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1,2,3,4,8,9,10,11-Octahydrobenzo[*j*]phenanthridine-7,12-diones as New Leads against *Mycobacterium tuberculosis*

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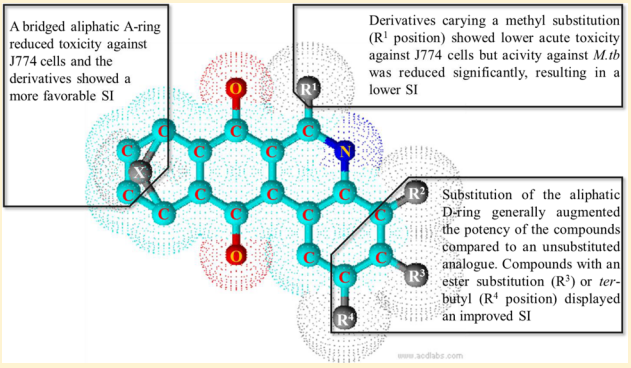
Supporting Information

ABSTRACT: Tuberculosis (TB) continues to be a worldwide health problem with over 1.4 million deaths each year. Despite efforts to develop more effective vaccines, more reliable diagnostics, and chemotherapeutics, tuberculosis remains a threat to global health, fueled by the HIV pandemic and the rapid generation of drug resistance. The exploration of novel drugs to serve as a companion drug for existing drugs is of paramount importance. As part of our program to design new 2-aza-anthraquinones with antimycobacterial activity, various tetrahydro- and octahydrobenzo[*j*]phenanthridinediones were synthesized. These compounds showed high in vitro potency against *Mycobacterium tuberculosis*, the etiological agent of TB and against other clinically relevant mycobacterial species at submicromolar concentrations. The susceptibility of a multidrug resistant strain toward these compounds and their ability to target intracellular replicating *Mycobacterium tuberculosis* was demonstrated. Next to the acute toxicity, the genotoxicity of these compounds was investigated. Often overlooked in studies, genotoxicity could be dismissed for the investigated compounds, making them a promising scaffold in TB drug research.



INTRODUCTION

The history of tuberculosis drug development is a story of great success and failure. Despite the undeniable impact of tuberculosis on global mortality, the discovery and development of antibiotics against *Mycobacterium tuberculosis* (*M.tb*) has been insufficient to eradicate the disease completely. Due to close host−pathogen coevolution, *M.tb* has grown to a highly resilient organism.[1−3] In an arms race with its host, mycobacteria have developed a thick lipid-rich cell wall as a defense against xenobiotic and harmful effector molecules unleashed by their host. It is the same mycobacterial cell wall that poses an impermeable barrier and is responsible for the high tolerance of *M.tb* to many commonly used antibiotics.[4−6] After promoting its own phagocytosis within the host, *M.tb* can proliferate within macrophages or enter into a dormant state depending on the conditions. Adapting metabolic quiescence not only helps the bacilli to survive harmful microenvironments within the host but also makes them virtually invulnerable for any known antibiotic that usually targets an active metabolism and replication.[7−9] This not only complicates the development of new drugs but also current treatment, which takes considerable time and relies on access to high quality antibiotics.

Lack of compliance results in the generation of drug resistant strains: multidrug resistant (MDR) strains with resistance to both isoniazid and rifampin and extensively drug resistant (XDR) strains with additional resistance to fluoroquinolones and one of the injectable aminoglycoside or polypeptide antibiotics.[10−12] Fueled by the HIV/AIDS pandemic, tuberculosis is not a mere disease from the past, and this fact can be found in the report and figures presented by the World Health Organization (WHO) in 2013. This report stated that 8.6 million people were infected with TB in 2012, of which 450 000

