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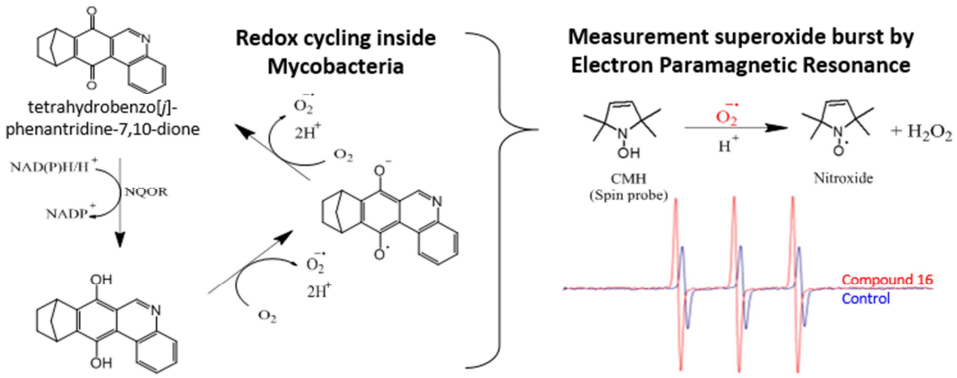
Graphical Abstract



The synthesis and *in vitro* biological evaluation of novel fluorinated tetrahydrobenzo[*j*]phenanthridine-7,12-diones against *Mycobacterium tuberculosis*

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Keywords
Tetrahydrobenzo[*j*]phenanthridine-7,12-dione, *Mycobacterium tuberculosis*, anti-mycobacterial activity, fluorinated derivatives, metabolic stability

Highlights

- Fluorinated benzo[*j*]phenanthridinediones were synthetized and characterized.
- *In vitro* activity against *Mtb* H37Ra with a MIC from 3.4 µM.
- Moderate cytotoxicity and no early signs of genotoxic effects.
- Metabolically stable in the presence of murine and human microsomes.

Abbreviations

4-hydroxy-TEMPO, 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl; AIDS, acquired immunodeficiency virus; CC_{50}, fifty percentage cytotoxicity concentration; CMH, 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine; CYP_{450}, cytochrome P450; DMEM, Dulbecco's modified Eagle's medium; ESI, electron spray ionization; HIV, human immunodeficiency virus; IC_{50}, half maximal inhibitory concentration; iFCS, heat-inactivated fetal calf serum; KHB, Krebs HEPES buffer; LB, Luria-Bertani; MDR, multi-drug resistant; MIC, minimal inhibitory concentration; MOI, multiplicity of infection; *Mtb*, *Mycobacterium tuberculosis*; Mtr, mycothione reductase; MXF, moxifloxacin; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NRU, neutral red uptake; OADC: oleic acid-albumin-dextrose-catalase; OQD, octahydrobenzo[*j*]phenanthridine-7,12-dione; PBS, phosphate-buffered saline; RLU, relative light units; ROS, reactive oxygen species; S/N, signal to noise; TB, Tuberculosis; TQD, tetrahydrobenzo[*j*]phenanthridine-7,12-diones; UDPGA, uridine diphosphate glucuronic acid; UGT, uridine glucuronosyl transferase; XDR, extensively drug resistant.

Abstract

Tuberculosis (TB) still has a major impact on public health. In order to efficiently eradicate this life-threatening disease, the exploration of novel anti-TB drugs is of paramount importance. As part of our program to design new 2-azaanthraquinones with anti-mycobacterial activity, various "out-of-

plane" tetrahydro- and octahydrobenzo[*j*]phenanthridinediones were synthesized. In this study, the scaffold of the most promising hits was further optimized in an attempt to improve the bioactivity and to decrease enzymatic degradation. The rudiment bio-evaluation of a small library of fluorinated tetrahydrobenzo[*j*]phenanthridine-7,12-dione derivatives indicated no significant improvement of the bio-activity against intracellular and extracellular *Mtb*. Though, the derivatives showed an acceptable toxicity against J774A.1 macrophages and early signs of genotoxicity were absent. All derivatives showed to be metabolic stabile in the presence of both phase I and phase II murine or human microsomes. Finally, the onset of reactive oxygen species within *Mtb* after exposure to the derivatives was measured by electron paramagnetic resonance (EPR). Results showed that the most promising fluorinated derivative is still a possible candidate for the subversive inhibition of mycothione reductase.

1. Introduction

Tuberculosis (TB), an infectious pulmonary disease caused by the weakly Gram-positive bacillus *Mycobacterium tuberculosis* (*Mtb*), has existed for centuries and still poses a major global health threat. In 2016, approximately 10.4 million people developed active TB disease and over 1.3 million persons died as a result. Accordingly, for the past few years TB has been the leading cause of death from a single infectious agent, surpassing even the human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) pandemic[1]. The efficiency of current anti-TB chemotherapeutics is constantly undermined by the emergence of multi-drug resistant (MDR) and extensively drug resistant (XDR) *Mtb* strains fueled by the current HIV/AIDS pandemic. Consequently, there is an urgent and continuing demand for the identification of innovative anti-TB compounds and biological targets. Towards this goal, the anti-tubercular properties of benzo[*j*]phenanthridine-7,12-diones, tetracyclic derivatives of the naturally occurring benz[*g*]isoquinoline-5,10-dione, have been studied by our group. This natural 2-azaanthraquinone is of considerable interest in organic synthesis due to its potential biological activity against the multi-drug resistant *Plasmodium falciparum*,[2] the yeast *Candida albicans*, *Cryptococcus neoformans*, the acid-fast *Mycobacterium intracellulare* and the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*.[3] Besides antimicrobial activities, the quinone nucleus is also often associated with antitumor activity[4]. As previously observed for the closely related group of naphthoquinones,[5] it was hypothesized that the benzo[*j*]phenanthridine-7,12-diones would also show subversive substrate properties with mycothione reductase (Mtr), a key enzyme in the redox homeostasis of *Mtb*.[6] This due to maintenance of the quinone motif, which was demonstrated to contribute to the Mtr-mediated generation of toxic superoxide anion radicals and, thus, biological activity.[5] As this unexploited bacillary target could lead to the identification of novel anti-TB candidates with an innovative mode of action, we were encouraged to test various substituted benzo[*j*]phenanthridine-7,12-dione derivatives[7] for their anti-mycobacterial activity. The study showed that 3-methylbenzo[*j*]phenanthridine-7,12-dione presented promising antimicrobial activity against both susceptible and MDR *Mtb* strains, possibly confirming the Mtr-mediated speculation. Nevertheless, the selectivity index of this favorable derivative was considerably low due to its relatively high cytotoxic effects.[7] To further investigate the importance of the nature and position of substitution, and to lower the cytotoxicity, which in turn could arise from the intercalating effects of these rather planar structures, Cappoen *et al*.[8] envisaged and evaluated a series of newly substituted "out-of-plane" tetrahydrobenzo[*j*]phenanthridine-7,12-dione (TQD) and octahydrobenzo[*j*]phenanthridine-7,12-dione (OQD) derivatives. Although anti-TB potency was enhanced and acute cytotoxicity successfully reduced, the anti-mycobacterial activity of the most favorable derivatives decreased in turn when subjected to enzymatic degradation[8]. To further improve the metabolic stability and allow the evaluation of the compound class in *in vivo* PK/PD and dose-response studies, further optimization of the benzophenanthridine-7,12-dione scaffold by the

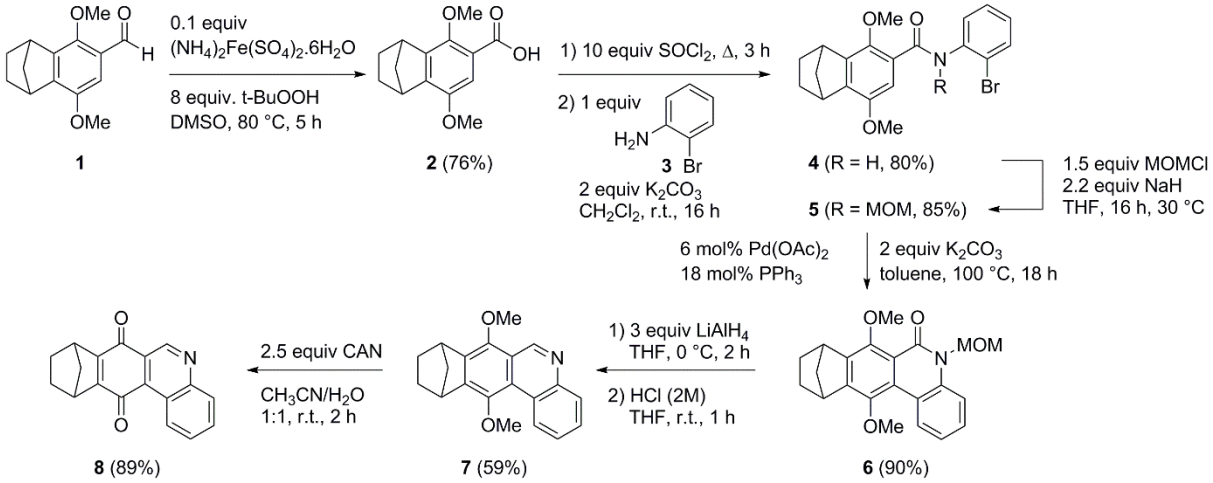
addition of fluorine or fluorine-containing groups was envisaged[9]. Therefore, besides 8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine-7,12-dione **8**, five fluoro- and trifluoromethyl-substituted TQD derivatives **14**-**18** were synthesized in the present study.

2. Results and Discussion

2.1. Chemistry

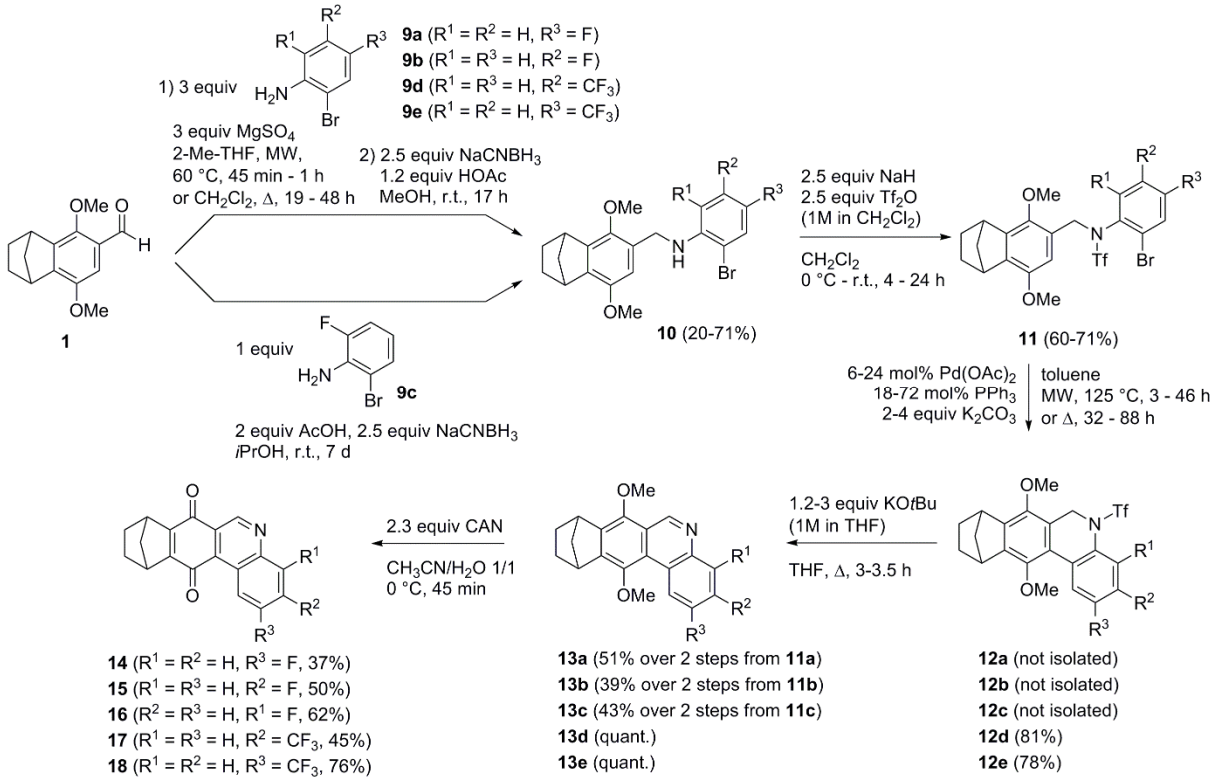
For the synthesis of the non-fluorinated TQD derivative **8** (Scheme 1), the starting carboxylic acid **2** was prepared by means of an iron-catalysed oxidation of 5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxaldehyde **1** and reacted with 2-bromoaniline **3** to give amide **4**. The amide nitrogen was MOM-protected and subsequent cyclisation of intermediate **5** by means of a palladium-catalysed intramolecular arylation afforded lactam **6**. This lactam **6** was reduced with $LiAlH_4$ followed by treatment with HCl to convert the intermediate hemi-aminals into the corresponding pyridine **7**. This compound **7** was then oxidatively demethylated towards 8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine-7,12-dione **8**.

Scheme 1. Synthesis of 8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine-7,12-dione 8.



For the fluorinated TQD derivatives **14**-**18** (Scheme 2), aldehyde **1** was converted into different amines **10** *via* reductive amination with various fluorinated 2-bromoanilines **9** which demonstrated a low nucleophilicity. The reductive amination was performed in a one-pot fashion in isopropanol in the presence of acetic acid and $NaCNBH_3$ or stepwise by imination either in 2-methyltetrahydrofuran under microwave irradiation or via heating in dichloromethane followed by reduction using $NaCNBH_3$. In the next step, amines **10** were treated with triflic anhydride in the presence of NaH in dichloromethane resulting in trifluoromethanesulfonamides **11**. Palladium-catalyzed ring closure of amides **11** followed by aromatization upon treatment with KO*t*Bu afforded phenanthridines **13**, which were subsequently oxidatively demethylated by treatment with CAN toward 8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine-7,12-diones **14**-**18.**

Scheme 2. Synthesis of fluorinated tetrahydrobenzo[*j*]phenanthridine-7,12-dione derivatives 14-18.



