Pharm.* **78**, 171-193.
5. Ramadoss, N.S., Alumasa, J.N., Cheng, L., Wang, Y., Li, S., Chambers, B.S., Chang, H., Chatterjee, A.K., Brinker, A., Engels, I.H., and Keiler, K.C. (2013) Small molecule inhibitors of trans-translation have broad-spectrum antibiotic activity. *Proc. Natl. Acad. Sci. USA* **110**, 10282-10287.
6. Alumasa, J.N., Keiler, K.C. (2015) Clicking on trans-translation drug targets. *Front. Microbiol.* **6**, 498.
7. Giudice, E., Macé, K., Gillet, R. (2014) Trans-translation exposed: understanding the structures and functions of tmRNA-SmpB. *Front. Microbiol.* **5**, 113.
8. Chadani, Y., Ono, K., Ozawa, S., Takahashi, Y., Takai, K., Nanamiya, H., Tozawa, Y., Kutsukake, K. and Abo T. (2010) Ribosome rescue by *Escherichia coli* ArfA (YhdL) in the absence of trans-translation system, *Mol. Microbiol.* **78**, 796-808.
9. Starosta, A.L., Lassak, J., Jung, K., Wilson, D.N. (2014) The bacterial translation stress response. *FEMS Microbiol. Rev.* **38**, 1172-1201.
10. Keiler, K.C., Feaga, H.A. (2014) Resolving nonstop translation complexes is a matter of life or death. *J. Bacteriol.* **196**, 2123-2130.
11. Brunel, R., Charpentier, X. (2016) Trans-translation is essential in the human pathogen *Legionella pneumophila*. *Sci. Rep.* **6**, 37935.
12. Julio, S.M., Heithoff, D.M., Mahan, M.J. (2000) *ssrA* (tmRNA) plays a role in *Salmonella enterica* serovar Typhimurium pathogenesis. *J. Bacteriol.* **182**, 1558-1563.
13. Huang, C., Wolfgang, M.C., Withey, J., Koomey, M., Friedman, D.I. (2000) Charged tmRNA but not tmRNA-mediated proteolysis is essential for *Neisseria gonorrhoeae* viability. *EMBO J.* **19**, 1098-1107.
14. Okan, N.A., Mena, P., Benach, J.L., Bliska, J.B., Karzai, A.W. (2010) The *smpB-ssrA* mutant of *Yersinia pestis* functions as a live attenuated vaccine to protect mice against pulmonary plague infection. *Infect. Immun.* **78**, 1284-1293.

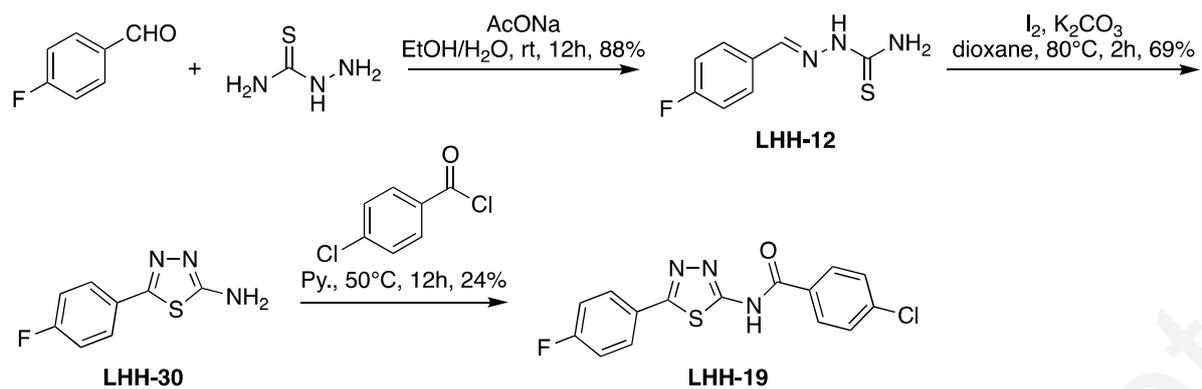
15. Svetlanov, A., Puri, N., Mena, P., Koller, A., Karzai, A.W. (2012) *Francisella tularensis* tmRNA system mutants are vulnerable to stress, avirulent in mice, and provide effective immune protection. *Mol. Microbiol.* **85**, 122–141.
16. Vioque, A., de la Cruz, J. (2003) Trans-translation and protein synthesis inhibitors. *FEMS Microbiol. Lett.* **218**, 9–14.
17. Li, J., Ji, L., Shi, W., Xie, J., Zhang, Y. (2013) Trans-translation mediates tolerance to multiple antibiotics and stresses in *Escherichia coli*. *J. Antimicrob. Chemother.* **68**, 2477–2481.
18. Macé, K., Demay, F., Guyomar, C., Georgeault, S., Giudice, E., Goude, R., Trautwetter, A., Ermel, G., Blanco, C., Gillet, R. (2017) A Genetic Tool to Quantify trans-Translation Activity in Vivo. *J. Mol. Biol.* **429**, 3617-3625.
19. Chadani Y, Ono K, Kutsukake K, Abo T. (2011) *Escherichia coli* YaeJ protein mediates a novel ribosome-rescue pathway distinct from SsrA- and ArfA-mediated pathways, *Mol Microbiol.* **80**, 772-785.
20. Armarego, W. L. F., and Chai, C. L. L. (2003). Purification of laboratory chemicals. Amsterdam, Butterworth-Heinemann.



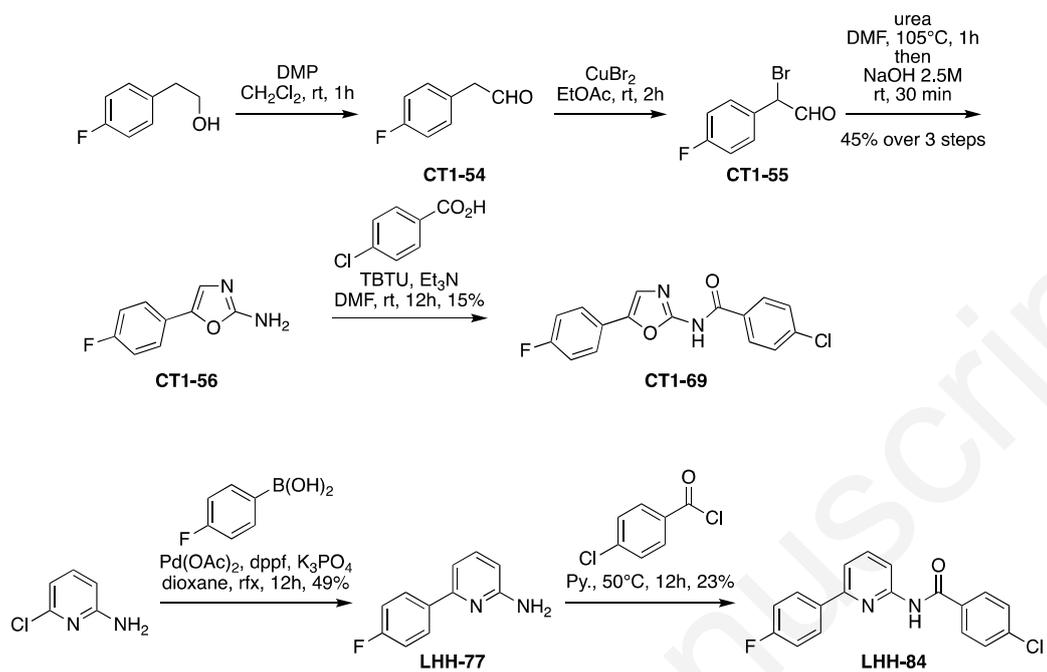
Scheme 1. Structural modifications of **KKL-35**