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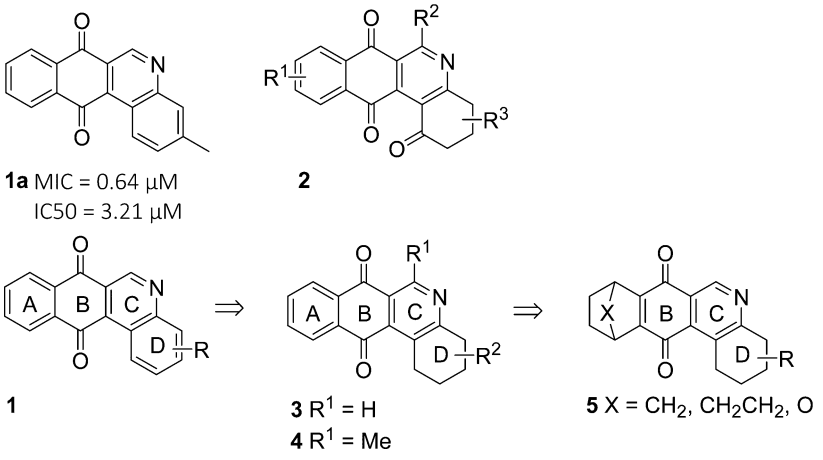
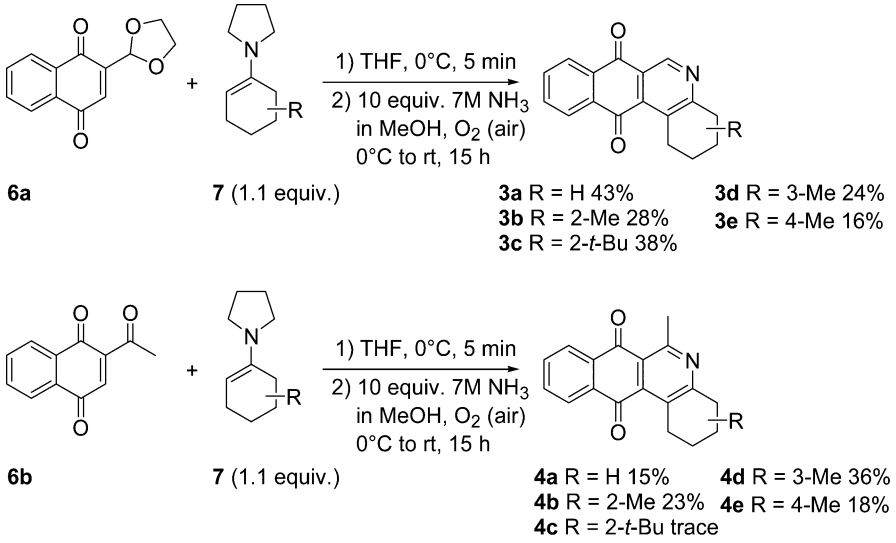
Scheme 1. Benzophenanthridinediones 1, Tetrahydrobenzophenanthridinediones 2, 3 & 4 and Octahydrobenzophenanthridinediones 5



Scheme 2. Synthesis of Tetrahydrobenzophenanthridinediones 3 and 4



had multidrug-resistant pulmonary TB. Of those infected, 1.3 million patients died, including 320 000 people that were TB/HIV coinfected.[13,14]

During a previous study, various benzo[*j*]phenantridine-7–12-diones **1** were tested against *Mycobacterium tuberculosis*. It was found that 3-methylbenzo[*j*]phenanthridine-7,12-dione **1a** showed promising antimycobacterial activity (MIC = 0.64 μM). However, this activity was accompanied with a relatively high cytotoxicity (IC_{50} = 3.21 μM), resulting in a selectivity index of only 15. As it is thought that this cytotoxicity is due to potential intercalation resulting from the flat nature of compounds **1**, the synthesis of more "out of plane" derivatives such as 1,2,3,4-tetrahydrobenzo[*j*]phenanthridinediones **3** and **4** with an aliphatic D-ring was envisaged. Structurally similar compounds **2**, bearing a carbonyl function on the 1-position, have been evaluated as antitumor compounds.[16] These compounds were synthesized by means of an oxidative addition[17] or a hetero Diels–Alder methodology.[18] In a next step, the synthesis of octahydrobenzo[*j*]phenathridinediones **5** with an aliphatic A- and D-ring was envisaged (Scheme 1).