2.2. Biology

Following the synthesis, both the unsubstituted scaffold **8** and the series of substituted TQDs **14**-**18** were evaluated for their *in vitro* biological properties. First, the derivatives were screened against a broader panel of clinically relevant bacteria, which consisted of the Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, the Gram-positive *Enterococcus faecium*, *S. aureus* and *Streptococcus pneumoniae*, several of which are member of the ESKAPE bacteria, associated with bacterial resistance and the acid-fast *Mtb* H37Ra (Table 1).

Table 1. *In vitro* antimicrobial specificity of the TQDs 8, 14-18.

Compound	IC_{50} (μM)[a]						
	E. coli	***P. aeruginosa***	***K. pneumoniae***	***E. faecium***	***S. aureus***	***S. pneumoniae***	***Mtb* H37Ra**
8	> 64.0	> 64.0	> 64.0	64.0 >x> 32.0	64.0 >x> 32.0	64.0 >x> 32.0	2.0 >x > 1.0
14	> 64.0	> 64.0	> 64.0	> 64.0	> 64.0	64.0 >x> 32.0	8.0 >x > 4.0
15	> 64.0	> 64.0	> 64.0	> 64.0	> 64.0	64.0 >x> 32.0	2.0 >x > 1.0
16	> 64.0	> 64.0	> 64.0	> 64.0	> 32.0	64.0 >x> 32.0	2.0 >x > 1.0
17	> 64.0	> 64.0	> 64.0	> 64.0	> 64.0	64.0 >x> 32.0	4.0 >x > 2.0
18	> 64.0	> 64.0	> 64.0	> 64.0	> 64.0	> 64.00	4.0 >x > 2.0
Norfloxacin	> 0.25	> 0.5	ND	ND	ND	ND	ND
Ciprofloxacin	ND	ND	< 0.25	ND	ND	ND	ND
Vancomycin	ND	ND	ND	> 0.50	ND	ND	ND
Doxycycline	ND	ND	ND	ND	< 0.25	> 0.25	ND

Isoniazid	ND	ND	ND	ND	ND	ND	< 0.25

ND, not done. [a] Fifty percentage inhibitory concentration.

Results showed that the novel TQDs derivatives maintained the specific antimicrobial activity against *Mtb* H37Ra as the tested Gram-negative and Gram-positive bacteria showed low susceptibility towards the TQDs, in contrast to the susceptibility of *Mtb* towards the derivatives. This observation could be explained by the fact that the activity of the TQDs is limited to targets present in *Mtb* but absent in the bacterial screening panel[6].

The anti-tubercular properties of these newly synthesized derivatives were then further studied in detail by a luminometric assay using a luminescent *Mtb* H37Ra^{lux} strain. Anti-mycobacterial potency was expressed as the fifty percentage inhibitory concentration (IC_{50}) and the minimal inhibitory concentration (MIC) at which the mycobacterial growth is reduced by 90% (Table 2). In parallel, the acute cytotoxicity of the TQDs against the eukaryotic J774A.1 macrophage cell line was studied using a neutral red uptake (NRU) assay. The cytotoxic concentration (CC_{50}) of a compound is defined as the concentration at which the NRU by the cells is reduced with 50% (Table 2). The selectivity index of the derivatives was calculated by dividing the CC_{50} with the MIC (Table 2).

Table 2. Anti-mycobacterial activity and acute cytotoxicity of the TQDs 8, 14-18.

Compound	*Mtb* H37Ra^{lux}		J774A.1	SI[d]
	IC_{50} (µM)[a]	MIC (µM)[b]	CC_{50} (µM)[c]	
8	1.28	6.33	64.50	10.19
14	6.93	57.28	> 128.00	-
15	1.80	10.22	35.17	3.44
16	1.54	4.43	85.50	19.30
17	3.05	7.95	122.12	15.36
18	2.51	9.23	108.42	11.75
Isoniazid	0.11	0.31	ND	ND
Tamoxifen	ND	ND	11.06	ND

ND, not done. [a] Fifty percentage inhibitory concentration. [b] Minimum inhibitory concentration. [c] Fifty percentage cytotoxicity concentration. [d] Selectivity index (CC_{50}/MIC).

Results showed that these "out-of-plane" derivatives have a reduced acute cytotoxicity in comparison with the planar derivatives from the initial study.[7] Moreover, the introduction of these fluoro and trifluoromethyl groups resulted even in a further decrease of acute cytotoxicity compared to the previous selected derivatives (CC_{50} = 51.35, 8.49 and 49.76 µM).[8] In contrast, anti-mycobacterial potency of the derivatives within the present study decreased compared with previously reported derivatives (MIC = 0.59, 0.22 and 0.26 µM), which in turn led to a lower SI in comparison with the previous hits (SI = 87.03, 38.59 and 191.38)[8]. Although fluorinated compounds are often synthesized to improve biological activity,[10] TQD **14** (R^3 = F) and **15** (R^2 = F) only showed a MIC of 57.28 and 10.22 µM, respectively. Though, when substituted on the R^1 position (compound **16**), an improved MIC value (= 4.43 µM) and, thus, SI (= 19.30) was observed, even surpassing the SI of the unsubstituted scaffold **8**. The trifluoromethylated derivatives **17** and **18** also showed a higher SI (= 15.36 and 11.75, respectively), mostly due to the decreased acute cytotoxicity. Considering the knowledge-based estimation of *Mtb* cell wall permeability probability of small organic compounds described by Merget *et al.*[11], both the unsubstituted scaffold and fluorinated derivatives are not considered as impermeable. Hereby, impaired *Mtb* cell wall permeability is not considered the sole reason of decreased antimycobacterial activity. The introduction of fluorine-containing substituents

might also decrease Mtr subversive substrate properties, the probable target of this promising compound class.

Furthermore, the TQD derivatives also showed anti-tubercular potencies against other, clinically relevant mycobacterial species and NTM such as *M. marinum*, which can cause a debilitating skin infection, *M. avium* subsp. *avium*, the causative agent of opportunistic infections in AIDS patients, and *M. bovis* BCG, member of the *Mycobacterium tuberculosis* complex (Table 3).

Table 3. Antimicrobial activity of the TQDs 8, 14-18 against other mycobacterial strains.

	IC_{50} (μM)[a]						
	8	**14**	**15**	**16**	**17**	**18**	**Moxifloxacin**
M. marinum	7.73	5.04	3.22	3.30	9.00	45.49	0.14
M. avium subsp. avium	0.12	0.24	0.43	0.10	3.24	8.51	0.19
M. bovis BCG	7.90	10.35	17.39	5.03	14.95	12.53	0.03

[a] Fifty percentage inhibitory concentration.

Next, the effect of the strategically introduced fluorine and fluorine-containing substitutions[10,12] on metabolic stability was evaluated by a microsomal stability assay using male mouse and human liver microsomes. Phase-I and Phase-II metabolism were studied through the cytochrome P450 (CYP_{450}) superfamily and uridine glucuronosyl transferase (UGT) enzymes, respectively. As an acceptable cut-off for favorable metabolic stability, a percentage of remaining parent compound higher than 50% upon 30 minutes of exposure was used to evaluate the results. Neither the unsubstituted scaffold **8** nor the newly synthesized TQD derivatives **14**-**18** demonstrated extensive Phase-I or Phase-II metabolism in both mouse and human microsomes (Table 4).

Table 4. Microsomal stability assay results for TQD 8, 14-18.

Microsomes	**Phase I/II**	**Time (min)**	**Remaining parent compound (%)**						
			8	**14**	**15**	**16**	**17**	**18**	**Diclophenac**
Human	**Phase I CYP450 – NADPH**	**0**	100	100	100	100	100	100	100
		15	100	100	100	100	100	100	45
		30	100	100	100	100	100	100	23
		60	100	100	100	100	100	100	5
	Phase II UGT enzymes	**0**	100	100	100	100	100	100	100
		15	83	92	79	78	71	100	22
		30	76	79	76	71	63	74	16
		60	68	64	60	65	63	69	13
Mouse	**Phase I CYP450 – NADPH**	**0**	100	100	100	100	100	100	100
		15	100	100	100	100	100	100	93
		30	100	100	100	100	100	100	81
		60	94	100	100	100	100	100	54
	Phase II UGT enzymes	**0**	100	100	100	100	100	100	100
		15	69	96	89	77	62	87	64
		30	86	92	88	70	70	81	40

	60	78	72	73	61	51	78	37

As there is no difference in the improvement of the metabolic stability between the unsubstituted scaffold **8** and the fluorinated TQDs **14-18**, this observation confirms that both the deletion of the substituents of the previous hits (R^x = *t*-Bu or CO_2Et)[8] and the exchange of these with protective groups improves metabolic stability.

Since the introduction of these fluorinated groups can modulate the lipophilicity and, thus, membrane permeability[10,12], the intracellular activity of the most potent TQD derivative **16** and unsubstituted scaffold **8** was tested. Therefore, J774A.1 macrophages were infected with the *Mtb* H37Ra^{lux} strain and treated with both derivatives. After 24, 48 and 72 hours, the cell monolayer was lysed and the number of intracellular bacteria was determined by luminometry (Figure 1).

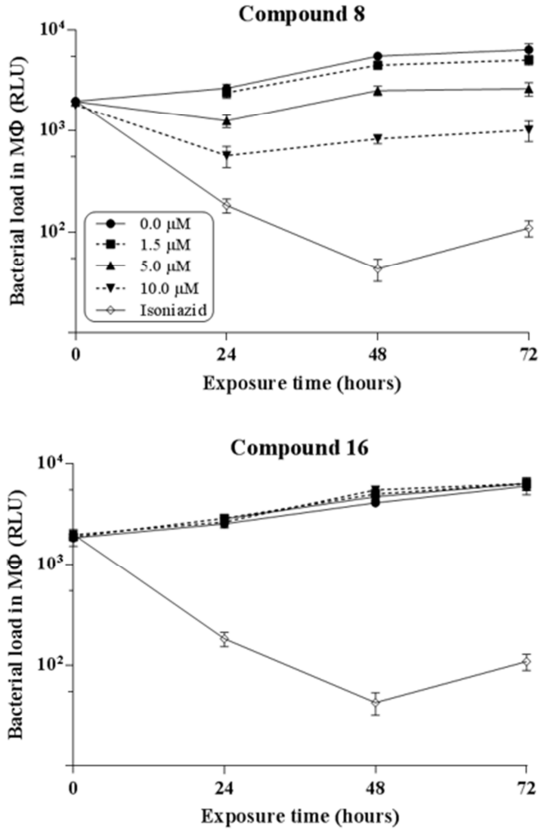


Figure 1. Growth inhibition of *Mtb* H37Ra^{lux} inside J774A.1 macrophages. Results are presented in relative light units (RLU) and plotted as the mean of triplicates ± SD. Isoniazid was used as a positive control at 0.1 µM.

Results, presented as relative light units (RLU), showed that the unsubstituted scaffold **8** was still able to inhibit intracellular *Mtb* replication. At 1.5, 5.0 and 10.0 µM, the bacterial replication was reduced by 20.68 ± 8.40%, 58.94 ± 6.24% and 84.14 ± 3.66%, respectively. In contrast to the unsubstituted scaffold **8** and the previous substituted hits[8], the fluoro-substituted TQD **16** (R^1 = F) was not able to reach the bacteria through the macrophage membrane. This indicates the fluorine substitution possibly hindered the ability to target intracellular replicating bacteria.

The capacity of compounds in early screening to cause genotoxicity should not be overlooked. Therefore, the mutagenic potential of TQD **8** and **16** or their metabolites was evaluated using the VITOTOX^{TM} assay.[13] This bacterial reporter model is closely correlated with the AMES test and can

detect early signs of genotoxicity by studying the induction of the regulatory SOS operon, a key element in the repair of early cellular DNA damage. Briefly, two recombinant *Salmonella typhimurium* reporter strains, the TA104 recN2-4 (Genox strain) and pr1 (Cytox strain), were used. The Genox strain carries an integrated lux operon under the transcriptional control of the recN promotor. Genotoxic compounds will activate the latter promotor, induce a luminescent signal and give rise to a signal to noise (S/N) ratio higher than 1.5. The Cytox strain on the other hand expresses a bacterial luciferase which is under the control of a constitutive pr1 promotor. The rise of a S/N ratio lower than 0.8 indicates direct toxicity for *S. typhimurium*, whereas a direct effect on the bacterial luciferase or luminescent signal itself is shown by an S/N ratio higher than 1.5. As both recombinant reporter strains lack the oxidative machinery to metabolize the TQDs, rat liver S9 fraction was added to assess the genotoxicity of the metabolites. The luminescence is measured for 4 hours with a 5 minute interval.

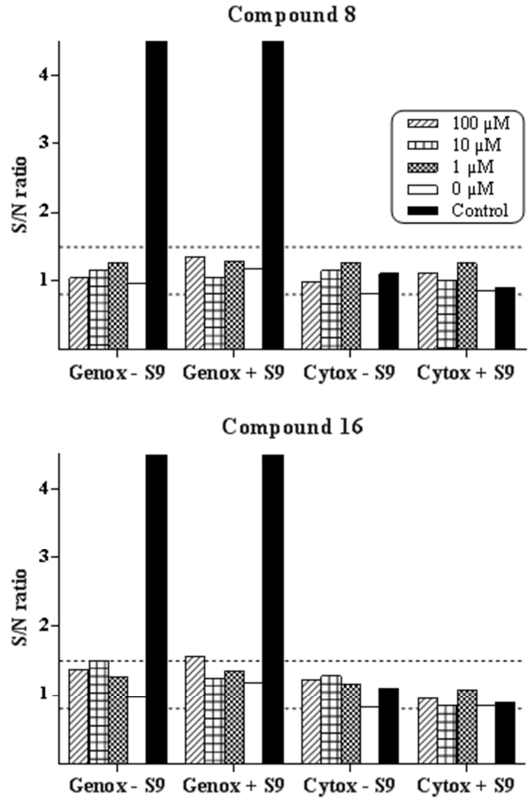


Figure 2. Maximum (Genox -/+ S9) and minimum (Cytox -/+ S9) recorded S/N ratios in a time span of 4 hours. In samples without S9, 4-nitroquinolone-1-oxide was used as a positive control, whereas benzo[a]pyrene was used in samples with S9 liver extract.