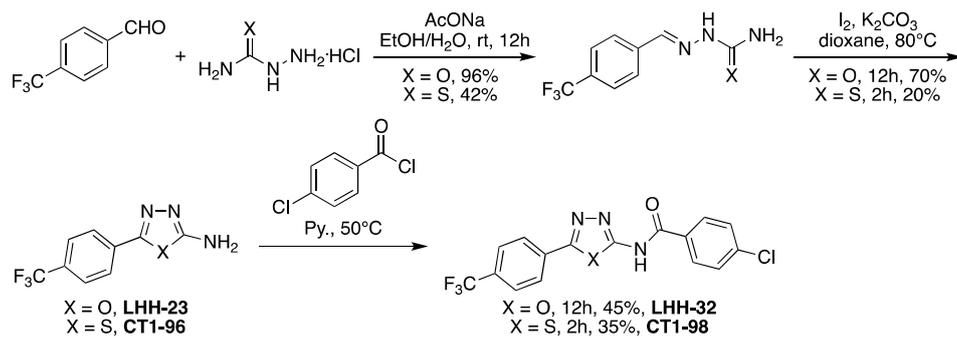
Scheme 2. Preparation of **LHH-55**



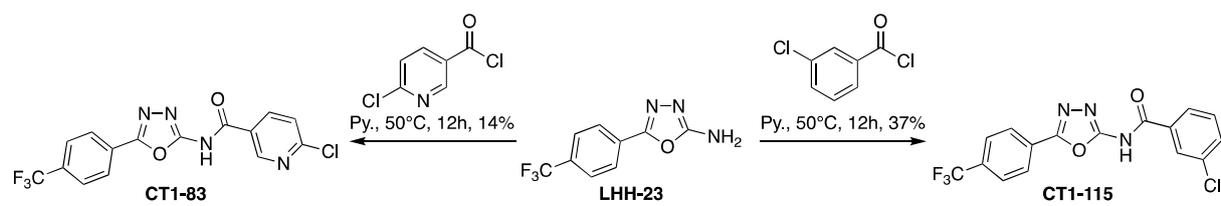
Scheme 3. Preparation of **LHH-19**



Scheme 4. Synthesis of **CT1-69** and **LHH-84**



Scheme 5. Synthesis of **LHH-32** and **CT1-98**



Scheme 6. Synthesis of CT1-83 and CT1-115

Accepted manuscript

Table 1: Measurement of the antimicrobial activity of various synthetic oxadiazole molecules against a panel of 24 bacterial strains (Diameter of zone of inhibition in mm)

Strains					
	KKL-35	CT1-69	CT1-83	CT1-115	LHH-32
<i>Staphylococcus aureus</i>	17	20	30	28	ND
<i>S. aureus</i> ATCC 29213	12	16	22	24	9
<i>Staphylococcus epidermidis</i>	15	18	28	27	ND
<i>Streptococcus pyogenes</i>	6	6	10	9	6
<i>Bacillus subtilis</i>	12	15	24	24	13
<i>Clostridium perfringens</i>	6	6	13	11	6
<i>E. faecalis</i> ATCC 29212	6	11	23	20	10
<i>E. faecium</i> AUS0004	6	12	23	21	9
<i>E. faecium</i> HM1070	8	13	24	23	11
<i>Kocuria rhizophila</i>	11	18	28	30	14
<i>Listeria monocytogenes</i>	13	17	31	28	16
<i>Acinetobacter baumannii</i>	6	6	6	6	6
<i>B. fragilis</i> ATCC 25285	6	8	16	11	9
<i>Enterobacter cloacae</i>	6	6	6	6	6
<i>Escherichia coli</i>	6	6	6	6	6
<i>Escherichia coli</i> ATCC 25922	6	8	6	6	6
<i>Klebsiella pneumoniae</i>	6	6	6	6	6
<i>Morganella morganii</i> EB6	6	6	6	6	ND
<i>Morganella morganii</i> EB8	6	6	6	6	ND
<i>Proteus mirabilis</i>	6	6	6	6	ND
<i>Proteus vulgaris</i>	6	10	8	6	ND
<i>Pseudomonas aeruginosa</i>	6	6	6	6	6
<i>Mycobacterium abscessus</i>	6	7	14	9	ND
<i>Mycobacterium fortuitum</i>	9	12	19	23	ND

ND, Not Determined

Table 2: Minimum inhibitory concentrations (mg/L) of various oxadiazole molecules against a group of bacterial strains

Strains	Molecules			
	KKL-35	CT1-69	CT1-83	CT1-115
<i>Staphylococcus aureus</i> ATCC 29213	[0.5-4]	[0.5-4]	[2-4]	2
<i>Staphylococcus epidermidis</i>	[0.5-4]	0.25	[1-2]	[0.0625-0.25]^a
<i>Streptococcus pyogenes</i>	[32-64]	[32->64]	32	[4-32]
<i>Clostridium perfringens</i>	4	ND ^b	4	[2-4]
<i>Enterococcus faecalis</i> ATCC 29212	16	[2-16]	[2-4]	[0.125-1]
<i>Enterococcus faecium</i> AUS0004	16	[1-4]	2	[0.0625-0.25]
<i>Listeria monocytogenes</i>	4	[32-64]	8	8
<i>Bacteroides fragilis</i> ATCC 25285	[2-4]	ND	2	[1-2]
<i>Mycobacterium abscessus</i>	64	ND	[16-32]	[32-64]
<i>Mycobacterium fortuitum</i>	32	[32->64]	8	[16-32]

^a Bold values are significantly different than the MICs observed with the **KKL-35** reference molecule.

^b ND, no detectable antimicrobial activity (see Table 1).

Table 3: Effects of the combination of oxadiazole molecules and antibiotics on the growth inhibition of three gram-positive bacterial strains

Molecules	Bacterial strains															
	<i>Enterococcus faecalis</i> ATCC 29212					<i>Enterococcus faecium</i> AUS0004						<i>Listeria monocytogenes</i>				
	Antibiotic ^a					Antibiotic						Antibiotic				
	No	RI	ER	HL	GM	No	RI	IP	AP	ER	HL	GM	No	MO	FO	OF
AT	F 5	Y	S	E 30	AT	F 5	M	I 2	Y	S	E 30	AT	X 30	S	X 5	
B		15	300		B		10		15	300		B		200		
None		<i>21</i>	<i>20</i>	<i>16</i>	<i>14</i>		<i>9</i>	<i>6</i>	<i>6</i>	<i>6</i>	<i>22</i>	<i>17</i>		<i>15</i>	<i>6</i>	<i>21</i>
DMSO	<i>6^b</i>	20	20	18	13	<i>6</i>	10	6	6	6	22	17	<i>6</i>	13	6	19
CT1-69	<i>9</i>	20	20	19	16	<i>11</i>	15	13	13	12	21	18	<i>16</i>	17	15	15^c
CT1-83	<i>20</i>	23	23	22	23	<i>20</i>	22	24	24	22	26	25	<i>29</i>	30	29	30
CT1-115	<i>17</i>	23	24	24	23	<i>22</i>	26	24	24	22	26	25	<i>28</i>	33	30	29

^aAntibiotics (ATBs) used: RIF (rifampicin); ERY (erythromycin); HLS (streptomycin); GME (gentamicin); IMP (imipenem); API (ampicillin); MOX (moxalactam); FOS (fosfomicin); and OFX (ofloxacin). The numbers underneath their abbreviations indicate the disk loads (μg).