RESULTS AND DISCUSSION

Chemistry. Pyranonaphthoquinones can be synthesized by means of the addition of a Stork enamine to a 2-(1-hydroxyalkyl)-1,4-naphthoquinone followed by aerobic oxidation.[19] Thus, the synthesis of tetrahydrobenzophenanthridinediones **3** and **4** was envisaged starting from the addition of Stork enamines **7** to 2-(1,3-dioxolan-2-yl)-1,4-naphthoquinone **6a**[20] and 2-acetyl-1,4-naphthoquinone **6b** followed by ammonia-mediated conversion of the enamine adduct and aerobic oxidation toward the corresponding 2-aza-anthraquinones **3** or **4**.[21] Even though a precedent of this methodology exists in the literature,[20] we could not reproduce this protocol, even upon several trials. Therefore, several enamines and conditions for the ammonia-mediated ring opening–ring closure were investigated, but no satisfying conditions were found.[22] The best results were obtained using 10 equivalents of 7 M NH_3 in MeOH as the nitrogen source. Using these conditions, a set of D-ring-substituted derivatives **3** and **4**, bearing either a methyl substituent or no substituent at C-6 were synthesized in low yields (Scheme 2). When these compounds were tested against *Mycobacterium tuberculosis*, no significant activity was observed. However, it was found that the compounds **3** with no substitution at C-6 were more active than the corresponding

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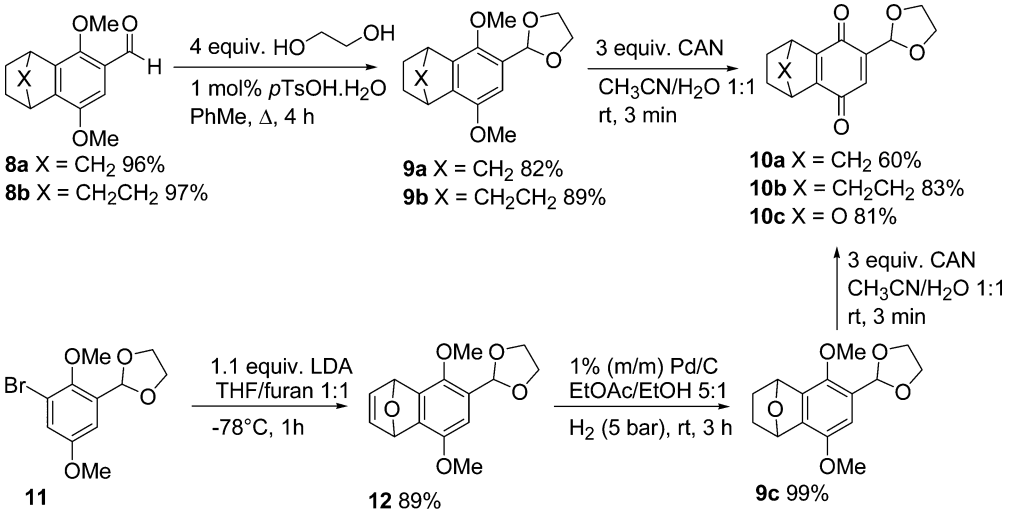
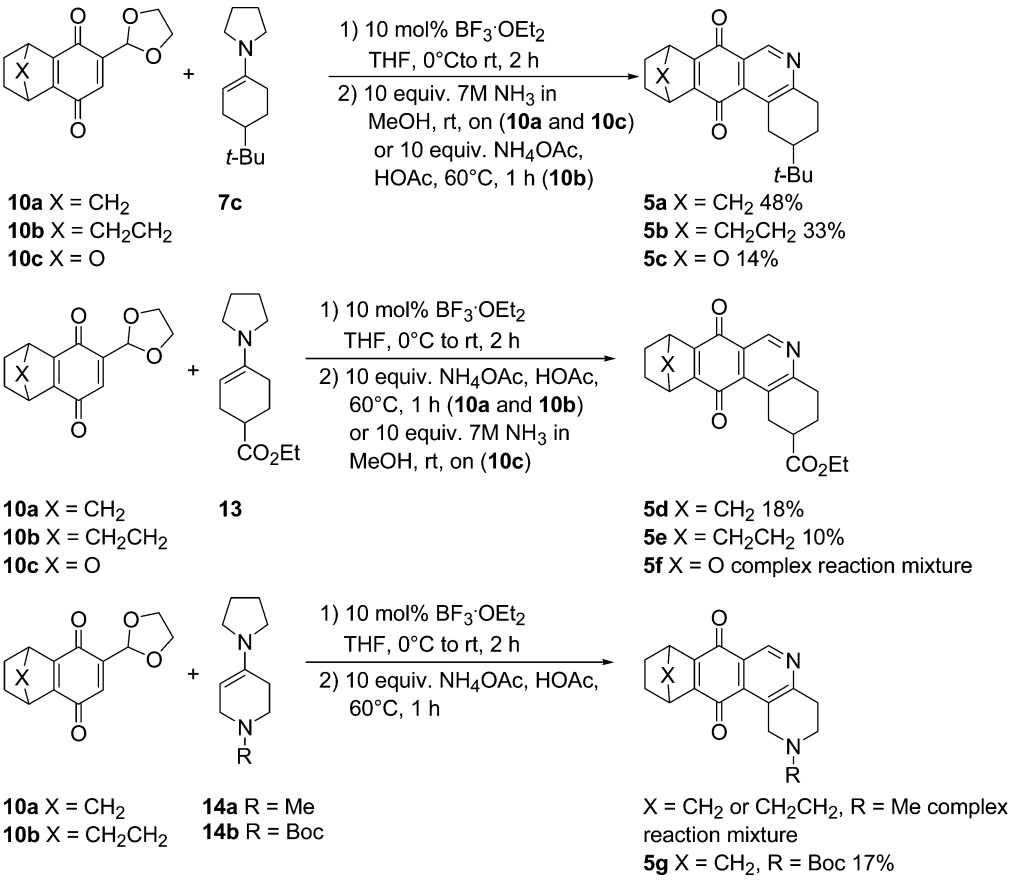
Scheme 3. Synthesis of the Starting 2-(1,3-Dioxolan-2-yl)-1,4-naphthoquinone 10