As shown in Figure 2, the S/N ratio of the different Cytox cultures showed to be not lower than 0.8 before and after the addition of S9 liver extract at the tested concentrations (1, 10 and 100 µM) of both compound**s**. This indicated the lack of direct toxicity of TQD **8** and **16** toward the *S. typhimurium* model and enabled to test for genotoxicity. Moreover, results could not be interpreted as false positives as there was no direct effect on luminescence either (S/N ratios were not > 1.5). For both compound **8** and **16**, activation of the SOS DNA repair operon or provocation of early signs of genotoxicity could not be demonstrated as the S/N ratio of the treated Genox cultures did not exceed 1.5. This was also the case after the addition of S9 liver extract.

In order to determine whether the most potent, fluorinated TQD **16** would still be a possible candidate for the subversive inhibition of Mtr, as earlier demonstrated for the related group of naphthoquinones,[5] the formation of reactive oxygen species (ROS) was studied by electron paramagnetic resonance (EPR). EPR is used in combination with spin traps or spin probes to yield detectable radical adducts as free radicals have very short half-lives. In this study, the 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH) spin probe, which can quickly react with superoxide, was used. In brief, *Mtb* H37Ra cultures were exposed to derivative **16** and, 50 minutes before the designated time points, additionally incubated with the spin probe solution. At 3, 6, 24 and 48 hours of exposure, 50 µL of the test samples were measured on a Magnettech MiniScope MS 200 spectrophotometer. The simulated spectra were presented as absolute concentrations of CMH radical (Figure 3). Upon exposure to the redox-cycling TQD derivative **16**, a significant increase in superoxide radicals was observed in comparison to the rifampicin and non-treated control. At each time point, the signal increased with an average of 2.85 ± 0.46 times. The observed concentration of ROS significantly differed from the concentration evoked by the moxifloxacin-treated group, indicating that the increase in ROS upon exposure is an important feature of this novel compound class.

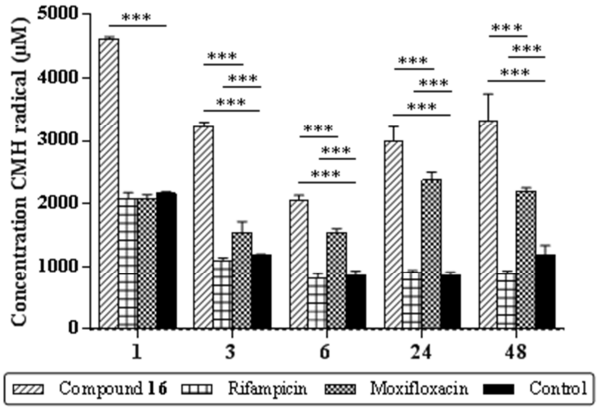


Figure 3. Intracellular ROS production by compound 16. Rifampicin was used as a negative control, whereas moxifloxacin was used as a positive. Non-treated control cultures were included as well. Results are presented as absolute concentrations of CMH radical and plotted as the mean of triplicates ± SD. *** P<0.001 compared to the untreated control or moxifloxacin group by two-way ANOVA with Tukey test.

3. Conclusion

Together, these data provided more insight in the optimization of this promising anti-TB compound class. With the generation of fluorinated TQDs, metabolically stable derivatives were synthesized, opening the possibility for evaluation by *in vivo* infection models. The introduction of fluorine and trifluoromethyl-containing substituents interfered with activity against both extracellular and intracellular growing *Mtb* H37Ra. However, the introduction of these groups resulted in an even further decrease of acute cytotoxicity and no early signs of genotoxicity were assessed. Although the investigation of the mechanism of action is well beyond the scope of this study, EPR data suggests that the most potent derivative of this study, TQD **16** induces the early onset of ROS within *Mtb* which ultimately leads to *Mtb*'s demise. This observation increases the value of the compound class as they become attractive candidates to be teamed up with compounds that target the bacillary electron transport such as the FDA approved bedaquiline. Further optimization, synergistic effects within combination therapy and investigation of the precise mechanism of action of these promising derivatives is of importance in future investigation.

4. Experimental section

4.1. Chemistry

General Methods

The purification of reaction mixtures was performed by column chromatography using a glass column filled with silica gel (Acros, particle size 35-70 µm, pore diameter ca. 6 nm). Solvent systems were determined *via* initial TLC analysis on glass plates, coated with silica gel (Merck, Kieselgel 60 F254, precoated 0.25 mm) using UV light (254 nm and 366 nm), iodine vapor or $KMnO_4$ oxidation as detection methods. Automated flash chromatography was performed on a Reveleris® X2 Flash Chromatography System. ^{1}H NMR spectra were recorded at 300 MHz (JEOL ECLIPSE+) or 400 MHz (Bruker Avance III) with $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane as internal standard. ^{13}C NMR spectra were recorded at 75 MHz (JEOL ECLIPSE+) or 100.6 MHz (Bruker Advance III) with $CDCl_3$ as solvent and tetramethylsilane as internal standard. ^{19}F NMR spectra were recorded at 282 MHz (JEOL ECLIPSE+) or 376.5 MHz (Bruker Advance III) with $CDCl_3$ as solvent and $CFCl_3$ as internal standard. Low resolution mass spectra were recorded via direct injection on an Agilent 1100 Series LC/MSD type SL mass spectrometer with Electron Spray Ionisation Geometry (ESI, 4000 V) and using a Mass Selective Detector (quadrupole). High resolution mass spectra were obtained with an Agilent Technologies 6210 Time-of-Flight Mass Spectrometer (TOFMS), equipped with ESI/APCI-multimode source. Infrared spectra were recorded on a Perkin Elmer Spectrum BX FT-IR Spectrometer. All compounds were analyzed in neat form with an ATR (Attenuated Total Reflectance) accessory. Only selected absorbances (ν_{max}, cm^{-1}) were reported. Melting points of crystalline compounds were determined using a Büchi B-540 apparatus or a Kofler bench, type WME Heizbank of Wagner & Munz and are uncorrected. LC and LC-MS analyses were performed on an Agilent 1200 Series liquid chromatograph using a reversed phase column (Eclipse plus C18 column, 50 x 4.6 mm, particle size 3.5 µm, or a Supelco Ascentis Express C18 column, 30 x 4.6 mm, particle size 2.7 µm) with an UV-VIS detector and an Agilent 1100 series LC/MSD type SL mass spectrometer (ESI, 4000 V) using a mass selective single quadrupole detector. Gradient elution was used (30% acetonitrile in water to 100% acetonitrile over 6 minutes). All microwave reactions were performed in a *CEM Focused MicrowaveTM Synthesis System, Model Discover* with a continuous power output from 0 to 300 W and a self-adjusting, single mode microwave cavity. Reactions were performed in 10 mL thick-walled Pyrex reaction vials, closed with a snap-cap and equipped with a small magnetic stirring bar. The temperature was increased from room temperature to the desired temperature using a ramp time of maximum 5 minutes. The desired temperature was maintained during the course of the reaction. The temperature control system used an external infrared sensor to measure the temperature on the bottom of the vessel and was used in a feedback loop with the on-board computer to regulate the temperature from 25 to 250 °C by adjusting the power output (1 W increments). The pressure control, *IntelliVent*TM *Pressure Control System*, used an indirect measurement of the pressure by sensing changes in the external deflection of the septa on the top of the sealed pressure vessel. Stirring was performed by a rotating magnetic plate located below the bottom of the microwave cavity. After the desired reaction time, the vial was cooled down by a stream of air which decreased the temperature of the vial from approximately 150 °C to 40 °C in less than 120 s. Dry dichloromethane was obtained via distillation over calcium hydride. Dry tetrahydrofuran, dry diethyl ether and dry toluene were freshly distilled over sodium/benzophenone ketyl before use. Other solvents and reagents were used as received from the supplier, unless specified otherwise and all glassware was oven-dried prior to use.

Synthesis of 5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxylic acid 2

In a 250 mL flask, 5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxaldehyde **1** (27.2 mmol, 6.30 g) and $(NH_4)_2Fe(II)(SO_4)_2.6H_2O$ (2.72 mmol, 1.04 g) were mixed with in DMSO (70 mL). To this mixture was added *t*-butyl hydroperoxide (70% in water, 5.44 mmol, 9.72 mL) and the reaction mixture was stirred at 80 °C. Every 30 minutes again *t*-butyl hydroperoxide (9.72 mL) was added until a complete conversion (according to LC-MS analysis) was obtained. Upon full conversion of aldehyde **1**, the reaction mixture was basified with a saturated solution of $NaHCO_3$ and extracted with ethyl acetate. The aqueous layer was subsequently acidified with 2M HCl and again extracted

with ethyl acetate. The organic layer was evaporated and upon purification via column chromatography (silica gel, dichloromethane/methanol 98/2) 5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxylic acid **2** (20.6 mmol, 5.10 g, yield 76%) was obtained as colorless crystals.

*5,8-Dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxylic acid **2***. Yield 76%, colorless crystals, mp 102 °C. R_f (SiO_2) = 0.28, dichloromethane/methanol 98/2. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.98-1.14 (2H, m), 1.46 (1H, br d, *J* = 8.8 Hz), 1.59 (1H, br d, *J* = 8.8 Hz), 1.83-1.98 (2H, m), 3.51 (1H, s), 3.56 (1H, s), 3.73 (3H, s), 3.76 (3H, s), 7.02 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 26.3, 26.9, 40.1, 42.6, 49.0, 56.1, 62.2, 111.6, 123.7, 141.0, 142.5, 147.7, 148.8, 167.9. IR (cm^{-1}): ν_{OH} = 2967, $\nu_{C=O}$ = 1672, ν_{CHAr} = 1589. MS (ES$^+$) *m/z* (%): 249 (M+H$^+$, 100). HRMS (ESI) calcd for $[C_{14}H_{17}O_4]^+$: 249.1127, found: 249.1127.

Synthesis of N-(2-bromophenyl)-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxamide 4

In an oven-dried 25 mL flask was 5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxylic acid **2** (2.82 mmol, 700 mg) dissolved in $SOCl_2$ (28.2 mmol, 2.06 mL). The reaction mixture was heated under reflux for three hours. Subsequently, the residual $SOCl_2$ was evaporated, two times benzene (1 mL) was added and again evaporated. The formed acid chloride was subsequently dissolved in dichloromethane (15 mL) and K_2CO_3 (5.64 mmol, 780 mg) was added to the solution. The reaction mixture was cooled to 0 °C and 2-bromoaniline **3** (2.82 mmol) dissolved in dichloromethane (10 mL) was added dropwise to the reaction mixture. The reaction mixture was slowly warmed up to room temperature and stirred overnight. Subsequently, the reaction mixture was washed two times with brine (20 mL), two times with a 2M HCl solution (20 mL) and finally again two times with brine (20 mL). Upon evaporation of the solvent, *N*-(2-bromophenyl)-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxamide **4** (2.26 mmol, 907 mg, yield 80%) was obtained in sufficiently pure form as a brown powder.

*N-(2-bromophenyl)-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxamide **4**.* Yield 80%, brown powder, mp 108 °C. R_f (SiO_2) = 0.41, petroleum ether/ethyl acetate 4/1. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.19-1.34 (2H, m), 1.55 (1H, br d, *J* = 8.8 Hz), 1.77 (1H, br d, *J* = 8.8 Hz); 1.91-2.07 (2H, m), 3.64 (1H, s), 3.68 (1H, s), 3.88 (3H, s), 4.00 (3H, s), 6.98 (1H, t, *J* = 7.7 Hz), 7.34 (1H, t, *J* = 7.7 Hz), 7.57 (1H, s), 7.58 (1H, d, *J* = 7.7 Hz), 8.67 (1H, d, *J* = 7.7 Hz), 10.71 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.1, 26.9, 40.0, 41.6, 49.2, 55.8, 62.5, 111.7, 113.4, 122.4, 123.0, 124.8, 128.3, 132.5, 137.3, 141.1, 142.4, 146.5, 149.6, 163.8. IR (cm^{-1}): ν_{NH} = 3268, $\nu_{C=O}$ = 1671, ν_{C-N} = 1589, ν_{CHAr} = 1582 and 1521, ν_{C-O} = 1307. MS (ES$^+$) *m/z* (%): 402/404 (M+H$^+$, 100). HRMS (ESI) calcd for $[C_{20}H_{21}BrNO_3]^+$: 402.0705, found: 402.0700.

Synthesis of N-(2-bromophenyl)-N-methoxymethyl-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxamide 5

In a 25 mL flask under nitrogen atmosphere, *N*-(2-bromophenyl)-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxamide **4** (2.41 mmol, 967 mg) was dissolved in THF (25 mL) together with sodium hydride (60% in oil, 5.30 mmol, 212 mg). The reaction mixture was cooled to 0 °C and MOMCl (3.61 mmol) was added dropwise. The reaction mixture was stirred overnight and conversion was monitored via LC-MS analysis. If no complete conversion was obtained, an additional amount of MOMCl (1.80 mmol) was added and the reaction mixture was additionally stirred for four hours. Subsequently, the reaction mixture was quenched upon addition of a small amount of water and filtered over a sintered glass filter. The THF was evaporated and the residual product was redissolved in ethyl acetate (25 mL) and washed with brine (25 mL). Following evaporation of the organic layer, the reaction mixture was further purified via column chromatography (silica gel, petroleum ether/ethyl acetate 5/1) to obtain pure *N*-(2-bromophenyl)-*N*-methoxymethyl-5,8-

dimethoxy-1,2,3,4-tetrahydro-1,4- methanonaphthalene-6-carboxamide **5** (2.04 mmol, 912 mg, yield 85%) as a white powder.