^bThe growth inhibition diameters (mm) observed when the molecule or antibiotic is used alone are italicized.

^cThe growth inhibition diameters (mm) that indicate significant synergistic effects are bold.

Table 4: IC50 values (μM) of cytotoxicity tests of oxadiazole derivatives on various mammalian cell lines

Molecules	Huh7 <i>liver</i>	Caco-2 <i>colon</i>	MDA-MB-231 <i>Breast</i>	HCT116 <i>colon</i>	PC-3 <i>prostate</i>	MCF-7 <i>breast</i>	NCI <i>lung</i>	Fibro <i>skin</i>
KKL-35	20	14	25	17	21	22	18	>25
CT1-69	3	3	5	2	2	4		>25
CT1-83	>25	>25	>25	>25	>25	>25	>25	>25
CT1-115	20	11	23	10	17	15	10	>25

Table 5. Minimum inhibitory concentrations of oxadiazole compounds on wild type and Δ tolC strains of *Escherichia coli* BW25113

Mol 10 mM	\emptyset inhibition (mm) BW25113	MIC BW25113	MIC Δ tolC	MBC Δ tolC
KKL-35	6	>128	2	>32
CT1-69	6	>128	ND	ND
CT1-83	6	>128	1	>32
CT1-115	6	>128	0.25	16

MIC. Minimum inhibitory concentration

MBC. Minimum bactericidal concentration

ND. Not determined

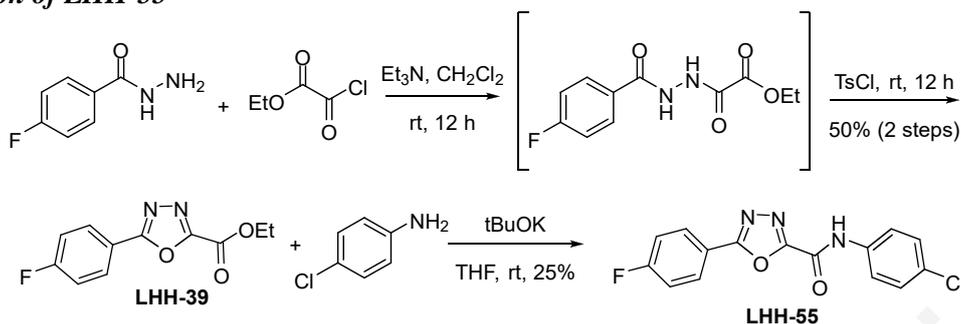
Table 6. Antimicrobial activity of the lead compounds against *E. coli* mutants

Bacterial strain	KKL-35	CT1 69	CT1 83	CT1 115
BW25113 Δ tolC	13 \pm 1	11.33 \pm 0.6	23.6 \pm 0.6	21.3 \pm 3.2
BW25113 Δ arfA arfB tolC	12.3 \pm 3.5	14 \pm 1.8	22.5 \pm 0.7	22.6 \pm 3.2
BW25113 Δ clpP tolC	14.3 \pm 2.1	13.3 \pm 1.1	27 \pm 2.6	22.3 \pm 2.5
BW25113 Δ ssrA tolC	13 \pm 1.4	13 \pm 2	27 \pm 2.6	22.3 \pm 2.3

Disc diffusion assay results are in mm. Results are the mean of three independent experiments and the standard deviations are reported.

Supporting Information

Preparation of LHH-55



Ethyl 5-(4-fluorophenyl)-1,3,4-oxadiazole-2-carboxylate (LHH-39)

To a stirred solution of 4-fluorobenzohydrazide (1.50 g, 9.73 mmol), and Et_3N (4.07 mL, 29.0 mmol, 3 eq.) in anhydrous CH_2Cl_2 (49 mL), under inert atmosphere, was added ethyloxalyl chloride (1.08 mL, 9.73 mmol, 1 eq.) dropwise at 0 °C. The reaction mixture was slowly allowed to reach r.t. and was stirred overnight. It was then treated with TsCl (1.86 g, 9.73 mmol, 1 eq.) and stirred for 12 h. The resulting mixture was diluted with EtOAc and the organic layer was washed with water, sat. aq. NaHCO_3 solution, brine, dried over MgSO_4 , filtered, and concentrated under reduced pressure. The residue was recrystallized from MeOH to give **LHH-39** (1.15 g, 4.9 mmol, 50% for 2 steps).

$^1\text{H NMR}$ (DMSO-d_6 , 300 MHz): δ 8.14 (ap. dd, $J = 8.8, 5.3$ Hz, 2H), 7.50 (ap. t, $J = 8.8$ Hz, 2H), 4.44 (q, $J = 7.1$ Hz, 2H), 1.37 (t, $J = 7.1$ Hz, 3H).

$^{13}\text{C NMR}$ (DMSO-d_6 , 75 MHz): δ 164.7 (d, $J = 251.7$ Hz), 164.6, 156.5, 154.0, 130.0 (d, $J = 9.8$ Hz, 2 \times CH), 129.3 (d, $J = 2.9$ Hz), 116.9 (d, $J = 22.4$ Hz, 2 \times CH), 63.0, 13.9.

$^{19}\text{F NMR}$ (DMSO-d_6 , 376 MHz): δ -105.9 (s, 1F).

Mp: 103-104 °C.

HRMS-ESI calculated for $\text{C}_{11}\text{H}_9\text{N}_2\text{O}_3\text{FNa}$: m/z 259.0495 ($[\text{M}+\text{Na}]^+$), found: m/z 259.0497 ($[\text{M}+\text{Na}]^+$).

N-(4-Chlorophenyl)-5-(4-fluorophenyl)-1,3,4-oxadiazole-2-carboxamide (LHH-55)

To a stirred solution of 4-chloroaniline (178 mg, 1.39 mmol, 1.5 eq.) and *t*-BuOK (208 mg, 1.86 mmol, 2 eq.) in anhydrous THF (6 mL), under inert atmosphere, was slowly added a solution of **LHH-39** (219 mg, 0.93 mmol) in anhydrous THF (4 mL). The resulting mixture was stirred at r.t. for 45 min, time after which solvent was evaporated under reduced pressure. Water was then added and the residue was triturated. The formed precipitate was collected by filtration and washed with cold MeOH and Et_2O to afford **LHH-55** (72 mg, 0.23 mmol, 25%) as a white solid.

$^1\text{H NMR}$ (DMSO-d_6 , 300 MHz): δ 8.18 (ap. dd, $J = 8.6, 5.5$ Hz, 2H), 7.86 (d, $J = 8.8$ Hz, 2H), 7.51 (t, $J = 8.6$ Hz, 2H), 7.48 (ap. t, $J = 8.8$ Hz, 2H).

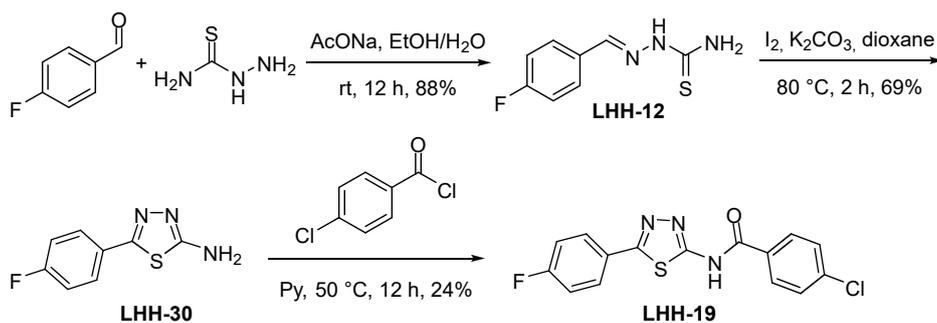
$^{13}\text{C NMR}$ (DMSO-d_6 , 125 MHz): δ 165.1 (d, $J = 251.8$ Hz), 165.0, 159.1, 152.1, 137.4, 130.4 (d, $J = 9.5$ Hz, 2 \times CH), 129.2 (2 \times CH), 129.0, 123.0 (2 \times CH), 120.0 (d, $J = 2.9$ Hz), 117.4 (d, $J = 22.9$ Hz, 2 \times CH).