Scheme 4. Synthesis of Octahydrobenzo[*j*]phenanthridinediones 5a–5g



methyl-substituted derivatives **4**. Therefore, the synthesis of 2-aza-anthraquinones with an aliphatic A- and D-ring and no substitution at C-6 was envisaged.

The synthesis of methano-, ethano-, and epoxy-bridged octahydrobenzo[*j*]phenanthridinediones **5** was envisaged using the above-mentioned methodology. The starting 2-(1,3-dioxolan-2-yl)-1,4-naphthoquinone **10** were prepared starting from 1,4-dimethoxy-5,6,7,8-tetrahydronaphthalene-2-carboxaldehydes **8**.[23] These aldehydes **8** were converted to acetal by reaction with ethylene glycol followed by oxidative demethylation with cerium(IV) ammonium nitrate (CAN) to afford 6-(1,3-dioxolan-2-yl)-1,2,3,4-tetrahydronaphthalene-5,8-diones **10a** and **10b**. As these compounds only had a limited stability, they had to be used immediately in the next step. 2-(5,8-Dimethoxy-1,4-dihydro-1,4-epoxynaphthalen-6-yl)-1,3-dioxolane **10c** could not be prepared using this methodology and was prepared by means of a Diels–Alder reaction of 2-(3-bromo-2,5-dimethoxyphenyl)-1,3-dioxolane with furan **11**. Hydrogenation of the isolated double bound followed by oxidative

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demethylation with CAN yielded the desired quinone **10c** in 81% yield (Scheme 3).