*N-(2-bromophenyl)-N-methoxymethyl-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxamide **5**.* Yield 85%, white powder, mp 112 °C. R_f (SiO_2) = 0.30, petroleum ether/ethyl acetate 5/1. ^{1}H NMR (300 MHz, $CDCl_3$): major isomer: δ 0.76-2.00 (6H, m), 3.38-3.52 (2H, m), 3.60 (3H, br s), 3.66 (3H, br s), 3.86 (3H, s), 4.63 (1H, dxd, *J* = 10.5, 5.2 Hz), 5.85 (1H, d, *J* = 10.5 Hz), 6.55 (1H, s), 6.96-7.15 (2H, m), 7.28-7.33 (1H, br t, *J* = 7.7 Hz), 7.40-7.48 (1H, br t, *J* = 7.7 Hz). minor isomer: δ 0.76-2.00 (6H, m), 3.14-3.23 (3H, m), 3.60-3.66 (2H, m), 3.83 (3H, br s), 3.92 (3H, br s), 3.86 (3H, s), 4.69-4.92 (2H, m), 6.55 (1H, s), 7.20-7.28 (2H, m), 7.38-7.45 (2H, m), 7.40-7.48 (1H, m), 7.69-7.74 (1H, br t, *J* = 7,7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): major isomer δ 26.2, 26.9, 39.6, 41.0, 49.1, 55.7, 56.9, 62.0, 77.3, 106.3, 122.9, 127.9, 128.2, 129.6, 132.1, 133.0, 138.1, 139.8, 140.3, 143.6, 148.3, 170.6. minor isomer: δ 26.3, 26.6, 39.8, 41.1, 48.3, 56.1, 56.3, 62.7, 82.3, 106.7, 123.1, 127.5, 128.3, 129.4, 131.8, 133.6, 138.0, 139.9, 140.2, 143.7, 148.4, 170.6. IR (cm^{-1}): $\nu_{C=O}$ = 1662, ν_{C-N} = 1592, ν_{CHAr} = 1478. MS (ES^+) *m/z* (%): 231 (100), 446/448 ($M+H^+$, 3). Due to the low abundance of the pseudomolecular ion, no HRMS could be recorded.

Synthesis of 7,12-dimethoxy-N-methoxymethyl-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine-6-one 6

N-(2-bromophenyl)-*N*-methoxymethyl-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxamide **5** (2.31 mmol, 1.03 g) together with K_2CO_3 (4.62 mmol, 562 mg), triphenylphosphine (0.14 mmol, 36.25 mg) and palladium(II)acetate (0.05 mmol, 10.35 mg) were added to an oven-dried flask under nitrogen atmosphere. To this mixture, toluene (25 mL) was added and the reaction mixture was heated to 95-100 °C. Every four hours the conversion of the reaction was analyzed with LC-MS and additional catalyst (0.14 mmol triphenylphosphine; 0.05 mmol, palladium(II)acetate) was added if necessary. After 18 hours of reaction, the catalyst was filtered over Celite®, the solvent was evaporated and the product was purified via column chromatography (silica gel, petroleum ether/ethyl acetate 5/1) to obtain 7,12-dimethoxy-*N*-methoxymethyl-8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine-6-one **6** (2.08 mmol, 0.761 g, yield 90%) as a yellow powder.

*7,12-dimethoxy-N-methoxymethyl-8,9,10,11-tetrahydro-8,11- methanobenzo[j]phenanthridine-6-one **6***. Yield 90%, yellow powder, mp 118 °C. R_f (SiO_2) = 0.23, petroleum ether/ethyl acetate 5/1. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (2H, br d, *J* = 8.8 Hz), 1.62 (1H, br d, *J* = 8.8 Hz), 1.81 (1H, br d, *J* = 8.8 Hz), 2.06 (2H, br d, *J* = 8.8 Hz), 3.50 (3H, s), 3.79 (2H, s), 3.79 (3H, s), 3.95 (3H, s), 5.77 (2H, s), 7.25 (1H, t, *J* = 8.3 Hz), 7.45 (1H, t, *J* = 8.3 Hz), 7.56 (1H, d, *J* = 8.3 Hz), 9.18 (1H, d, *J* = 8.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.9, 40.6, 41.4, 48.5, 56.7, 60.7, 62.1, 73.9, 115.2, 118.5, 119.0, 122.7, 127.5, 127.7, 128.8, 136.6, 142.4, 142.4, 147.2, 147.9, 151.5, 160.7. IR (cm^{-1}): $\nu_{C=O}$ = 1654, ν_{C-N} = 1615. MS (ES^+) *m/z* (%): 366 ($M+H^+$, 100). HRMS (ESI) calcd for $[C_{22}H_{24}NO_4]^+$: 366.1705, found: 366.1708.

Synthesis of 7,12-dimethoxy-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine 7

In a 50 mL flask, 7,12-dimethoxy-*N*-methoxymethyl-8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine-6-one **6** (1.67 mmol, 610 mg) was dissolved in dry THF (25 mL) under nitrogen atmosphere. The solution was cooled to 0 °C and $LiAlH_4$ (5 mmol, 190 mg) was added. After two hours of reaction at room temperature, the reaction was quenched by careful addition of water. Subsequently, 2M HCl (20 mL) was added to the reaction mixture and stirred for one hour at room temperature. The reaction mixture was made basic by addition of 2M NaOH and extracted twice with ethyl acetate (25 mL). The organic layer was evaporated and following purification via column chromatography (silica gel, dichloromethane) pure 7,12-dimethoxy-8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine **7** (0.98 mmol, 298 mg, yield 59%) was obtained as an orange viscous oil.

7,12-dimethoxy-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine ***7****.* Yield 59%, orange viscous oil. R_f (SiO_2) = 0.10, dichloromethane. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (2H, br d, *J* = 10.4 Hz), 1.68 (1H, br d, *J* = 8.8 Hz), 1.87 (1H, br d, *J* = 8.8 Hz), 2.11 (2H, br d, *J* = 8.8 Hz), 3.88 (2H, s), 3.92 (3H, s), 4.09 (3H, s), 7.63 (1H, t, *J* = 8.3 Hz), 7.71 (1H, t, *J* = 8.3 Hz), 8.17 (1H, d, *J* = 8.3 Hz), 9.39 (1H, d, *J* = 8.3 Hz), 9.59 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.2, 27.2, 40.9, 41.3, 48.7, 60.9, 62.7, 120.6, 123.9, 125.5, 126.7, 126.9, 128.0, 129.8, 137.1, 145.0, 145.9, 146.6, 147.0, 148.5. IR (cm^{-1}): $\nu_{C=C}$ = 1622, ν_{C-O} = 1324. MS (ES$^+$) *m/z* (%): 306 (M+H$^+$, 100). HRMS (ESI) calcd for $[C_{20}H_{20}NO_2]^+$: 306.1494, found: 306.1499.

Synthesis of 8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine-7,12-dione 8

In a 50 mL flask under nitrogen atmosphere, 7,12-dimethoxy-8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine **7** (0.33 mmol, 100 mg) was dissolved in acetonitrile (15 mL). A solution of CAN (0.82 mmol, 446 mg) in water (15 mL) was added. After two hours of reaction at room temperature, the reaction mixture was extracted twice with dichloromethane (20 mL). After evaporation of the organic layer, pure 8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine-7,12-dione **8** (0.29 mmol, 80 mg, yield 89%) was obtained as an orange powder.

8,9,10,11-Tetrahydro-8,11-methanobenzo[j]phenanthridine-7,12-dione ***8****.* Yield 89%, orange powder, mp 126 °C. R_f = 0.55, dichloromethane/methanol 19/1. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (2H, br d, *J* = 8.8 Hz), 1.51 (1H, br d, *J* = 8.8 Hz), 1.75 (1H, br d, *J* = 8.8 Hz), 2.04 (2H, br d, *J* = 8.8 Hz), 3.69 (2H, s), 7.76 (1H, t, *J* = 8.3 Hz), 7.86 (1H, t, *J* = 8.3 Hz), 8.17 (1H, d, *J* = 8.3 Hz), 9.42 (1H, d, *J* = 8.5 Hz), 9.60 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.4, 25.5, 41.0, 41.5, 47.3, 122.8, 123.8, 127.7, 130.2, 131.8, 133.0, 147.5, 151.9, 152.1, 155.3, 182.4, 185.6. IR (cm^{-1}): $\nu_{C=O}$ = 1648. MS (ES$^+$) *m/z* (%): 276 (M+H$^+$, 100). HRMS (ESI) calcd for $[C_{18}H_{14}NO_2]^+$: 276.1025, found: 276.1025.

Synthesis of 2-bromo-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]anilines 10a and 10b

The synthesis of 2-bromo-*N*-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-4-fluoroaniline **10a** is described as representative. A solution of 5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carbaldehyde **1** (0.22 g, 0.93 mmol), 2-bromo-4-fluoroaniline **9a** (3 equiv, 0.53 g, 2.78 mmol) and $MgSO_4$ (3 equiv, 0.33 g, 2.78 mmol) in 2-methyltetrahydrofuran (4 mL) was stirred under microwave irradiation at 60 °C for 45 minutes. Subsequently, the drying agent was filtered off and the solvent was removed *in vacuo*, affording the corresponding imine, which was used without further purification in the next step. The imine was dissolved in MeOH (10 mL), and $NaCNBH_3$ (2.5 equiv, 0.15 g, 2.32 mmol) and acetic acid (1.2 equiv, 0.069 g, 1.11 mmol) were added. After stirring the reaction mixture for 17 hours at room temperature, saturated aqueous Na_2CO_3 (15 mL) was added and an extraction with EtOAc was performed (3x 10 mL). After drying of the combined organic phases ($MgSO_4$), filtration and evaporation, crude aniline **10a** was obtained, which was purified by reverse phase automated flash chromatography (0.26 g, 0.62 mmol).

2-Bromo-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-4-fluoroaniline ***10a.*** Pale yellow crystals, mp 93-94 °C. R_f = 0.03, petroleum ether/EtOAc 9/1. Gradient used for purification: during 3 CV: 40% CH_3CN, during 30 CV: 40 -> 100% CH_3CN, during 3 CV: 100% CH_3CN. Yield 67%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28-1.36 (2H, m), 1.56 (1H, d, *J* = 8.7 Hz), 1.80 (1H, d, *J* = 8.7 Hz), 1.94-2.07 (2H, m), 3.66 (1H, br s), 3.71 (1H, br s), 3.82 (3H, s), 3.91 (3H, s), 4.37 (2H, br s), 4.60 (1H, br s), 6.69 (1H, s), 6.72 (1H, dd, *J* = 9.0 Hz, $J_{H,F}$ = 5.2 Hz), 6.97 (1H, ddd, *J* = 9.0 Hz, $J_{H,F}$ = 8.1 Hz, *J* = 2.9 Hz), 7.27 (1H, dd, $J_{H,F}$ = 8.0 Hz, *J* = 2.9 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 26.5, 27.0, 39.7, 41.3, 44.1, 49.1, 55.9, 61.4, 108.9 (d, *J* = 9.8 Hz), 109.6, 111.7 (d, *J* = 7.5 Hz), 115.0 (d, *J* = 11.6 Hz), 119.3 (d, *J* = 25.5 Hz), 128.3, 136.5, 140.5, 142.0 (d, *J* = 2.1 Hz), 146.1, 149.1, 154.5 (d, *J* = 238.8 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -127.76 (1F, ddd, $J_{H,F}$ = 8.1 Hz, 8.0 Hz, 5.2 Hz). IR (ATR, cm^{-1}): ν_{max} = 1506, 1490, 1315, 1023, 848, 796. MS (ES$^+$): *m/z* (%): 217 (100). HRMS (ES$^+$): calcd for $C_{14}H_{17}O_2^+$ (M - $C_6H_4BrFN^-$): 217.1223, found 217.1224.

2-Bromo-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-5-fluoroaniline ***10b***. Pale yellow crystals, mp 80-81 °C. Gradient used for purification: during 3 CV: 40% CH_3CN, during 30 CV: 40 -> 100% CH_3CN, during 3 CV: 100% CH_3CN. Yield 53%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20-1.28 (2H, m), 1.49 (1H, d, *J* = 8.8 Hz), 1.70-1.73 (1H, m), 1.87-1.99 (2H, m), 3.56- 3.59 (1H, m), 3.62-3.64 (1H, m), 3.76 (3H, s), 3.83 (3H, s), 4.29 (2H, br s), 4.78 (1H, br s), 6.28 (1H, ddd, *J* = 8.5 Hz, $J_{H,F}$ = 8.2 Hz, *J* = 2.8 Hz), 6.44 (1H, dd, $J_{H,F}$ = 11.3 Hz, *J* = 2.8 Hz), 7.32 (1H, dd, *J* = 8.5 Hz, $J_{H,F}$ = 6.0 Hz). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 26.5, 27.1, 39.8, 41.4, 43.7, 49.2, 56.1, 61.6, 99.1 (d, *J* = 27.8 Hz), 103.7 (d, *J* = 2.5 Hz), 104.3 (1H, d, *J* = 23.1 Hz), 109.8, 127.8, 132.9 (d, *J* = 9.9 Hz), 136.8, 140.7, 146.3, 146.5 (d, *J* = 11.3 Hz), 149.2, 163.5 (d, *J* = 243.0 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -113.62 (1F, ddd, $J_{H,F}$ = 11.3 Hz, 8.2 Hz, 6.0 Hz). IR (ATR, cm^{-1}): ν_{max} = 1613, 1486, 1308, 1170, 1021, 824. MS (ES^+): *m/z* (%): 217 (100). HRMS (ES^+): calcd for $C_{14}H_{17}O_2^+$ (M - $C_6H_4BrFN^-$): 217.1223, found 217.1227.

Synthesis of 2-bromo-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-6-fluoroaniline 10c

A mixture of 5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carbaldehyde **1** (0.23 g, 0.97 mmol), 2-bromo-6-fluoroaniline **9c** (1 equiv, 0.18 g, 0.97 mmol), $NaCNBH_3$ (2.5 equiv, 0.15 g, 2.4 mmol) and acetic acid (2 equiv, 0.12 g, 1.9 mmol) in *iso*-propanol (20 mL) was stirred at room temperature for seven days. After this time, a saturated aqueous solution of Na_2CO_3 (15 mL) was added and an extraction with EtOAc was performed (3x 15 mL). After drying of the combined organic phases ($MgSO_4$), filtration and evaporation, crude aniline **10c** was obtained, which was purified by reverse phase automated flash chromatography (0.080 g, 0.19 mmol).