$^{19}\text{F NMR}$ (DMSO-d_6 , 376 MHz): δ -106.2 (s, 1F).

Mp: 239-240 °C.

HRMS-ESI calculated for $\text{C}_{15}\text{H}_9\text{N}_3\text{O}_2\text{FCINa}$: m/z 340.0265 ($[\text{M}+\text{Na}]^+$), found: m/z 340.0267 ($[\text{M}+\text{Na}]^+$).

Preparation of LHH-19



(*E*)-2-(4-Fluorobenzylidene)hydrazine-1-carbothioamide (LHH-12)

Thiosemicarbazide (3.67 g, 40.0 mmol, 1 eq.) was dissolved in water (100 mL). A solution of sodium acetate (3.28 g, 40.0 mmol, 1 eq.) in water (25 mL) was added. The reaction mixture was stirred at r.t. for 5 min, then a third solution containing 4-fluorobenzaldehyde (4.32 mL, 40.0 mmol) in EtOH (50 mL) was slowly added, and the resulting mixture was stirred at r.t. overnight. The reaction mixture was poured into crushed ice and the formed precipitate was collected by filtration, washed with water and air-dried to yield **LHH-12** (6.94 g, 35.2 mmol, 88%) as a white solid.

Note: The product can also be recrystallized from EtOH, but was obtained clean, without purification.

¹H NMR (DMSO-*d*₆, 300 MHz): δ 11.44 (s, 1H), 8.21 (br s, 1H), 8.03 (br s, 2H), 7.87 (ap. dd, *J* = 8.9, 5.7 Hz, 2H), 7.24 (ap. t, *J* = 8.9 Hz, 2H).

Mp : 194-195 °C.

5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-amine (LHH-30)

To a stirred solution of **LHH-12** (500 mg, 2.53 mmol) in 1,4-dioxane (25 mL), under inert atmosphere, were added K₂CO₃ (1.05 g, 7.60 mmol, 3 eq.) and iodine (760 mg, 3.00 mmol, 1.2 eq.). The reaction mixture was heated to 80 °C and stirred for 2 h. After being cooled to r.t., it was treated with aq. half-saturated Na₂S₂O₃ solution and extracted with CH₂Cl₂ (5 times). The combined organic layers were dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was recrystallized from EtOH to yield **LHH-30** (339 mg, 1.74 mmol, 69 %) as a pale yellow solid.

Note: The extraction can be carried out with a 10:1 mixture of CH₂Cl₂/MeOH and the product can probably be recrystallized from *i*PrOH for better results. The reaction time has to be followed carefully (see LHH20 / CT1-107).

¹H NMR (DMSO-*d*₆, 300 MHz): δ 7.79 (ap. dd, *J* = 8.9, 5.6 Hz, 2H), 7.42 (br s, 2H), 7.30 (ap. t, *J* = 8.9 Hz, 2H).

¹³C NMR (DMSO-*d*₆, 75 MHz): δ 168.7, 162.5 (d, *J* = 247.5 Hz), 155.3, 128.5 (d, *J* = 8.8 Hz, 2×CH), 127.6 (d, *J* = 2.7 Hz), 116.0 (d, *J* = 22.5 Hz, 2×CH).

¹⁹F NMR (DMSO-*d*₆, 376 MHz) δ -111.4 (s, 1F).

Mp : 233-234 °C.

HRMS-ESI calculated for C₈H₆N₃FNas: *m/z* 218.0164 ([M+Na]⁺), found: *m/z* 218.0164 ([M+Na]⁺).

4-Chloro-*N*-(5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)benzamide (LHH-19)

To a stirred solution of **LHH30** (110 mg, 0.56 mmol) in freshly distilled pyridine (2 mL), under inert atmosphere, was added 4-chlorobenzoyl chloride (215 mg, 1.2 mmol, 2.1 eq.) at r.t. The obtained suspension was then placed in a pre-heated oil bath at 50 °C and stirred overnight. The resulting solution was cooled down to r.t. and poured into ice-cold water. The formed precipitate was collected by filtration, washed with water, then with a small amount of hot MeOH, and finally air-dried to afford the desired compound **LHH-19** (45 mg, 0.14 mmol, 24%) as a white solid.

¹H NMR (DMSO-*d*₆, 300 MHz): δ 13.30 (br s, 1H), 8.14 (d, *J* = 8.5 Hz, 2H), 8.05 (ap. dd, *J* = 8.9, 5.3 Hz, 2H), 7.66 (d, *J* = 8.5 Hz, 2H), 7.39 (ap. t, *J* = 8.9 Hz, 2H).

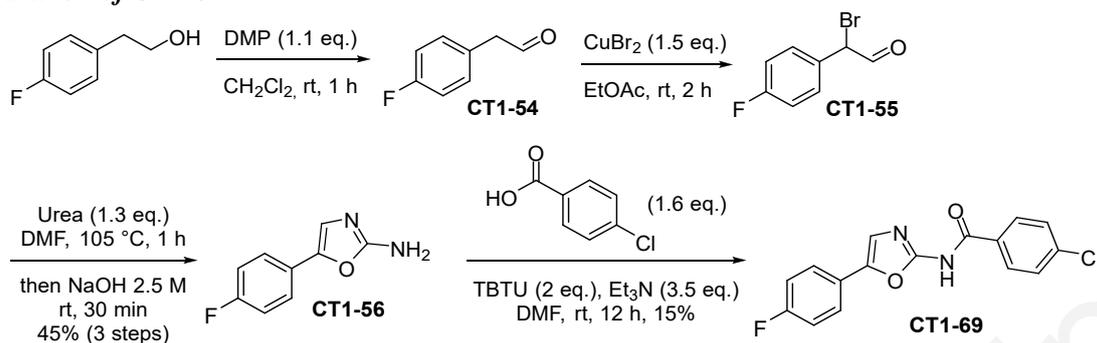
¹³C NMR (DMSO-*d*₆, 125 MHz): δ 164.2 (br s), 163.4 (d, *J* = 248.8 Hz), 161.7 (br s), 159.9 (br s), 138.0, 130.9 (br s), 130.4 (2×CH), 129.3 (d, *J* = 8.3 Hz, 2×CH), 128.8 (2×CH), 126.8 (d, *J* = 3.7 Hz), 116.5 (d, *J* = 24.3 Hz, 2×CH).

¹⁹F NMR (DMSO-*d*₆, 376 MHz) δ -109.8 (s, 1F).

HRMS-ESI calculated for C₁₅H₉N₃OFCINas: *m/z* 356.0037 ([M+Na]⁺), found: *m/z* 356.0038 ([M+Na]⁺).

Mp : >266 °C.