No reaction was observed when enamine **7c** was added to 6-(1,3-dioxolan-2-yl-1,2,3,4-tetrahydro-1,4-methanonaphthalene-5,8-dione **10a** under the above-mentioned conditions. After investigation of the reaction conditions,[22] it was found that octahydrobenzophenanthridinediones **4** could be synthesized upon addition of 10 mol % of $BF_3{\cdot}OEt_2$ to the reaction mixture. In the case of 6-(1,3-dioxan-2-yl)-1,2,3,4-tetrahydro-1,4-methanonaphthalene-5,8-dione **10b**, ammonium acetate in HOAc had to be used to form the desired octahydrobenzophenanthridine **5b**. For O-bridged *tert*-butyl-substituted derivative **4c**, the ring-opening step was performed with 7 M NH_3 in MeOH as the reaction in acetic acid led to ring-opening of the epoxy-bridge (Scheme 4). Starting from ethyl 4-(pyrrolidin-1-yl)cyclohex-3-enecarboxylate **13**, two ester-substituted derivatives **5d** and **5e** were synthesized. The reaction with 6-(1,3-dioxolan-2-yl)-1,2,3,4-tetrahydro-1,4-epoxynaphthalene-5,8-dione **10c** resulted in a complex reaction mixture. Next, substitution of a carbon with nitrogen was envisaged. When the enamine addition was performed with 1-methyl-4-(pyrrolidin-1-yl)-1,2,3,6-tetrahydropyridine **14a**, a complex reaction mixture was obtained for both quinones **10a** and **10b**. Upon treatment of quinone **10a** with 1-*tert*-butoxycarbonyl-4-(pyrrolidin-1-yl)-1,2,3,6-tetrahydropyridine **14b**, 2-*tert*-butoxycarbonyl-1,2,3,4,8,9,10,11-octahydro-2-aza-8,11-methanobenzo[*j*]phenanthridine-7,12-dione **5g** was isolated in 17% yield. Due to the low yields of the addition reaction, no further derivatization of the obtained compounds was attempted.

Biology. Following the synthesis described here, the series of pentacyclic quinones **3a**−**3e**, **4a**, **4d**, **4e**, and **5a**−**5g** were tested for their antimycobacterial activity against *Mycobacterium tuberculosis*. A model based on a luminescent *M.tb* H37Rv strain ($H37Rv^{lux}$) was used. As reported previously in the literature, this technique offers a sensitive and reproducible tool, able to replace enumeration of bacteria by fastidious plating on agar.[24−27] Antitubercular properties of the synthesized compounds are reported by a reduction of luminescence emitted by a culture exposed to the compound, compared with a negative control culture. After 6 days of exposure of *M.tb* $H37Rv^{lux}$ to serial dilutions of the compounds, the potency was calculated as the minimal inhibitory concentration (MIC) at which the mycobacterial growth is reduced by 99%. In parallel, the acute toxicity of the analogues against eukaryotic J774 A.1 cells, a murine macrophage like monocyte cell line was studied in a neutral uptake assay. The macrophage model was chosen as macrophages are the primary host cell for *M.tb* in a tuberculosis infection. The neutral red uptake assay relies on the ability of viable cells to bind and incorporate the neutral red dye.[28] The acute toxic concentration (IC_{50}) of a compound is defined as the concentration at which the uptake of the neutral red dye by the cells is reduced by 50%. By dividing the IC_{50} with the MIC, the selectivity index (SI) can be calculated.

Tetrahydrobenzophenanthridinedione **3a** (MIC = 9.88 μM, IC_{50} = 7.34 μM, SI = 0.74) with an aliphatic D-ring did not show an increase in SI over the previous reported benzo[*j*]-phenantridine-7−12-diones (Table 1).[15] Although a decreased toxicity was observed, the activity against *M.tb* was lower as well. By alkyl substitution of the cyclohexane ring, two compounds with improved activity were found: derivative **3c** with a 2-*t*-Bu substitution at the para position (MIC = 9.64 μM, IC_{50} = 11.49 μM, SI = 1.19) and derivative **3d** with a methyl substitution at the meta position (MIC = 5.30 μM, IC_{50} = 8.13 μM, SI = 1.53). This reinforced the notion of importance for substitution of the annellated ring of the 2-aza-anthraquinones.

Table 1. Growth Inhibition of *M.tb* by Octahydrobenzo[*j*]phenanthridinediones and Their Acute Toxicity against Macrophages

compd	MIC[a]	IC_{50}[b]	SI[c]
	(μM)		
3a	9.88	7.34	0.74
3b	9.61	1.31	0.14
3c	9.64	11.49	1.19
3d	5.3	8.13	1.53
3e	14.43	14.91	1.03
4a	17.79	10.9	0.61
4d	9.85	25.68	2.61
4e	17.68	30.79	1.74
5a	0.59	51.35	87.03
5b	8.56	22.95	2.68
5c	7.21	53.23	7.38
5d	0.22	8.49	38.59
5e	0.26	49.76	191.38
5g	12.88	7.33	0.57
INH[g]	0.13	>100	>769.23
RIF[e]	0.19	>100	>526.31

[a]Minimal inhibitory concentration (MIC) at which 99% growth inhibition of the *M.tb* $H37Rv^{lux}$ lab strain was observed as calculated from triplicate cultures (SD values <10%). [b]Inhibitory concentration (IC_{50}) at which viability of the J774 A.1 macrophages was reduced by 50% as calculated from triplicate cultures (SD values <10%). [c]Selectivity index (SI), calculated as IC_{50}/MIC. [g]INH, [e]RIF the first line *M.tb* antibiotics, isoniazid and rifampin, used as a positive control.