2-Bromo-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-6-fluoroaniline ***10c***. Pale yellow oil. Gradient used for purification: during 3 CV: 40% CH_3CN, during 30 CV: 40 -> 100% CH_3CN, during 3 CV: 100% CH_3CN. Yield 20%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17-1.28 (2H, m), 1.48 (1H, ddd, *J* = 8.7 Hz, 1.4 Hz, 1.4 Hz), 1.69-1.72 (1H, m), 1.86-1.98 (2H, m), 3.55-3.57 (1H, m), 3.62-3.64 (1H, m), 3.76 (3H, s), 3.86 (3H, s), 4.38 (1H, br s), 4.47 (2H, d, *J* = 4.4 Hz), 6.61 (1H, ddd, *J* = 8.2 Hz, 8.1 Hz, $J_{H,F}$ = 4.9 Hz), 6.95 (1H, ddd, $J_{H,F}$ = 12.4 Hz, *J* = 8.2 Hz, 1.4 Hz), 7.22 (1H, ddd, *J* = 8.1 Hz, 1.4 Hz, 1.3 Hz). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 26.6, 27.1, 39.7, 41.5, 46.7 (d, *J* = 9.6 Hz), 49.1, 56.0, 61.5, 110.2, 113.9 (d, *J* = 5.5 Hz), 116.0 (d, *J* = 21.3 Hz), 119.6 (d, *J* = 8.5 Hz), 128.2 (d, *J* = 3.1 Hz), 129.5, 135.4 (d, *J* = 12.0 Hz), 136.5, 140.3, 146.3, 148.9, 153.4 (d, *J* = 245.0 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -124.10 (1F, dd, $J_{H,F}$ = 12.4 Hz, 4.9 Hz). MS (ES^+): *m/z* (%): 217 (100).

Synthesis of 2-bromo-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]anilines 10d and 10e

The synthesis of 2-bromo-*N*-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-5-(trifluoromethyl)aniline **10d** is described as representative. A mixture of 5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalene-6-carboxaldehyde **1** (0.67 g, 2.88 mmol), 2-bromo-5-trifluoromethylaniline **9d** (3 equiv, 2.07 g, 8.63 mmol) and $MgSO_4$ (3 equiv, 1.04 g, 8.63 mmol) in dry dichloromethane (40 mL) was stirred at reflux temperature for 19 hours. After this time, the drying agent was filtered off and the solvent was removed *in vacuo*, affording the corresponding imine which was used without further purification in the next step. The imine was dissolved in MeOH (30 mL) and $NaCNBH_3$ (2.5 equiv, 0.45 g, 7.2 mmol) and acetic acid (1.2 equiv, 0.21 g, 3.46 mmol) were added. After stirring the reaction mixture for 17 hours at room temperature, saturated aqueous Na_2CO_3 (30 mL) was added and an extraction with EtOAc was performed (3x 20 mL). After drying of the combined organic phases ($MgSO_4$), filtration and evaporation, crude aniline **10d** was obtained, which was purified by reverse phase automated flash chromatography (0.86 g, 1.90 mmol).

2-Bromo-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-5-(trifluoromethyl)aniline ***10d***. Pale yellow oil. Gradient used for purification: during 3 CV: 40% CH_3CN,

during 30 CV: 40 -> 100% CH_3CN, during 3 CV: 100% CH_3CN. Yield 66%. ^1H NMR (400 MHz, $CDCl_3$): δ 1.19-1.28 (2H, m), 1.50 (1H, d, *J* = 8.8 Hz), 1.71-1.74 (1H, m), 1.88-2.00 (2H, m), 3.58 (1H, br s), 3.64 (1H, br s), 3.77 (3H, s), 3.85 (3H, s), 4.34 (2H, d, *J* = 5.3 Hz), 4.89 (1H, t, *J* = 5.3 Hz), 6.61 (1H, s), 6.80 (1H, dd, *J* = 8.2 Hz, 1.5 Hz), 6.94 (1H, d, *J* = 1.5 Hz), 7.51 (1H, d, *J* = 8.2 Hz). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 26.5, 27.1, 39.8, 41.5, 43.8, 49.2, 56.1, 61.6, 107.9 (q, *J* = 3.9 Hz), 110.2, 113.0, 114.0 (q, *J* = 3.9 Hz), 124.3 (q, *J* = 272.4 Hz), 127.6, 131.0 (q, *J* = 32.1 Hz), 132.8, 137.1, 140.7, 145.4, 146.5, 149.1. ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -63.36 (1F, s). IR (ATR, cm^{-1}): ν_{max} = 1165, 1119, 1080, 1054, 1019. MS (ES^+): *m/z* (%): 217 (100). HRMS (ES^+): calcd for $C_{14}H_{17}O_2^+$ ($M - C_7H_4BrF_3N^-$): 217.1223, found 217.1227.

2-Bromo-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-4-(trifluoromethyl)aniline ***10e****.* Pale yellow oil. Gradient used for purification: during 3 CV: 40% CH_3CN, during 30 CV: 40 -> 100% CH_3CN, during 3 CV: 100% CH_3CN. Yield 71%. ^1H NMR (400 MHz, $CDCl_3$): δ 1.19-1.29 (2H, m), 1.50 (1H, d, *J* = 8.8 Hz), 1.72 (1H, d, *J* = 8.8 Hz), 1.88-2.00 (2H, m), 3.58 (1H, br s), 3.64 (1H, br s), 3.76 (3H, s), 3.84 (3H, s), 4.36 (2H, d, *J* = 5.5 Hz), 5.03 (1H, t, *J* = 5.5 Hz), 6.58 (1H, s), 6.73 (1H, d, *J* = 8.5 Hz), 7.41 (1H, dd, *J* = 8.5 Hz, 1.2 Hz), 7.67 (1H, d, *J* = 1.2 Hz). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 26.5, 27.0, 39.8, 41.5, 43.5, 49.2, 56.1, 61.5, 108.7, 109.8, 110.6, 119.2 (q, *J* = 33.3 Hz), 124.2 (q, *J* = 270.7 Hz), 125.9 (q, *J* = 3.6 Hz), 127.5, 129.6 (q, *J* = 3.8 Hz), 137.0, 140.7, 146.3, 147.5, 149.2. ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -61.48 (1F, s). IR (ATR, cm^{-1}): ν_{NH} = 3408, ν_{max} = 1610, 1319, 1107, 1076, 1056. MS (ES^+): *m/z* (%): 217 (100). HRMS (ES^+): calcd for $C_{14}H_{17}O_2^+$ ($M - C_7H_4BrF_3N^-$): 217.1223, found 217.1222.

Synthesis of trifluoromethanesulfonamides 11

The synthesis of *N*-(2-bromo-5-fluorophenyl)-*N*-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-1,1,1-trifluoromethanesulfonamide **11b** is described as representative. To a solution of aniline **10b** (0.158 g, 0.39 mmol) in dry CH_2Cl_2 (10 mL), were added NaH (2.5 equiv, 23 mg, 0.97 mmol) and Tf_2O (1M in CH_2Cl_2, 2.5 equiv, 0.97 mL, 0.97 mmol) at 0 °C under N_2 atmosphere. This mixture was stirred at room temperature for seven hours, after which it was quenched with saturated aqueous NH_4Cl (10 mL) and extracted with EtOAc (3x 10 mL). The combined organic phases were dried ($MgSO_4$), filtered and evaporated *in vacuo*. After purification *via* column chromatography, pure trifluoromethanesulfonamide **11b** was obtained in 71% yield (0.149 g, 0.28 mmol).

N-(2-Bromo-4-fluorophenyl)-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-1,1,1-trifluoromethanesulfonamide ***11a****.* This compound exists as two rotamers in a ratio R1/R2 1/1, due to hindered rotation of the sulfonamide bond. Viscous oil. R_f = 0.27, petroleum ether/EtOAc 95/5. Yield 61%. ^1H NMR (400 MHz, $CDCl_3$): δ 1.01-1.14 (2H, m, R1 and R2), 1.42-1.45 (1H, m, R1 and R2), 1.58-1.62 (1H, m, R1 and R2), 1.84-1.93 (2H, m, R1 and R2), 3.52 (2H, br s, R1 and R2), 3.571 and 3.574 (2x 1.5H, 2x s, R1 and R2), 3.71 and 3.73 (2x 1.5H, 2x s, R1 and R2), 4.88-4.99 (2H, m, R1 and R2), 6.51 and 6.59 (2x 0.5H, 2x s, R1 and R2), 6.79 and 6.81 (2x 0.5H, 2x ddd, 2x *J* = 8.8 Hz, 7.2 Hz, 2.8 Hz, R1 and R2), 6.93 and 6.96 (2x 0.5H, 2x dd, 2x *J* = 8.8 Hz, 5.5 Hz, R1 and R2), 7.31 and 7.32 (2x 0.5H, 2x dd, 2x *J* = 7.8 Hz, 2.8 Hz, R1 and R2). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 26.36, 26.41, 26.9 and 27.0 (R1 and R2), 39.7, 39.8, 41.58 and 41.62 (R1 and R2), 48.8 and 49.0 (R1 and R2), 51.0 and 51.2 (R1 and R2), 55.9 (R1 and R2), 60.9 and 61.1 (R1 and R2), 111.77 and 111.84 (R1 and R2), 114.7 and 114.8 (2x d, 2x *J* = 22.2 Hz, R1 and R2), 120.2 (q, *J* = 323.0 Hz, R1 and R2), 120.8 (d, *J* = 25.5 Hz, R1 and R2), 122.7 (d, *J* = 16.5 Hz, R1 and R2), 126.0-126.4 (m, R1 and R2), 131.8 and 131.9 (2x d, 2x *J* = 11.9 Hz, R1 and R2), 134.15 (d, *J* = 7.5 Hz, R1 and R2), 138.89 and 138.96 (R1 and R2), 139.6 and 139.7 (R1 and R2), 147.0 and 147.1 (R1 and R2), 148.7 and 148.9 (R1 and R2), 162.2 (d, *J* = 254.5 Hz, R1 and R2). ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -75.23 (3F, s), -109.55 to -109.67 (1F, m). IR (ATR, cm^{-1}): ν_{max} = 1485, 1392, 1223, 1185, 1142, 1040, 1024, 731. MS (ES^+): *m/z* (%): 217 (100). HRMS (ES^+): calcd for $C_{14}H_{17}O_2^+$ ($M - C_7H_3BrF_4NO_2S^-$): 217.1223, found 217.1216.

N-(2-Bromo-5-fluorophenyl)-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-1,1,1-trifluoromethanesulfonamide ***11b****.* This compound exists as two rotamers in a ratio R1/R2 1/1, due to hindered rotation of the sulfonamide bond. Viscous oil. R_f = 0.28, petroleum ether/EtOAc 9/1. Yield: 71%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00-1.18 (2H, m, R1 and R2), 1.43 (1H, d, *J* = 8.6 Hz, R1 and R2), 1.58-1.65 (1H, m, R1 and R2), 1.85-1.91 (2H, m, R1 and R2), 3.52 (2H, br s, R1 and R2), 3.59 and 3.62 (2x 1.5H, 2x s, R1 and R2), 3.708 and 3.711 (2x 1.5H, 2x s, R1 and R2), 4.95 (2H, s, R1 and R2), 6.51 and 6.54 (2x 0.5H, 2x s, R1 and R2), 6.70 and 6.75 (2x 0.5H, 2x dd, 2x *J* = 8.9 Hz, 2.9 Hz, R1 and R2), 6.87-6.92 (1H, m, R1 and R2), 7.52 and 7.53 (2x 0.5H, 2x dd, 2x *J* = 8.9 Hz, 5.7 Hz, R1 and R2). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 26.3, 26.4, 26.9 and 27.0 (R1 and R2), 39.7, 39.8, 41.66 and 41.67 (R1 and R2), 48.8 and 49.0 (R1 and R2), 51.0 and 51.3 (R1 and R2), 56.0 (R1 and R2), 60.9 and 61.1 (R1 and R2), 111.8 and 112.0 (R1 and R2), 117.99 and 118.04 (2x d, 2x *J* = 22.1 Hz, R1 and R2), 120.1 (R1 and R2), 120.1 (q, *J* = 313.1 Hz, R1 and R2), 120.6 (d, *J* = 21.1 Hz, R1 and R2), 122.5 and 122.6 (R1 and R2), 134.1 (d, *J* = 8.5 Hz, R1 and R2), 136.6 (d, *J* = 10.6 Hz, R1 or R2), 136.8 (d, *J* = 11.4 Hz, R1 or R2), 139.06 and 139.11 (R1 and R2), 139.6 and 139.7 (R1 and R2), 146.99 and 147.00 (R1 and R2), 148.7 and 148.8 (R1 and R2), 161.1 and 161.2 (2x d, 2x *J* = 249.7 Hz, R1 and R2). ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -75.35 (3F, s), -113.71 to -113.87 (1F, m). MS (ES^+) *m/z* (%): 555/57 ($M + NH_4^+$, 25), 217 (100). HRMS (ES^+): calcd for $C_{14}H_{17}O_2^+$ ($M - C_7H_3BrF_4NO_2S^-$): 217.1223, found 217.1218.