Preparation of CT1-69



2-(4-Fluorophenyl)acetaldehyde (CT1-54)

To a stirred solution of commercially available 2-(4-fluorophenyl)ethanol (1 g, 7.14 mmol) in CH₂Cl₂ (39 mL), was added freshly prepared Dess-Martin periodinane (DMP, 3.33 g, 7.85 mmol, 1.1 eq.). The reaction mixture was stirred for 1 h, then poured into a mixture 1:1 of saturated aqueous NaHCO₃ and saturated aqueous Na₂S₂O₃ solutions, and the resulting mixture was stirred at r.t. for 30 min. The organic layer was separated, washed with brine, filtered, and concentrated under reduced pressure to afford the desired aldehyde **CT1-54** as a pale yellow oil. This was used in the next step without further purification.

Note: the obtained aldehyde is unstable and should be used as fast as possible in the next step, or can be stored overnight in a refrigerator.

¹H NMR (CDCl₃, 300 MHz): δ 9.76 (t, *J* = 2.2 Hz, 1H), 7.19 (ap. dd, *J* = 8.8, 5.3 Hz, 2H), 7.07 (ap. t, *J* = 8.8 Hz, 2H), 3.69 (d, *J* = 2.2 Hz, 2H).

2-Bromo-2-(4-fluorophenyl)acetaldehyde (CT1-55)

To a stirred solution of previously prepared crude aldehyde **CT1-54** (7.14 mmol) in EtOAc (24 mL), under N₂ atmosphere, was added CuBr₂ (2.39 g, 10.7 mmol, 1.5 eq.). The resulting mixture was stirred at r.t. for 2 h, then diluted with Et₂O, filtered through a celite pad and concentrated under reduced pressure to yield α-bromoaldehyde **CT1-55** as a pale yellow oil. This compound was used in the next step without further purification.

Note: the obtained α-bromoaldehyde is highly unstable and cannot be stored, so it has to be used for the next step as fast as possible.

¹H NMR (CDCl₃, 300 MHz): δ 9.55 (d, *J* = 3.1 Hz, 1H), 7.42 (ap. dd, *J* = 8.7, 5.3 Hz, 2H), 7.11 (ap. t, *J* = 8.7 Hz, 2H), 5.26 (d, *J* = 3.1 Hz, 1H).

5-(4-Fluorophenyl)oxazol-2-amine (CT1-56)

To a stirred solution of previously prepared crude α-bromoaldehyde **CT1-55** (7.14 mmol) in DMF (4.3 mL), under N₂ atmosphere, was added urea (556 mg, 9.27 mmol, 1.3 eq.). The resulting mixture was placed in a pre-heated oil bath at 105 °C and stirred at this temperature for 1h. The reaction mixture was then cooled down to r.t., solvent was removed under reduced pressure, and an aqueous NaOH solution (2.5 M) was added to the residue. The resulting mixture was stirred at r.t. for 30 min, after which the formed precipitate was collected by filtration and washed with ice-cold water. Trituration of the crude solid with PE then ice-cold CH₂Cl₂ yielded the desired 2-aminooxazole as a beige powder (580 mg, 3.25 mmol, 45% over three steps).

Note: This oxazole is partially soluble in CH₂Cl₂.

¹H NMR (DMSO-*d*₆, 300 MHz): δ 7.48 (ap. dd, *J* = 8.9, 5.3 Hz, 2H), 7.21 (ap. t, *J* = 8.9 Hz, 2H), 7.15 (s, 1H), 6.83 (br. s, 2H).

¹³C NMR (DMSO-*d*₆, 75 MHz): δ 161.3, 160.6 (d, *J* = 243.7 Hz), 142.2, 125.4 (d, *J* = 2.9 Hz), 123.9 (d, *J* = 8.1 Hz, 2×CH), 122.7, 115.9 (d, *J* = 21.8 Hz, 2×CH).

¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ -109.8 (s, 1F).

Mp: 240-242 °C.

HRMS-ESI calculated for C₉H₈N₂OF: *m/z* 179.0621 ([M+H]⁺), found : *m/z* 179.0620 ([M+H]⁺).

4-Chloro-N-(5-(4-fluorophenyl)oxazol-2-yl)benzamide (CT1-69)

To a stirred solution of commercially available 4-chlorobenzoic acid (211 mg, 1.35 mmol, 1.6 eq.) in anhydrous DMF (3.3 mL), under N₂ atmosphere, was added TBTU (540 mg, 1.68 mmol, 2 eq.). The

resulting mixture was stirred at r.t. for 20 min, then **CT1-56** (150 mg, 0.84 mmol) was added followed by freshly distilled Et₃N (398 μ L, 2.95 mmol, 3.5 eq.), and the reaction mixture was stirred at r.t. overnight. At the end of the reaction, the crude mixture was poured into ice-cold water and the formed precipitate was collected by filtration and washed with water. The crude solid was triturated in petroleum ether, quickly washed with ice-cold CH₂Cl₂, and finally recrystallized from MeOH to yield **CT1-69** (38 mg, 0.12 mmol, 15%) as beige powder.

¹H NMR (DMSO-d₆, 300 MHz): δ 8.04 (d, J = 8.4 Hz, 2H), 7.73 (ap. dd, J = 8.9, 5.3 Hz, 2H), 7.66 (s, 1H), 7.61 (d, J = 8.4 Hz, 2H), 7.33 (ap. t, J = 8.9 Hz, 2H).

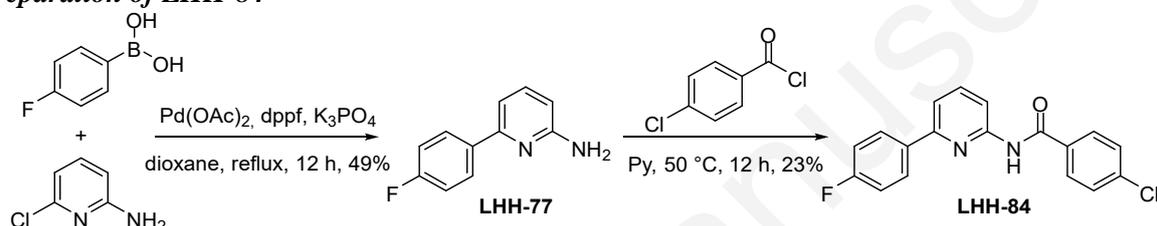
¹³C NMR (DMSO-d₆, 125 MHz): δ 165.5 (br s), 161.7 (d, J = 246.0 Hz), 153.9 (br s), 145.8 (br s), 137.2, 132.2 (br s), 130.1 (2 \times CH), 128.5 (2 \times CH), 125.6 (d, J = 7.7 Hz, 2 \times CH), 124.0 (d, 2.7 Hz), 120.5 (br s), 116.1 (d, J = 21.9 Hz, 2 \times CH).

¹⁹F NMR (DMSO-d₆, 376 MHz): δ -112.8 (s, 1F).

Mp: 214 $^{\circ}$ C.

HRMS-ESI calculated for C₁₆H₁₀N₂O₂FCINa : m/z 339.0312 ([M+Na]⁺), found : m/z 339.0313 ([M+Na]⁺).

Preparation of LHH-84



6-(4-Fluorophenyl)pyridin-2-amine (LHH-77)

A two-neck bottom flask equipped with a stirring bar and a condenser was charged with 6-chloropyridin-2-amine (500 mg, 3.90 mmol), 4-fluorophenylboronic acid (820 mg, 5.85 mmol, 1.5 eq.); and tripotassium phosphate (1.65 g, 7.80 mmol, 2 eq.). The flask was capped, evacuated, and flushed with argon for 5 min. Anhydrous 1,4-dioxane (15 mL) was then added, followed by palladium (II) acetate (44.0 mg, 0.195 mmol, 5 mol%) and 1,1'-bis(diphenylphosphino)ferrocene (108 mg, 0.195 mmol, 5 mol%). The resulting suspension was placed in a pre-heated oil bath at 110 $^{\circ}$ C and stirred at this temperature overnight. After cooling down to r.t., the reaction mixture was partitioned between H₂O and EtOAc, the layers were separated, and the aqueous layer was extracted once more with EtOAc. The combined organic layers were washed with 1 M NaOH solution, brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH₂Cl₂/MeOH 19:1) to yield the title compound **LHH-37** (360 mg, 1.91 mmol, 49%) as a light brown powder.