Further attempts to optimize the structure by methyl substitution at the C-6 backbone position were unsuccessful. Compounds **4a**−**4e** showed further decreased toxicity, but this went along with a significantly lower activity against *M.tb*. Compound **4d** was the most active one, again bearing a methyl substitution at the meta position of the annellated aliphatic ring (MIC = 9.85 μM, IC_{50} = 25.68 μM, SI = 2.61).

In the next step, methano-, ethano-, and epoxy-bridged octahydrobenzo[*j*]phenanthridinediones **5** were submitted to biotesting. Bridging the ring in compounds **5a**−**5c** did have an impact on the acute toxicity of compound **3c** with a 2-*t*-Bu substitution at the para position. The methano-bridged derivative **5a** showed not only increased activity but also lower acute toxicity, resulting in a more favorable SI (MIC = 0.59 μM, IC_{50} = 51.35 μM, SI = 87.03). In the case of the ethano-bridged compound **5b**, both toxicity and activity were lower (MIC = 8.56 μM, IC_{50} = 22.95 μM, SI = 2.68). This was also the case for epoxy-bridged derivative **5c** (MIC = 7.21 μM, IC_{50} = 53.23 μM, SI = 7.38). Two ester analogues **5d** and **5e** of the bridged compounds were tested as well. Compound **5d** with the methano-bridge (MIC = 0.22 μM, IC_{50} = 8.49 μM, SI = 38.59) showed an in vitro potency against *M.tb* close to the potency of isoniazid (INH), tested as a positive control in the present model and a well-known first line drug in tuberculosis chemotherapy. Although acute toxicity could be observed at relatively low concentrations, the potency at low concentrations resulted in an acceptable SI. Ethano-bridged derivative **5e** displayed a lower activity than **5d** but with a mild toxicity for the J774 A.1 macrophages (MIC = 0.22 μM, IC_{50} = 49.76 μM, SI = 191.38). In summary, compound **5e** was the most promising compound showing the most favorable properties.

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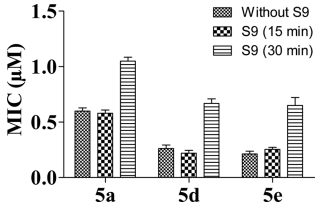
Compound **5g**, a methano-bridged analogue bearing a tert-butoxycarbonyl protecting group at the para position, only showed low activity against *M.tb*, and IC_{50} was also very low (MIC = 12.88 μM, IC_{50} = 7.33 μM, SI = 0.57).

In order to obtain information on the metabolic stability, an in vitro assay was set up in which the compounds **5a**, **5d**, and **5e** were exposed to S9 liver extract mixture. After 15 and 30 min of incubation, serial dilutions of the compounds exposed to the S9 mixture were incubated with *M.tb*. After 6 days, the antitubercular activity was investigated. As shown in Figure 1,



[†]Minimal inhibitory concentration of compounds **5a**, **5d**, and **5e** against *M.tb* measured after 6 days following 15 and 30 min exposure to S9 mixture. Presented values in the graph are the mean of eight replications with ± SD.

[1]MIC against *M.tb* of compounds **5a**, **5d**, **5e** after exposure to S9 mixture[†]

no significant reduction in potency of compounds **5a**, **5d**, and **5e** was observed after an incubation of 15 min with the S9 mixture. However, after 30 min, the MIC of compounds **5a**, **5d**, and **5e** increased significantly from 0.60 μM to 1.05 μM, from 0.21 μM to 0.66 μM, and from 0.26 to 0.65 μM, respectively. The results indicate that the compounds were subjected to enzymatic degradation by the S9 liver extract, resulting in a decreased potency. As a negative control, we confirmed that the growth of *M.tb* H37Rv was not inhibited by the presence of the S9 mixture itself.