N-(2-Bromo-6-fluorophenyl)-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-1,1,1-trifluoromethanesulfonamide ***11c****.* This compound exists as two rotamers in a ratio major/minor 0.56/0.44, due to hindered rotation of the sulfonamide bond. Viscous oil. R_f = 0.35, petroleum ether/EtOAc 9/1. Yield 61%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.01-1.13 (2H, m), 1.41-1.43 (1H, m), 1.60-1.64 (1H, m), 1.82-1.90 (2H, m), 3.50 (1H, br s), 3.52 (1H, br s), 3.54 (1.8H, s, major), 3.57 (1.2H, s, minor), 3.68 (1.2H, s, minor), 3.70 (1.8H, s, major), 4.82 (0.4H, d, *J* = 13.3 Hz, minor), 4.85 (0.6H, d, *J* = 13.4 Hz, major), 5.03 (0.6H, d, *J* = 13.4 Hz, major), 5.07 (0.4H, d, *J* = 13.3 Hz, minor), 6.55 (0.4H, s, minor), 6.61 (0.6H, s, major), 6.97-7.03 (1H, m), 7.17 (1H, ddd, *J* = 8.2 Hz, 8.2 Hz, 5.5 Hz), 7.34-7.37 (1H, m). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 26.4 and 26.9 (minor), 26.5 and 27.0 (major), 39.7, 41.68 and 41.71 (minor+major), 48.8 (major), 48.9 (minor), 50.4 (minor), 50.5 (major), 55.9 (minor), 56.0 (major), 61.0 (major), 61.1 (minor), 112.6 (minor), 112.7 (major), 115.5 (d, *J* = 21.6 Hz, minor), 115.6 (d, *J* = 21.6 Hz, major), 120.0 (q, *J* = 323.3 Hz), 122.3 (minor), 122.4 (major), 124.7 (d, *J* = 15.1 Hz, minor), 124.9 (d, *J* = 14.7 Hz, major), 127.5, 129.1 (d, *J* = 3.8 Hz, major), 129.2 (d, *J* = 4.3 Hz, minor), 131.6 (d, *J* = 9.4 Hz), 139.0, 139.1 (major), 139.2 (minor), 147.2, 148.5 (minor), 148.6 (major), 160.79 (d, *J* = 256.1 Hz, minor), 160.83 (d, *J* = 256.3 Hz, major). ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -75.51 (d, *J* = 11.4 Hz, major or minor), -75.54 (d, *J* = 15.5 Hz, major or minor), -112.18 (1F, br s). IR (ATR, cm^{-1}): ν_{max} = 1394, 1187, 1138, 1024, 869, 782. MS (ES^+): *m/z* (%): 217 (100). HRMS (ES^+): calcd for $C_{14}H_{17}O_2^+$ ($M - C_7H_3BrF_4NO_2S^-$): 217.1223, found 217.1210.

N-[2-Bromo-5-(trifluoromethyl)phenyl]-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-1,1,1-trifluoromethanesulfonamide ***11d****.* This compound exists as two rotamers in a ratio R1/R2 1/1, due to hindered rotation of the sulfonamide bond. Viscous oil. R_f = 0.24, petroleum ether/EtOAc 9/1. Yield 60%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00-1.12 (2H, m, R1 and R2), 1.40-1.42 (1H, m, R1 and R2), 1.55-1.60 (1H, m, R1 and R2), 1.81-1.90 (2H, m, R1 and R2), 3.47-3.52 (2H, m, R1 and R2), 3.577 and 3.580 (2x 1.5H, 2x s, R1 and R2), 3.708 and 3.715 (2x 1.5H, 2x s, R1 and R2), 4.91-5.01 (2H, m, R1 and R2), 6.51 and 6.54 (2x 0.5H, 2x s, R1 and R2), 7.12 and 7.18 (2x 0.5H, 2x d, 2x *J* = 1.7 Hz, R1 and R2), 7.36 and 7.40 (2x 0.5H, 2x dd, 2x *J* = 8.6 Hz, 1.7 Hz, R1 and R2), 7.70 and 7.72 (2x 0.5H, 2x d, 2x *J* = 8.6 Hz, R1 and R2). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 26.2, 26.3, 26.8 and 26.9 (R1 and R2), 39.65, 39.70, 41.68 and 41.70 (R1 and R2), 48.8 and 48.9 (R1 and R2), 51.2 and 51.4 (R1 and R2), 56.01 and 56.03 (R1 and R2), 60.7 and 60.8 (R1 and R2), 111.8 and 112.0 (R1 and R2), 120.1 (q, *J* = 322.8 Hz, R1 and R2), 122.08 and 122.11 (R1 and R2), 123.0 (q, *J* = 270.2 Hz, R1 and R2), 127.0-127.2 (m, R1 and R2), 129.8-130.5 (R1 and R2), 130.3 and 130.4 (2x d, 2x *J* = 33.6 Hz, R1 and R2), 134.1 and 134.2 (R1 and R2), 136.3 and 136.8 (R1 and R2), 139.3, 139.4, 139.5 and 139.6

(R1 and R2), 146.8 and 147.0 (R1 and R2), 148.7 and 148.8 (R1 and R2). ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -63.43 and -63.48 (2x 1.5F, 2x s, R1 and R2), -75.42 and -75.45 (2x 1.5F, 2x s, R1 and R2). IR (ATR, cm^{-1}): ν_{max} = 1395, 1325, 1225, 1189, 1173, 1133. MS (ES^+): *m/z* (%): 217 (100).

N-[2-Bromo-4-(trifluoromethyl)phenyl]-N-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-1,1,1-trifluoromethanesulfonamide ***11e****.* This compound exists as two rotamers in a ratio R1/R2 1/1, due to hindered rotation of the sulfonamide bond. Yellow oil. R_f = 0.53, petroleum ether/EtOAc 9/1. Yield 62%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97-1.06 (2H, m, R1 and R2), 1.41-1.44 (1H, m, R1 and R2), 1.57-1.61 (1H, m, R1 and R2), 1.81-1.92 (2H, m, R1 and R2), 3.49 and 3.50 (2H, 2x br s, R1 and R2), 3.55 and 3.57 (2x 1.5H, 2x s, R1 and R2), 3.70 and 3.72 (2x 1.5H, 2x s, R1 and R2), 4.95 (1H, d, *J* = 13.5 Hz, R1 and R2), 5.01 (1H, d, *J* = 13.5 Hz, R1 and R2), 6.52 and 6.57 (2x 0.5H, 2x s, R1 and R2), 7.08 and 7.12 (2x 0.5H, 2x d, 2x *J* = 8.3 Hz, R1 and R2), 7.33-7.37 (1H, m, R1 and R2), 7.83-7.85 (1H, m, R1 and R2). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 26.3, 26.8 and 26.9 (R1 and R2), 39.67, 39.73, 41.6 and 41.7 (R1 and R2), 48.7 and 48.9 (R1 and R2), 51.0 and 51.3 (R1 and R2), 55.9 (R1 and R2), 60.7 and 60.9 (R1 and R2), 111.8 and 112.0 (R1 and R2), 120.1 (q, *J* = 322.9 Hz, R1 and R2), 122.2 and 122.4 (R1 and R2), 122.7 (q, *J* = 273.1 Hz, R1 and R2), 124.4-124.6 (m, R1 and R2), 125.9-126.3 (m, R1 and R2), 130.5-130.7 (m, R1 and R2), 132.5 and 132.6 (2x d, 2x *J* = 33.5 Hz), 133.7 (m, R1 and R2), 139.0, 139.1, 139.2, 139.3, 139.4 and 139.4 (R1 and R2), 147.0 (R1 and R2), 148.7 and 148.8 (R1 and R2). ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -63.50 and -63.53 (2x 1.5F, 2x s, R1 and R2), -75.29 and -75.32 (2x 1.5F, 2x s, R1 and R2). IR (ATR, cm^{-1}): ν_{max} = 1394, 1319, 1175, 1134, 1025. MS (ES^+): *m/z* (%): 217 (100). HRMS (ES^+): calcd. for $C_{14}H_{17}O_2^+$ (M - $C_8H_3BrF_6NO_2S^-$): 217.1223, found 217.1214.

Synthesis of 7,12-dimethoxy-5-(trifluoromethanesulfonyl)-5,6,8,9,10,11- hexahydro-8,11-methanobenzo[j]phenanthridines 12d-e

The synthesis of 7,12-dimethoxy-2-trifluoromethyl-5-(trifluoromethanesulfonyl)-5,6,8,9,10,11-hexahydro-8,11-methanobenzo[*j*]phenanthridine **12e** is described as representative. To a solution of *N*-(2-bromo-4-(trifluoromethyl)phenyl)-*N*-(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl)-1,1,1-trifluoromethanesulfonamide **11e** (0.32 g, 0.55 mmol) in THF (10 mL) under nitrogen atmosphere, were added $Pd(OAc)_2$ (0.06 equiv, 7.4 mg, 0.033 mmol), triphenylphosphine (0.18 equiv, 26 mg, 0.099 mmol) and K_2CO_3 (2 equiv, 0.152 g, 1.10 mmol) and this mixture was stirred at reflux temperature for 61 hours. After this time, extra $Pd(OAc)_2$ (0.06 equiv, 7.4 mg, 0.033 mmol), triphenylphosphine (0.18 equiv, 26 mg, 0.099 mmol) and K_2CO_3 (2 equiv, 0.152 g, 1.10 mmol) were added and stirring was continued for another 25 hours. After the addition of H_2O (10 mL), the reaction mixture was filtered over Celite® and extracted with EtOAc (3x 10 mL). After drying of the combined organic phases ($MgSO_4$), filtration and evaporation *in vacuo*, the crude product was purified *via* column chromatography yielding 7,12-dimethoxy-2-trifluoromethyl-5-(trifluoromethanesulfonyl)-5,6,8,9,10,11-hexahydro-8,11-methanobenzo[*j*]phenanthridine **12e** (0.217 g, 0.43 mmol).

7,12-Dimethoxy-2-trifluoromethyl-5-(trifluoromethanesulfonyl)-5,6,8,9,10,11-hexahydro-8,11-methanobenzo[j]phenanthridine ***12e****.* Pale yellow crystals, mp 118-119 °C. R_f = 0.38, petroleum ether/EtOAc 9/1. Yield 78%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29-1.34 (2H, m), 1.59 (1H, d, *J* = 9.0 Hz), 1.79 (1H, d, *J* = 9.0 Hz), 2.02-2.07 (2H, m), 3.63 (3H, s), 3.70 (2H, br s), 3.90 (3H, s), 4.66 (1H, br s), 4.97 (1H, br s), 7.57 (1H, dd, *J* = 8.5 Hz, 1.6 Hz), 7.72 (1H, d, *J* = 8.5 Hz), 8.77 (1H, d, *J* = 1.6 Hz). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 27.0, 40.7, 41.7, 45.6, 48.9, 60.9, 61.2, 120.0 (q, *J* = 324.5 Hz), 120.8, 124.0 (q, *J* = 272.4 Hz), 124.5 (q, *J* = 3.5 Hz), 125.3, 125.55, 125.61 (q, *J* = 4.0 Hz), 129.7 (q, *J* = 32.5 Hz), 130.2, 137.0, 141.2, 142.8, 145.6, 147.5. ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -63.19 (3F, s), -75.78 (3F, s). IR (ATR, cm^{-1}): ν_{max} = 1400, 1332, 1195, 1137, 1086. MS (ES^+): *m/z* (%): 374 (M - CHF_3SO_3 + H^+, 100). HRMS (ES^+): calcd. for $C_{21}H_{19}F_3NO_2^+$ (M - CHF_3SO_3 + H^+): 374.1362, found 374.1378.

7,12-Dimethoxy-3-trifluoromethyl-5-(trifluoromethanesulfonyl)-5,6,8,9,10,11-hexahydro-8,11-methanobenzo[j]phenanthridine ***12d****.* Pale yellow crystals, mp 143-144 °C. R_f = 0.42, petroleum ether/EtOAc 9/1. Yield 81%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28-1.35 (2H, m), 1.59 (1H, d, *J* = 9.0 Hz), 1.79 (1H, d, *J* = 9.0 Hz), 2.03-2.07 (2H, m), 3.62 (3H, s), 3.70 (2H, br s), 3.90 (3H, s), 4.64 (1H, br s), 4.99 (1H, br s), 7.61 (1H, d, *J* = 8.5 Hz), 7.83 (1H, s), 8.55 (1H, d, *J* = 8.5 Hz). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 27.0, 40.7, 41.7, 45.6, 48.8, 61.0, 61.1, 120.0 (q, *J* = 324.7 Hz), 120.9, 122.5 (q, *J* = 3.7 Hz), 123.7 (q, *J* = 272.3 Hz), 124.3 (q, *J* = 3.5 Hz), 125.7, 128.8, 129.6 (q, *J* = 33.2 Hz), 133.1, 134.5, 141.5, 142.8, 145.7, 147.7. ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -63.20 (3F, s), -75.70 (3F, s). IR (ATR, cm^{-1}): ν_{max} = 1398, 1327, 1311, 1193, 1121, 1075. MS (ES^+): *m/z* (%): 374 (M - CHF_3SO_3 + H^+, 100). HRMS (ES^+): calcd. for $C_{21}H_{19}F_3NO_2^+$ (M - CHF_3SO_3 + H^+): 374.1362, found 374.1368.

Synthesis of 7,12-dimethoxy-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridines 13a-c

The synthesis of 7,12-dimethoxy-2-fluoro-8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine **13a** is described as representative. To a mixture of *N*-(2-bromo-4-fluorophenyl)-*N*-[(5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)methyl]-1,1,1-trifluoromethanesulfonamide **11a** (0.250 g, 0.46 mmol) in dry toluene (4 mL) in a pressure vial under N_2-atmosphere, $Pd(OAc)_2$ (0.06 equiv, 6 mg, 0.027 mmol), triphenylphosphine (0.18 equiv, 22 mg, 0.084 mmol) and K_2CO_3 (2 equiv, 128 mg, 0.93 mmol) were added. This mixture was stirred under microwave irradiation at 125 °C for 3 hours, after which it was quenched with H_2O (4 mL). Subsequently, this mixture was filtered over Celite® and extracted with EtOAc (3x 6 mL). This organic phase was dried ($MgSO_4$), filtered and evaporated *in vacuo*, affording crude 7,12-dimethoxy-2-fluoro-5-(trifluoromethanesulfonyl)-5,6,8,9,10,11-hexahydro-8,11-methanobenzo[*j*]phenanthridine **12a**, which was not purified but used as such in the following reaction. Therefore, crude **12a** was dissolved in dry THF (10 mL) and two equivalents of KO*t*Bu (1M in THF, 0.92 mL) were added and this mixture was stirred at reflux temperature for two hours. After the addition of a saturated aqueous solution of NH_4Cl (10 mL), an extraction with EtOAc was performed (3x 5 mL) and the combined organic phases were dried ($MgSO_4$), filtered and evaporated under reduced pressure. Purification using preparative TLC afforded pure 7,12-dimethoxy-2-fluoro-8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine **13a** (0.077 g, 0.24 mmol).