¹H NMR (DMSO-d₆, 300 MHz): δ 8.00 (ap. dd, J = 9.0, 5.7 Hz, 2H), 7.45 (t, J = 7.9 Hz, 1H), 7.24 (ap. t, J = 9.0 Hz, 2H), 7.01 (d, J = 7.7 Hz, 1H), 6.40 (d, J = 7.7 Hz, 1H), 6.00 (br s, 2H).

¹³C NMR (DMSO-d₆, 75 MHz): δ 162.4 (d, J = 245.4 Hz), 159.5, 153.2, 138.0, 135.8 (d, J = 2.9 Hz), 128.3 (d, J = 8.6 Hz, 2 \times CH), 115.2 (d, J = 21.3 Hz, 2 \times CH), 108.0, 107.0.

¹⁹F NMR (DMSO-d₆, 376 MHz): δ -114.2 (s, 1F).

Mp: 80 $^{\circ}$ C.

HRMS-ESI calculated for C₁₁H₁₀N₂F: m/z 189.0828 ([M+H]⁺), found: m/z 189.0828 ([M+H]⁺).

4-Chloro-N-(6-(4-fluorophenyl)pyridin-2-yl)benzamide (LHH-84)

To a stirred solution of **LHH-77** (100 mg, 0.53 mmol) in freshly distilled pyridine (2 mL), under inert atmosphere, was added 4-chlorobenzoyl chloride (100 μ L, 0.79 mmol, 1.5 eq.) at r.t. The obtained suspension was then placed in a pre-heated oil bath at 50 $^{\circ}$ C and stirred overnight. The resulting solution was cooled down to r.t. and poured into ice-cold water. The formed precipitate was collected by filtration, washed with water, air-dried and purified by flash chromatography (petroleum ether/EtOAc 8:2) to afford the desired compound **LHH-33** (40 mg, 0.12 mmol, 24%) as a white solid.

¹H NMR (DMSO-d₆, 300 MHz): δ 10.84 (br s, 1H), 8.20 (ap. dd, J = 8.9, 5.6 Hz, 2H), 8.11 (d, J = 8.2 Hz, 1H), 8.07 (d, J = 8.6 Hz, 2H), 7.94 (t, J = 7.9 Hz, 1H), 7.75 (d, J = 8.2 Hz, 1H), 7.61 (d, J = 8.6 Hz, 2H), 7.34 (ap. t, J = 8.9 Hz, 2H).

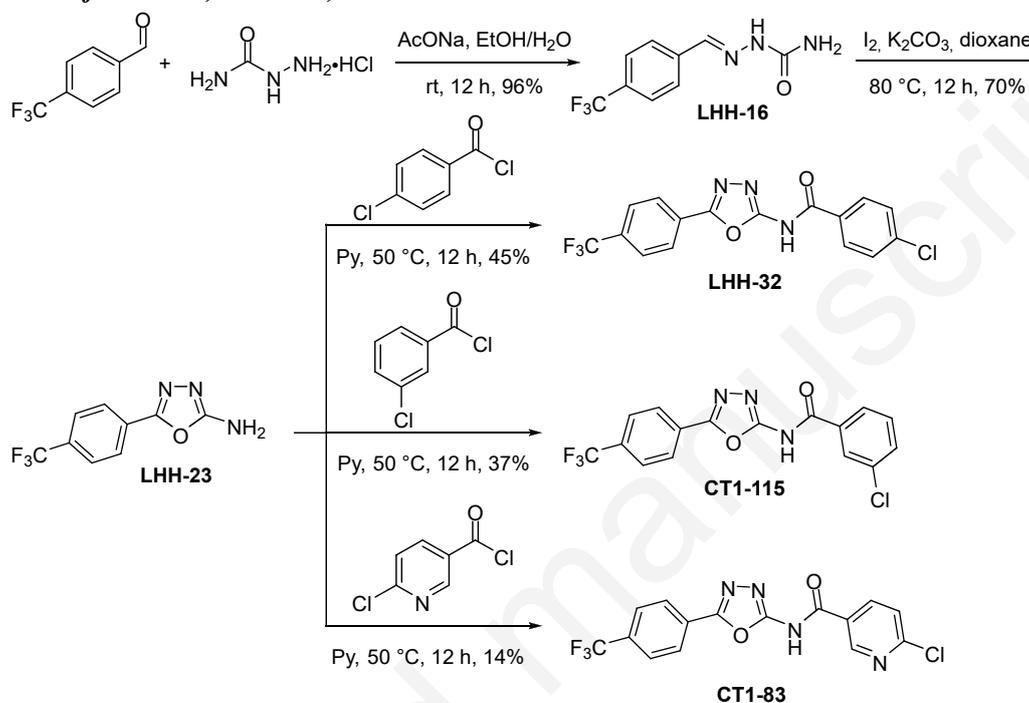
¹³C NMR (DMSO-d₆, 125 MHz): δ 165.1, 162.8 (d, *J* = 246.0), 153.6, 151.6, 139.2, 136.7, 134.5 (d, *J* = 2.9 Hz), 132.9, 130.0 (2×CH), 128.8 (d, *J* = 8.6 Hz, 2×CH), 128.3 (2×CH), 115.9, 115.4 (d, *J* = 21.0 Hz, 2×CH), 113.3.

¹⁹F NMR (DMSO-d₆, 376 MHz): δ -112.9 (s, 1F).

Mp: 150 °C.

HRMS-ESI calculated for C₁₈H₁₂N₂OFCINa: *m/z* 349.0520 ([M+Na]⁺), found: *m/z* 349.0516 ([M+Na]⁺).

Preparation of LHH-32, CT1-115, CT1-83



(*E*)-2-(4-(Trifluoromethyl)benzylidene)hydrazine-1-carboxamide (LHH-16)

Semicarbazide hydrochloride (1.92 g, 17.2 mmol, 1 eq.) was dissolved in water (43 mL). To this solution, was added a solution of sodium acetate (1.41 g, 17.2 mmol, 1 eq.) in water (11 mL). The reaction mixture was stirred at r.t. for 5 min, then a third solution of 4-fluorobenzaldehyde (2.35 mL, 17.2 mmol) in EtOH (21 mL) was slowly added and the resulting mixture was stirred at r.t. overnight. The reaction mixture was poured into crushed ice and the formed precipitate was collected by filtration, washed with water and air-dried to afford **LHH-16** (3.82 g, 16.5 mmol, 96%) as a white solid.

Note: The obtained product can also be recrystallized from EtOH or *i*-PrOH, but was obtained clean without purification.

¹H NMR (DMSO-d₆, 300 MHz): δ 10.48 (s, 1H), 7.95 (d, *J* = 8.1 Hz, 2H), 7.89 (s, 1H), 7.71 (d, *J* = 8.1 Hz, 2H), 6.62 (br s, 2H).

5-(4-(Trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-amine (LHH-23)

To a stirred solution of **LHH-16** (2.0 g, 8.65 mmol) in 1,4-dioxane (86 mL), under inert atmosphere, were added K₂CO₃ (3.58 g, 26.0 mmol, 3 eq.) and iodine (2.63 g, 10.4 mmol, 1.2 eq.). The reaction mixture was heated to 80 °C and stirred overnight. After being cooled to r.t., it was treated with aq. half-saturated Na₂S₂O₃ solution and extracted with a mixture CH₂Cl₂/MeOH 10:1 (5 times). The combined organic layers were dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was recrystallized from *i*-PrOH, then quickly washed with cold *i*-PrOH and cold MeOH to afford **LHH-23** (1.33 g, 5.80 mmol, 67%) as a white solid.

¹H NMR (DMSO-d₆, 300 MHz): δ 7.99 (d, *J* = 8.3 Hz, 2H), 7.89 (d, *J* = 8.3 Hz, 2H), 7.44 (br s, 2H).

¹³C NMR (DMSO-d₆, 75 MHz): δ 164.3, 156.3, 129.9 (q, *J* = 32.3 Hz), 128.1, 126.2 (q, *J* = 3.8 Hz, 2×CH), 125.6 (2×CH), 123.9 (q, *J* = 272.2 Hz).

¹⁹F NMR (DMSO-d₆, 376 MHz): δ -61.4 (s, 3F).

Mp: >266 °C.

HRMS-ESI calculated for C₉H₆N₃OF₃Na: *m/z* 252.0361 ([M+Na]⁺), found: *m/z* 252.0361 ([M+Na]⁺).

4-Chloro-*N*-(5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)benzamide (LHH-32)

To a stirred solution of **LHH-23** (100 mg, 0.44 mmol) in freshly distilled pyridine (1.7 mL), under inert atmosphere, was added 4-chlorobenzoyl chloride (78 μL, 0.61 mmol, 1.4 eq.) at r.t. The obtained suspension was then placed in a pre-heated oil bath at 50 °C and stirred overnight. The resulting solution was cooled down to r.t. and poured into ice-cold water. The formed precipitate was collected by filtration, washed with water, air-dried and finally washed with Et₂O to afford the desired compound **LHH-32** (72 mg, 0.19 mmol, 45%) as a white solid.

¹H NMR (DMSO-*d*₆, 300 MHz): δ 8.17 (d, *J* = 8.2 Hz, 2H), 8.05 (d, *J* = 8.4 Hz, 2H), (d, *J* = 8.2 Hz, 2H), 7.99 (d, *J* = 8.4 Hz, 2H), 7.64 ((d, *J* = 8.2 Hz, 2H).

¹³C NMR (DMSO-*d*₆, 125 MHz): δ 164.3 (br s), 160.4 (br s), 159.0 (br s), 137.9, 131.3 (q, *J* = 32.4 Hz), 131.0 (br s), 130.3 (2×CH), 128.8 (2×CH), 127.1, 126.9 (2×CH), 126.5 (q, *J* = 3.8 Hz, 2×CH), 123.8 (q, *J* = 272.7 Hz).

¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ -61.6 (s, 3F).

Mp: 264-265 °C.

HRMS-ESI calculated for C₁₆H₉N₃O₂F₃ClNa: *m/z* 390.0233 ([M+Na]⁺), found: *m/z* 390.0230 ([M+Na]⁺).

3-Chloro-*N*-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)benzamide (CT1-115)

To a stirred solution of **LHH-23** (150 mg, 0.65 mmol) in freshly distilled pyridine (3.8 mL), under inert atmosphere, was added 3-chlorobenzoyl chloride (141 μL, 1.11 mmol, 1.7 eq.) at 0 °C. The resulting suspension was heated to 50 °C and stirred overnight. The resulting solution was cooled to r.t. and poured into ice-cold water. The obtained aqueous suspension was extracted with EtOAc (3 times) and the combined organic extracts were washed with water (twice), brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was triturated in Et₂O and the white solid was collected by filtration to afford **CT1-115** (88 mg, 0.24 mmol, 37%) as a white solid.

¹H NMR (DMSO-*d*₆, 300 MHz): δ 8.17 (d, *J* = 8.1 Hz, 2H), 8.09 (s, 1H), 8.01-7.98 (3H including (d, *J* = 8.1 Hz, 2H)), 7.74 (d, *J* = 8.1 Hz, 1H), 7.61 (ap. t, *J* = 7.9 Hz, 1H).

¹³C NMR (DMSO-*d*₆, 125 MHz): δ 164.1 (br s), 159.4 (br s), 158.5 (br s), 134.5 (br s), 133.4, 132.6, 131.3 (q, *J* = 32.4 Hz), 130.6, 128.1, 127.1 (2×CH), 126.8 (2×CH), 126.4 (q, *J* = 3.8 Hz, 2×CH), 123.7 (q, *J* = 271.8 Hz).

¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ -61.6 (s, 3F).

Mp: 229 °C.

HRMS-ESI calculated for C₁₆H₉N₃O₂F₃ClNa: *m/z* 390.0228 ([M+Na]⁺), found: *m/z* 390.0228 ([M+Na]⁺).

6-Chloro-*N*-(5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)nicotinamide (CT1-83)

To a stirred solution of **LHH-23** (150 mg, 0.65 mmol) in freshly distilled pyridine (2.3 mL), under inert atmosphere, was added 6-chloronicotinoyl chloride (223 mg, 1.27 mmol, 1.9 eq.) at 0 °C. The resulting suspension was heated to 50 °C and stirred for 20 h. The resulting solution was cooled to r.t. and poured into ice-cold water. The formed precipitate was collected by filtration, washed with water, then recrystallized from MeOH to afford the desired compound **CT1-83** (63 mg, 0.17 mmol, 14%) as a white solid.

Note: The protocol described above is the one to follow with commercially available 6-chloronicotinoyl chloride. However, due to an issue from Alfa Aesar the purchased 6-chloronicotinoyl chloride appears to be the corresponding carboxylic acid. 6-Chloronicotinoyl chloride was thus prepared as previously described and was dissolved in pyridine prior to the addition of LHH23.

¹H NMR (DMSO-*d*₆, 300 MHz): δ 9.02 (s, 1H), 8.41 (d, *J* = 8.0 Hz, 1H), 8.17 (d, *J* = 8.1 Hz, 2H), 7.99 (d, *J* = 8.1 Hz, 2H), 7.74 (d, *J* = 8.1 Hz, 1H).

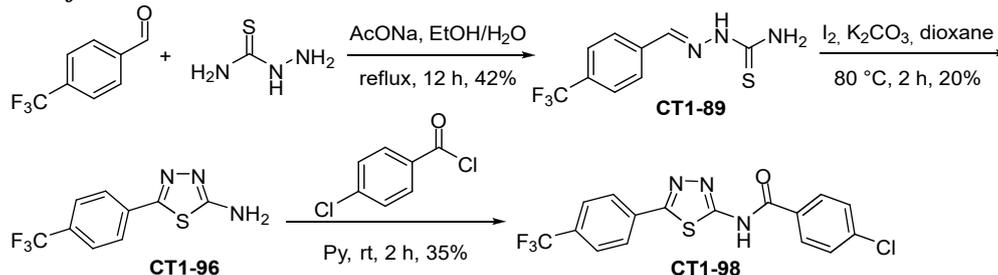
¹³C NMR (DMSO-*d*₆, 125 MHz): δ 163.8 (br s), 159.1 (br s), 158.8 (br s), 153.8, 150.1, 139.7, 131.3 (q, *J* = 32.4 Hz), 128.4 (br s), 127.1, 126.9 (2×CH), 126.5 (q, *J* = 3.8 Hz, 2×CH), 124.4, 123.8 (q, *J* = 271.8 Hz).

¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ -61.6 (s, 3F).