7,12-Dimethoxy-2-fluoro-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine ***13a****.* Yellow oil. R_f = 0.06, petroleum ether/EtOAc 9/1. Yield 51%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42-1.44 (2H, m), 1.71 (1H, d, *J* = 9.1 Hz), 1.87-1.90 (1H, m), 2.11-2.16 (2H, m), 3.89 (2H, br s), 3.96 (3H, s), 4.10 (3H, s), 7.45 (1H, ddd, *J* = 9.0 Hz, 7.7 Hz, 2.8 Hz), 8.14 (1H, dd, *J* = 9.0 Hz, 6.1 Hz), 9.07 (1H, dd, *J* = 12.3 Hz, 2.8 Hz), 9.54 (1H, s). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 27.21, 27.23, 41.0, 41.3, 48.8, 61.0, 62.2, 111.6 (d, *J* = 25.6 Hz), 116.9 (d, *J* = 24.7 Hz), 120.6, 124.9 (d, *J* = 10.7 Hz), 125.1 (d, *J* = 4.2 Hz), 131.6 (d, *J* = 9.4 Hz), 138.0, 142.0, 145.7, 146.7, 147.0, 147.8, 161.0 (d, *J* = 243.6 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -112.89 to -112.96 (1F, m). MS (ES^+): *m/z* (%): 324 (M + H^+, 100). HRMS (ES^+): calcd. for $C_{20}H_{19}FNO_2^+$: 324.1394, found 324.1408.

7,12-Dimethoxy-3-fluoro-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine ***13b****.* Yellow oil. R_f = 0.07, petroleum ether/EtOAc 9/1. Yield 39%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.40-1.44 (2H, m), 1.69 (1H, d, *J* = 9.1 Hz), 1.88 (1H, d, *J* = 9.1 Hz), 2.10-2.15 (2H, m), 3.89 (2H, br s), 3.92 (3H, s), 4.10 (3H, s), 7.38 (1H, ddd, *J* = 9.4 Hz, 8.0 Hz, 2.8 Hz), 7.79 (1H, dd, *J* = 9.8 Hz, 2.8 Hz), 9.41 (1H, dd, *J* = 9.4 Hz, 6.3 Hz), 9.59 (1H, s). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 27.1, 27.2, 40.9, 41.2, 48.6, 60.8, 62.0, 113.9 (d, *J* = 20.3 Hz), 115.7 (d, *J* = 22.7 Hz), 120.1, 120.5 (d, *J* = 2.1 Hz), 125.4, 128.7 (d, *J* = 8.8 Hz), 136.7, 146.0, 146.3, 146.4 (d, *J* = 11.3 Hz), 147.0, 149.6, 161.8 (d, *J* = 248.1 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -113.31 to -113.38 (1F, m). IR (ATR, cm^{-1}): ν_{max} = 1450, 1314, 1208, 1049. MS (ES^+): *m/z* (%): 324 (M + H^+, 100). HRMS (ES^+): calcd. for $C_{20}H_{19}FNO_2^+$: 324.1394, found 324.1399.

7,12-Dimethoxy-4-fluoro-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine ***13c****.* Yellow oil. R_f = 0.07, petroleum ether/EtOAc 9/1. Yield 43%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41-1.45 (2H, m), 1.71

(1H, d, *J* = 9.1 Hz), 1.88-1.91 (1H, m), 2.12-2.16 (2H, m), 3.90 (1H, br s), 3.91 (1H, br s), 3.93 (3H, s), 4.10 (3H, s), 7.42 (1H, ddd, $J_{H,F}$ = 10.0 Hz, *J* = 8.1 Hz, 1.2 Hz), 7.56 (1H, ddd, *J* = 8.4 Hz, 8.1 Hz, $J_{H,F}$ = 5.8 Hz), 9.18 (1H, d, *J* = 8.4 Hz), 9.63 (1H, s). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 27.06, 27.10, 40.9, 41.3, 48.6, 60.9, 62.1, 112.9 (d, *J* = 19.3 Hz), 120.7, 122.2 (d, *J* = 4.5 Hz), 124.9 (d, *J* = 2.6 Hz), 125.6, 126.5 (d, *J* = 8.6 Hz), 134.8 (d, *J* = 10.3 Hz), 137.7, 146.3, 146.5, 147.1, 148.7 (d, *J* = 1.3 Hz), 158.4 (d, *J* = 252.4 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -125.22 (1F, dd, $J_{H,F}$ = 10.0 Hz, 5.8 Hz). IR (ATR, cm^{-1}): ν_{max} = 1316, 1033, 963, 762. MS (ES^+): *m/z* (%): 324 (M + H^+, 100). HRMS (ES^+): calcd. for $C_{20}H_{19}FNO_2^+$: 324.1394, found 324.1406.

Synthesis of 7,12-dimethoxy-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridines 13d,e

The synthesis of 7,12-dimethoxy-2-trifluoromethyl-8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine **13e** is described as representative. To a solution of 7,12-dimethoxy-2-trifluoromethyl-5-(trifluoromethanesulfonyl)-5,6,8,9,10,11-hexahydro-8,11-methanobenzo[*j*]phenanthridine **12e** (0.155 g, 0.31 mmol) in THF (10 mL), was added KO*t*Bu (1M in THF, 1.2 equiv, 0.37 mmol) and this mixture was stirred at reflux temperature for three hours. After the addition of saturated aqueous NH_4Cl, the reaction mixture was extracted with EtOAc (3x 10 mL), dried ($MgSO_4$), filtered and evaporated under reduced pressure. Without the need for purification, 7,12-dimethoxy-2-trifluoromethyl-8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine **13e** was obtained in quantitative yield (0.113 g, 0.31 mmol).

7,12-Dimethoxy-2-trifluoromethyl-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine ***13e****.* Yellow oil. Yield quant. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39-1.44 (2H, m), 1.70 (1H, d, *J* = 9.2 Hz), 1.87-1.90 (1H, m), 2.11-2.16 (2H, m), 3.90 (2H, br s), 3.95 (3H, s), 4.11 (3H, s), 7.89 (1H, dd, *J* = 8.5 Hz, 1.8 Hz), 8.24 (1H, d, *J* = 8.5 Hz), 9.67 (1H, s), 9.79 (1H, br s). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 27.0, 27.1, 41.0, 41.4, 48.7, 60.8, 62.0, 120.6, 123.4, 123.9 (q, *J* = 3.1 Hz), 124.6 (q, *J* = 271.2 Hz), 124.7 (q, *J* = 4.6 Hz), 125.0, 128.2 (q, *J* = 31.9 Hz), 130.4, 137.9, 146.2, 146.8, 147.2, 150.6. ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -62.20 (3F, s). IR (ATR, cm^{-1}): ν_{max} = 1334, 1320, 1161, 1118, 1087. MS (ES^+): *m/z* (%): 374 (M + H^+, 100). HRMS (ES^+): calcd. for $C_{21}H_{19}F_3NO_2^+$: 374.1362, found 374.1378.

7,12-Dimethoxy-3-trifluoromethyl-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine ***13d****.* Yellow oil. Yield quant. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41-1.46 (2H, m), 1.72 (1H, d, *J* = 9.1 Hz), 1.88-1.92 (1H, m), 2.13-2.18 (2H, m), 3.92 (2H, br s), 3.95 (3H, s), 4.12 (3H, s), 7.82 (1H, dd, *J* = 8.9 Hz, 1.9 Hz), 8.44 (1H, br s), 9.52 (1H, d, *J* = 8.9 Hz), 9.66 (1H, s). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 26.9, 27.0, 40.9, 41.4, 48.6, 60.7, 61.8, 120.8, 122.3 (q, *J* = 3.1 Hz), 124.2 (q, *J* = 272.2 Hz), 125.5, 126.2, 127.0 (q, *J* = 4.1 Hz), 127.7, 129.3 (q, *J* = 32.6 Hz), 138.2, 144.2, 146.4, 146.6, 146.9, 149.8. ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -62.82 (3F, s). IR (ATR, cm^{-1}): ν_{max} = 1332, 1312, 1174, 1116, 1070, 1050. MS (ES^+): *m/z* (%): 374 (M + H^+, 100). HRMS (ES^+): calcd. for $C_{21}H_{19}F_3NO_2^+$: 374.1362, found 374.1379.

Synthesis of 8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine-7,12-diones 14-18

The synthesis of 2-fluoro-8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine-7,12-dione **14** is described as representative. To a solution of 7,12-dimethoxy-2-fluoro-8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine **13a** (0.030 g, 0.093 mmol) in CH_3CN (2 mL), was added a solution of CAN (2.3 equiv, 0.12 g, 0.21 mmol) in H_2O (2 mL) at 0 °C. This mixture was stirred at this temperature for 45 minutes, after which H_2O (4 mL) was added and an extraction was performed with EtOAc (3x 5 mL). The combined organic layers were dried ($MgSO_4$), filtered and evaporated under reduced pressure affording crude 2-fluoro-8,9,10,11-tetrahydro-8,11-methanobenzo[*j*]phenanthridine-7,12-dione **14**, which was purified *via* preparative TLC (0.010 g, 0.034 mmol).

2-Fluoro-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine-7,12-dione ***14****.* Orange crystals, mp 198-199 °C. R_f = 0.13, petroleum ether/EtOAc 9/1. Yield 37%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27-1.34 (2H, m), 1.52 (1H, d, *J* = 9.2 Hz), 1.74-1.77 (1H, m), 2.00-2.08 (2H, m), 3.69 (1H, br s), 3.70 (1H, br

s), 7.62 (1H, ddd, *J* = 9.3 Hz, $J_{H,F}$ = 7.5 Hz, *J* = 2.8 Hz), 8.17 (1H, dd, *J* = 9.3 Hz, $J_{H,F}$ = 5.7 Hz), 9.15 (1H, dd, $J_{H,F}$ = 11.4 Hz, *J* = 2.8 Hz), 9.55 (1H, s). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 25.2, 25.3, 40.9, 41.4, 47.3, 111.3 (d, *J* = 25.8 Hz), 122.1 (d, *J* = 26.4 Hz), 123.7 (d, *J* = 11.8 Hz), 124.1, 132.3 (d, *J* = 6.7 Hz), 132.5 (d, *J* = 9.5 Hz), 146.7 (d, *J* = 2.8 Hz), 149.4, 152.0, 155.3, 163.1 (d, *J* = 251.1 Hz), 182.1, 185.0. ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -107.83 (1F, ddd, $J_{H,F}$ = 11.4 Hz, 7.5 Hz, 5.7 Hz). IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1645, ν_{max} = 1320, 1271, 1196, 1006. MS (ES^+): *m/z* (%): 294 (M + H^+, 100). HRMS (ES^+): calcd. for $C_{18}H_{13}FNO_2^+$: 294.0925, found 294.0931.

3-Fluoro-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine-7,12-dione ***15****.* Orange crystals, mp 154-155 °C. R_f = 0.29, petroleum ether/EtOAc 9/1. Yield 50%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27-1.34 (2H, m), 1.52 (1H, d, *J* = 9.2 Hz), 1.73-1.77 (1H, m), 2.02-2.08 (2H, m), 3.69 (2H, br s), 7.54 (1H, ddd, *J* = 9.6 Hz, 8.0 Hz, 2.6 Hz), 7.89 (1H, dd, *J* = 9.4 Hz, 2.6 Hz), 9.48 (1H, dd, *J* = 9.6 Hz, 6.2 Hz), 9.60 (1H, s). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 25.3, 25.4, 40.9, 41.4, 47.2, 113.8 (d, *J* = 20.2 Hz), 119.6, 120.5 (d, *J* = 24.5 Hz), 123.3 (d, *J* = 2.7 Hz), 130.3 (d, *J* = 9.6 Hz), 133.0 (d, *J* = 1.8 Hz), 148.7, 152.0, 153.6 (d, *J* = 12.7 Hz), 155.1, 164.1 (d, *J* = 256.5 Hz), 182.0, 185.2. ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -105.86 (1F, ddd, *J* = 9.4 Hz, 8.0 Hz, 6.2 Hz). IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1655, ν_{max} = 1620, 1314, 1287. MS (ES^+): *m/z* (%): 294 (M + H^+, 100). HRMS (ES^+): calcd. for $C_{18}H_{13}FNO_2^+$ (M + H^+): 294.0925, found 294.0927.

4-Fluoro-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine-7,12-dione ***16****.* Orange crystals, mp 171-172 °C. Yield 62%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30-1.34 (2H, m), 1.53 (1H, d, *J* = 9.2 Hz), 1.75-1.78 (1H, m), 2.04-2.08 (2H, m), 3.70 (2H, br s), 7.54 (1H, ddd, $J_{H,F}$ = 9.9 Hz, *J* = 7.8 Hz, 1.2 Hz), 7.69 (1H, ddd, *J* = 8.8 Hz, 7.8 Hz, $J_{H,F}$ = 5.4 Hz), 9.20 (1H, d, *J* = 8.8 Hz), 9.62 (1H, s). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 25.3, 25.4, 41.0, 41.5, 47.2, 115.7 (d, *J* = 18.5 Hz), 123.5 (d, *J* = 5.3 Hz), 124.2, 124.4, 129.9 (d, *J* = 8.1 Hz), 132.8 (d, *J* = 2.5 Hz), 142.1 (d, *J* = 11.3 Hz), 147.6, 151.9, 155.4, 157.8 (d, *J* = 257.9 Hz), 181.9, 184.9. ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -123.53 (1F, dd, $J_{H,F}$ = 9.9 Hz, 5.4 Hz). IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1655, ν_{max} = 1326, 1287, 1274, 765. MS (ES^+): *m/z* (%): 294 (M + H^+, 100). HRMS (ES^+): calcd. for $C_{18}H_{13}FNO_2^+$: 294.0925, found 294.0931.

3-Trifluoromethyl-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine-7,12-dione ***17****.* Orange crystals, mp 158-159 °C. Yield 45%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28-1.35 (2H, m), 1.54 (1H, d, *J* = 9.2 Hz), 1.76-1.78 (1H, m), 2.03-2.10 (2H, m), 3.70 (1H, br s), 3.71 (1H, br s), 7.92 (1H, dd, *J* = 9.1 Hz, 1.7 Hz), 8.47 (1H, s), 9.58 (1H, d, *J* = 9.1 Hz), 9.69 (1H, s). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 25.2, 25.3, 41.0, 41.5, 47.3, 123.4 (q, *J* = 272.9 Hz), 124.3, 124.7, 125.3 (q, *J* = 2.9 Hz), 127.6 (q, *J* = 4.4 Hz), 129.1, 132.6, 132.9 (q, *J* = 33.2 Hz), 148.8, 150.9, 152.0, 155.4, 181.7, 184.8. ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -63.80 (3F, s). IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1655, ν_{max} = 1332, 1286, 1163, 1131. MS (ES^+): *m/z* (%): 344 (M + H^+, 100). HRMS (ES^+): calcd. for $C_{19}H_{13}F_3NO_2^+$: 344.0893, found 344.0901.