Mp: 253 °C.

HRMS-ESI calculated for C₁₅H₈N₄O₂F₃ClNa: *m/z* 391.0186 ([M+Na]⁺), found: *m/z* 391.0187 ([M+Na]⁺).

Preparation of CT1-98



(E)-2-(4-(Trifluoromethyl)benzylidene)hydrazine-1-carbothioamide (CT1-89)

Thiosemicarbazide (1.57 g, 17.2 mmol, 1 eq.) was dissolved in water (43 mL). To this solution, was added a solution of sodium acetate (1.41 g, 17.2 mmol, 1 eq.) in water (11 mL). The reaction mixture was stirred at r.t. for 5 min, then a third solution of 4-fluorobenzaldehyde (2.35 mL, 17.2 mmol) in EtOH (21 mL) was slowly added and the resulting mixture was stirred at r.t. overnight. The reaction mixture was poured into crushed ice and the formed precipitate was collected by filtration, washed with water, air-dried and recrystallized from *i*-PrOH to afford CT1-89 (1.80 g, 7.28 mmol, 43%) as a light yellow solid.

Note: For better results, the reaction should be performed at 60-70 °C overnight.

¹H NMR (DMSO-*d*₆, 300 MHz): δ 11.61 (s, 1H), 8.33 (br s, 1H), 8.17 (br s, 1H), 8.10 (s, 1H), 8.03 (d, J = 8.2 Hz, 2H), 7.72 (d, J = 8.2 Hz, 2H).

5-(4-(Trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-amine (CT1-96)

To a stirred solution of CT1-89 (800 mg, 3.23 mmol) in 1,4-dioxane (32 mL), under inert atmosphere, were added K₂CO₃ (1.34 g, 9.71 mmol, 3 eq.) and iodine (1.23 g, 4.85 mmol, 1.2 eq.). The reaction mixture was heated to 80 °C and stirred for 30 h. After being cooled to r.t., it was treated with aq. half-saturated Na₂S₂O₃ solution and extracted with a mixture CH₂Cl₂/MeOH 10:1 (5 times). The combined organic layers were dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (10% to 50% EtOAc in petroleum ether). The obtained orange solid was washed with the minimal amount of EtOAc until obtaining CT1-96 (120 mg, 0.49 mmol, 15%) as a beige solid.

Note: Reaction time too long, should be shorter to avoid the formation of a by-product.

¹H NMR (DMSO-*d*₆, 300 MHz): δ 7.96 (d, J = 8.1 Hz, 2H), 7.81 (d, J = 8.1 Hz, 2H), 7.60 (br s, 2H).

¹³C NMR (DMSO-*d*₆, 75 MHz): δ 169.4, 154.7, 134.7, 129.2 (q, J = 31.9 Hz), 126.8 (2×CH), 126.0 (q, J = 3.6 Hz, 2×CH), 124.0 (q, J = 271.9 Hz).

¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ -61.2 (s, 3F).

Mp: 241 °C.

HRMS-ESI calculated for C₉H₇N₃F₃S: m/z 246.0313 ([M+H]⁺), found: m/z 246.0310 ([M+H]⁺).

4-Chloro-*N*-(5-(4-(trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-yl)benzamide (CT1-98)

To a stirred solution of CT1-96 (80 mg, 0.33 mmol) in freshly distilled pyridine (1.5 mL), under inert atmosphere, was added 4-chlorobenzoyl chloride (62 μ L, 0.49 mmol, 1.5 eq.) at 0 °C. The resulting suspension was stirred at r.t. for 1.5 h, then poured into ice-cold water. The formed precipitate was collected by filtration, washed with water, then recrystallized from *i*-PrOH and washed with cold MeOH to afford the desired compound CT1-98 (44 mg, 0.12 mmol, 35%) as a light beige solid.

¹H NMR (DMSO-*d*₆, 300 MHz): δ 8.21 (d, J = 8.1 Hz, 2H), 8.16 (d, J = 8.6 Hz, 2H), 7.91 (d, J = 8.1 Hz, 2H), 7.66 (d, J = 8.6 Hz, 2H).

¹³C NMR (DMSO-*d*₆, 125 MHz): δ 164.6 (br s), 160.6 (br s), 160.2 (br s), 138.0, 133.9, 130.4 (3×C including (2×CH and 1Cq (q, J = 37.9 Hz))), 128.8 (2×CH), 127.7 (2×CH), 126.3 (q ap. s, 2×CH), 124.3, 123.9 (q, J = 271.8 Hz).

¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ -61.3 (s, 3F).

Mp: >266 °C.

HRMS-ESI calculated for C₁₆H₉N₃OF₃ClNaS: m/z 406.0005 ([M+Na]⁺), found: m/z 406.0001 ([M+Na]⁺).

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Table S1. Bacterial strains used in this study. The following characteristics were present across the selected strains: gram-positive and -negative; not colorable with the Gram-stain method; aerobic and anaerobic bacteria; high-CO₂ requirement.

Strains	Culture conditions	Origin
Gram-positive		
<i>Staphylococcus aureus</i>	Aerobic	Clinical isolate
<i>Staphylococcus aureus</i> ATCC 29213	Aerobic	Reference strain
<i>Staphylococcus epidermidis</i>	Aerobic	Clinical isolate
<i>Streptococcus pyogenes</i>	Aerobic + 5% CO ₂	Clinical isolate
<i>Bacillus subtilis</i>	Aerobic	Clinical isolate
<i>Clostridium perfringens</i>	Aerobic + humidity	Clinical isolate
<i>Enterococcus faecalis</i> ATCC 29212	Aerobic	Reference strain
<i>Enterococcus faecium</i> AUS0004	Aerobic	Lam <i>et al.</i> , 2012
<i>Enterococcus faecium</i> HM1070	Aerobic	Bozdogan & Leclercq, 1999
<i>Kocuria rhizophila</i>	Aerobic	Clinical isolate
<i>Listeria monocytogenes</i>	Aerobic	Clinical isolate
Gram-negative		
<i>Acinetobacter baumannii</i>	Aerobic	Clinical isolate
<i>Bacteroides fragilis</i> ATCC 25285	Anaerobic	Reference strain
<i>Enterobacter cloacae</i>	Aerobic	Clinical isolate
<i>Escherichia coli</i>	Aerobic	Clinical isolate
<i>Escherichia coli</i> ATCC 25922	Aerobic	Reference strain
<i>Klebsiella pneumoniae</i>	Aerobic	Clinical isolate
<i>Morganella morganii</i> EB6	Aerobic	Clinical isolate
<i>Morganella morganii</i> EB8	Aerobic	Clinical isolate
<i>Proteus mirabilis</i>	Aerobic	Clinical isolate
<i>Proteus vulgaris</i>	Aerobic	Clinical isolate
<i>Pseudomonas aeruginosa</i>	Aerobic	Clinical isolate
Mycobacteria		
<i>Mycobacterium abscessus</i>	Aerobic + humidity	Clinical isolate
<i>Mycobacterium fortuitum</i>	Aerobic + humidity	Clinical isolate

Lam MM, Seemann T, Bulach DM, Gladman SL, Chen H, Haring V, Moore RJ, Ballard S, Grayson ML, Johnson PD, Howden BP, Stinear TP. 2012. Comparative analysis of the first complete *Enterococcus faecium* genome. J. Bacteriol. 194:2334-2341.

Bozdogan B, Leclercq R. 1999. Effects of genes encoding resistance to streptogramins A and B on the activity of quinupristin-dalfopristin against *Enterococcus faecium*. Antimicrob. Agents Chemother. 43:2720-2725.