2-Trifluoromethyl-8,9,10,11-tetrahydro-8,11-methanobenzo[j]phenanthridine-7,12-dione ***18****.* Orange crystals, mp 151-152 °C. R_f = 0.17, petroleum ether/EtOAc 9/1. Yield 76%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27-1.35 (2H, m), 1.54 (1H, d, *J* = 9.2 Hz), 1.75-1.79 (1H, m), 2.03-2.10 (2H, m), 3.70 (1H, br s), 3.71 (1H, br s), 8.01 (1H, dd, *J* = 8.9 Hz, 2.0 Hz), 8.29 (1H, d, *J* = 8.9 Hz), 9.70 (1H, s), 9.83 (1H, s). ^{13}C NMR (100.6 MHz, ref = $CDCl_3$): δ 25.3, 25.5, 41.1, 41.6, 47.4, 121.9, 123.9 (q, *J* = 272.9 Hz), 124.4, 126.0 (q, *J* = 4.6 Hz), 127.3 (q, *J* = 3.2 Hz), 131.4, 131.7 (q, *J* = 32.7 Hz), 133.4, 149.7, 152.2, 152.6, 155.4, 181.8, 184.9. ^{19}F NMR (376.5 MHz, $CDCl_3$, ref = $CFCl_3$): δ -63.16 (3F, s). IR (ATR, cm^{-1}): $\nu_{C=O}$ = 1657, ν_{max} = 1303, 1287, 1273, 1130. MS (ES^+): *m/z* (%): 344 (M + H^+, 100). HRMS (ES^+): calcd. for $C_{19}H_{13}F_3NO_2^+$: 344.0893, found 344.0897.

4.2. Biology

Bacterial strains and growth conditions

Five mycobacterial strains used for screening were *Mycobacterium tuberculosis (Mtb)* H37Ra (ATCC® 25177™), *M. marinum* (NCTC 2275), *M. avium* subsp. *avium* (ATCC® 25291™) and *M. bovis* BCG (ATCC® 35737™). For luminometric assays, the *pSMT1* luciferase reporter plasmid[14] was used to

transform *Mtb* H37Ra (*Mtb* H37Ra^{lux}). For specificity assays, the Gram-negative *Escherichia coli* (ATCC® 8739™), *Pseudomonas aeruginosa* (ATCC® 9027™) and *Klebsiella pneumoniae* (ATCC® 13883™), and the Gram-positive *Enterococcus faecium* (ATCC® 19434™), *Staphylococcus aureus* (ATCC® 6538™) and *Streptococcus pneumonia* (ATCC® BAA-334™), were used. Stock vials were preserved at -80°C. Mycobacteria were cultivated in Middlebrook 7H9 medium (Sigma-Aldrich) supplemented with 0.5% (v/v) glycerol (Sigma-Aldrich) and 10% (v/v) oleic acid-albumin-dextrose-catalase (OADC) enrichment (BD) (complete liquid growth medium). For *M. bovis* BCG, glycerol was replaced with 0.05% (v/v) Tween 80 (Sigma-Aldrich). Media were supplemented with hygromycin (Roche) at a concentration of 100 µg/ml, when necessary. All liquid cultures were incubated under shaking conditions (150 rpm) at 37°C or, in case of *M. marinum*, at 32°C. To ensure single-cell suspension were used in all assays, cultures were sonicated for 15 minutes, mixed vigorously using a vortex, passed through a 27-gauge syringe needle for at least 20 times, and allowed to settle for 5 minutes prior taking an aliquot of the top layer of the culture. Other bacterial strains were grown in Luria-Bertani (LB) medium (Sigma-Aldrich) at 37°C.

***In vitro* antimicrobial screening**

Antimicrobial effects against a broader panel of Gram-negative and Gram-positive bacteria were evaluated by the resazurin microtiter assay. All compounds were solubilized in 100% dimethylsulfoxide (DMSO; Fisher Scientific) at stock concentration of 10 mM. Starting from a final concentration of 64 µM, twofold serial dilutions of each compound were made in the appropriate growth medium. Volumes of 100 µL of the serial dilutions were added in triplicate to transparent 96-well, flat-bottomed micro-well plates. As a positive control, norfloxacin (*E. coli*, *P. aeruginosa*; Sigma-Aldrich), ciprofloxacin (*K. pneumonia*; Sigma-Aldrich), vancomycin (*E. faecium*; Sigma-Aldrich), docycycline (*S. aureus*, *S. pneumonia*; Sigma-Aldrich) and isoniazid (*Mtb* H37Ra; Sigma-Aldrich) was included. Bacterial suspensions were made by thawing and dissolving frozen glycerol stocks in appropriate growth medium to obtain a suspension with an appropriate inoculum size. Next, volumes of 100 µL of the bacterial suspensions were added to the test plates. Outer perimeter wells were filled with at least 200 µL of deionized water to prevent evaporation of the inner test wells. After 24 hours or, in the case of *Mtb* H37Ra, 7 days of exposure to the tested compounds, 20 µL of 0.01% (w/v) resazurin (Sigma-Aldrich) was added to each well and test plates were incubated again at 37°C until a color change from blue to pink occurred. Bacterial replication was then analyzed by measuring the fluorescence (λ_{ex} = 550 nm, λ_{em} = 590 nm) of each test well with a spectrophotometer (Promega Discover). Results were presented as the mean of triplicate values.

Monitoring mycobacterial growth by luminometry

The half maximal inhibitory concentration (IC_{50}) and minimal inhibitory concentration (MIC) against *Mtb* H37Ra^{lux} were assed using a luminometric assay. Briefly, twofold serial dilutions of each compound were made in complete liquid growth medium. Volumes of 100 µL of the serial dilutions were added in triplicate to black 96-well, flat-bottomed micro-well plates. As a positive control, isoniazid was included. A mycobacterial suspension was made by diluting a thawed frozen glycerol stock in complete liquid growth medium in order to obtain a suspension with 10.000 relative light units (RLU) per mL. Subsequently, volumes of 100 µL of the bacterial suspension were added to the test plates. Outer perimeter wells were filled with at least 200 µL of deionized water. Upon 7 days of exposure to the compounds, bacterial replication was analyzed by luminometry. The luminescent signal was evoked by addition of 25 µL of 1% (v/v) n-decanal in ethanol (Sigma-Aldrich) and measured using a luminometer (Promega Discover). Results were presented as the mean of triplicate values.

Assessment of acute cytotoxicity

The 50% cytotoxic concentration towards the J774A.1 murine macrophage cell line (ATCC® TIB-67™) was determined by a neutral red uptake (NRU) assay. The J774A.1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher) supplemented with 10% (v/v) heat-inactivated

fetal calf serum (iFCS; Thermo Fisher) in a 5% CO_2 atmosphere at 37°C until a semi-confluent layer of cells was obtained. Next, the cells were harvested and seeded into transparent, flat-bottomed 96-well plates at a density of 40.000 cells per well and left for recovery at 37°C and 5% CO_2. The following day, twofold serial dilutions of the tested compounds were made in DMEM + 10% iFCS with a final starting concentration of 128 µM. As a positive control, tamoxifen (Sigma-Aldrich) was included. The J774A.1 cells were washed with sterile phosphate-buffered saline (PBS; Thermo Fisher) and exposed to the compounds by adding volumes of 100 µL of the serial dilutions. Test plates were left for 24 hours at 37°C and 5% CO_2. After 24 hours exposure to the compounds, the cells were washed two times with sterile PBS and 100 µL neutral red (Sigma-Aldrich) working solution was added per well. Subsequently, the test plates were incubated for 3 hours at 37°C and 5% CO_2. The cells were washed again with sterile PBS and 150 µL of an 1:1 ethanol/acetic acid (Merck) mixture was added in each well. The plates were left shaking until the color became homogenous purple, and the optical density was measured at 530 nm and 620 nm (reference wavelength) using a plate reader (Promega Discover). Results were presented as the mean of triplicate values.

Activity against other mycobacterial strains

Inhibitory effects of the compounds on four other mycobacterial strains was monitored by the resazurin microtiter assay, as described in the above. In case of *M. marinum*, test plates were incubated at 32°C. As a positive control, moxifloxacin (MXF; Sigma-Aldrich) was included. Results were presented as the mean of triplicate values.

Microsomal stability assay

The metabolic stability of each compound was evaluated by exposure to male mouse and human liver microsomes from commercial sources (BD Biosciences). The assay was carried out based on the BD Biosciences Guidelines for Use with minor adaptations. Phase-I and Phase-II metabolism were studied through the cytochrome P450 (CYP_{450}) superfamily (Corning) and uridine glucuronosyl transferase (UGT) enzymes (Corning), respectively. Both the compounds and Phase-I or Phase-II metabolic enzymes were incubated at 5 µM, together with 0.5 mg/mL rat or human liver microsomes in PBS. For the CYP_{450} and other reduced nicotinamide adenine dinucleotide phosphate (NADPH) dependent enzymes, the reaction was started by the addition of 1 mM NADPH (Corning). For the UGT enzymes, 2 mM uridine diphosphate glucuronic acid (UDPGA) cofactor (Corning) was used. For both Phase-I and Phase-II metabolism, diclophenac (Sigma-Aldrich) was included as a reference drug. At 0, 15, 30 and 60 minutes upon the start of each reaction, a sample of 20 µL was withdrawn from the reaction mixture. To inactivate the enzymes and precipitate the proteins, a volume of 80 µL ice-cold acetonitrile (VWR®) was added. The mixtures were vortexed for 30 seconds and centrifuged at 15.000 rpm for 5 minutes at 4°C. The supernatant of each sample was kept at -80°C until further analysis. To determine the corresponding loss of parent compound in each sample, ultra-performance liquid chromatography (Waters Aquity™) coupled with tandem quadrupole mass spectrophotometry (Waters Xevo™), equipped with an interface and operated in multiple reaction monitoring mode, was used. The optimal MS parameters and control of the chromatographic separation conditions was tuned for each compound in a preceding experiment. Results were presented as the mean of at least two replications and the errors are standard deviations.

Inhibition of intracellular mycobacterial growth

The intracellular activity of the non-substituted scaffold **8** and TQD **16** was tested on the murine J774A.1 macrophage cell line infected with *Mtb* $H37Ra^{lux}$. The J774A.1 cells were cultured, harvested and seeded into transparent, flat-bottomed 96-well plates as described above. Upon recovery, the cells were washed with sterile PBS and infected with $H37Ra^{lux}$ at a multiplicity of infection (MOI) of 10 for 2 hours at 37°C. J774A.1 cells were washed two times with sterile PBS, incubated with 100 µg/mL gentamicin (Sigma-Aldrich) for 1 hour to kill the residual extracellular bacteria and, again, washed with sterile PBS. Then, the infected J774A.1 cells were treated with a TQD (10, 5 or 1.5 µM) or isoniazid (0.1 µM). Uninfected cells were used as a control. At 24, 48 and 72 hours post exposure, the

infected J774A.1 cells were washed and lysed with 200 µL of 1% Triton X-100 (Sigma-Aldrich). To assess the intracellular bacterial replication, 25 µL of 1% (v/v) n-decanal in ethanol was added to 100 µL of the lysate and luminescence was measured using a luminometer (Promega Discover). Results were presented the mean of triplicate values and the errors are standard deviations.

Vitotox assay

Observations on early signs of genotoxicity were done with the VITOTOX™ test (Gentaur) and the recommended protocol was followed. In brief, the Genox (RecN2-4) and Cytox (pr1) *Salmonella typhimurium* strains were diluted 250 times and cultivated with shaking (150 rpm) in poor 869 medium for 16 hours at 36°C. After incubation, the bacterial cultures were diluted 10 times more, incubated shaking (150 rpm) for 1 hour at 36°C, and kept on ice. S9-mix was added to the designated S9+ cultures to test the genotoxic effects of the metabolites of the compounds. The bacterial suspensions were incubated at 36°C and the luminescent signal was measured for 4 hours with a 5 minute interval.

Electron paramagnetic resonance spectroscopy

Electron paramagnetic resonance spectroscopy (EPR) was used to measure the formation of reactive oxygen species (ROS). Briefly, *Mtb* H37Ra cultures were exposed to TQD **16**, rifampicin (RIF; Sigma-Aldrich) or moxifloxacin (2 x MIC). Non-treated cultures were used as a negative control. Samples were taken and mixed (1:1) with a superoxide spin probe solution containing 10 mM 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH; Noxygen Science Transfer & Diagnostics GmbH) dissolved in Krebs HEPES buffer (KHB; Noxygen Science Transfer & Diagnostics GmbH) 50 minutes before the designated time-points (3, 6, 24 and 48 hours). Upon the additional incubation with the spin probe solution, allowing the probe to react with the superoxide, a volume of 50 µl of each sample was loaded into a glass capillary (Hirschmann). All measurements were performed on a Magnettech MiniScope MS 200 spectrometer as follows: frequency, 9.4 GHz; power, 5 dBm (3.16 mW); modulation frequency, 100 kHz; modulation amplitude, 0.1 mT; sweep time, 40 s; time constant, 0.1; sweep width, 10 mT; and number of scans, 3. Calibration was performed with aqueous solutions of a stable nitroxide radical 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (4-hydroxy-TEMPO; Sigma-Aldrich) in the range of concentrations 1 to 100 µM. The signal intensity reported was obtained via double integration of the respective simulated spectra of 4-hydroxy-TEMPO (a_N = 1.68 mT). The simulations were performed using a NIEHS P.E.S.T. WinSIM ver. 0.96 (available online at https://www.niehs.nih.gov/research/resources/software/tox-pharm/tools/). The simulated spectra of the test samples were double integrated (a_N = 1.58 mT) and the absolute concentration values were obtained using the calibration data above. Statistical analysis was performed using ANOVA in Graphpad Prism 7 software. Results were plotted as the mean of at least three replications and the errors are standard deviations. Significant difference was defined by a value of P<0.05.

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Conflict of interests

The authors declare no conflict of interest.

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