The susceptibility of other clinically relevant mycobacterial species and an additional *M.tb* stain, *M.tb* Beijing 17919, to compounds **5a**, **5d** and **5e** is shown in Table 2. The *M.tb*

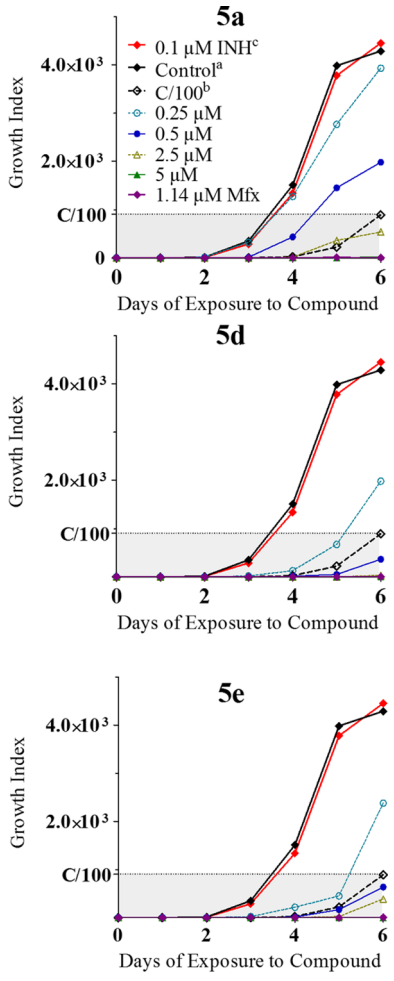
Table 2. In Vitro MIC of Compounds 5a, 5d, and 5e against Other Clinically Relevant Mycobacteria

	MIC (μM)			
	5a	**5d**	**5e**	**INH**
M. tuberculosis H37Rv^{lux}	0.56	0.11	0.22	0.13
M. tuberculosis Bejing 17919^{lux}	0.33	0.14	0.16	0.09
M.bovis AN5^{lux}	0.78	0.09	0.57	0.08
M. avium subsp. *paratuberculosis ATCC19698*^{lux}	0.69	0.44	0.21	0.99
M. avium subsp. *avium ATCC15769*^{lux}	0.8	1.22	0.37	0.76

In vitro growth inhibition was measured by luminometry after 6 days exposure to the compounds. Inhibition calculated from triplicates cultures (SD values <10%).

Beijing 17919 is a wild type strain originating from a clinical isolate. *M.tb* Beijing strains are generally considered to be more virulent than the *M.tb* H37Rv labstrain with possible implications for drug susceptibility.[29] *M. bovis*, together with *M. africanum* and *M. microti*, constitute the *M. tuberculosis* complex.[30] *M. avium* subsp. *avium* is pathogenic for birds but can cause opportunistic infections in AIDS patients,[31] whereas *M. avium* subsp. *paratuberculosis* is the etiological agent of Johne's disease or paratuberculosis (in ruminants) which may be linked to Crohn's disease in humans.[32] *M. ulcerans* causes Buruli ulcer, a necrotizing skin disease affecting mostly children in certain rural areas of West-Africa and may lead to irreversible disabilities when left untreated.[33] *M.tb* Beijing isolate 17919 showed the same susceptibility as *M.tb* H37Rv for the three tested compounds (Table 2). The same was true for the selected *M. bovis* strain. The two *M. avium* subspecies showed a somewhat lower susceptibility. Finally, the MIC of the three compounds against *M. ulcerans 1615*^{lux} was found to be significant higher than the MIC for *M. tuberculosis*, with IC_{90} values, respectively, 6-fold higher for compound **5a**, 10-fold higher for compound **5e**, and even 50-fold higher for compound **5d** (data not shown).

To study the potential of the compound class for the treatment of drug resistant tuberculosis, the susceptibility toward the compounds of a multidrug resistant *M.tb* LAM-1 strain was tested, as shown in Figure 2. The LAM-1 strain was spoligotyped, and resistance against isoniazid, rifampin, rifabutine, and prothionamide was confirmed by the National Reference Laboratory of Belgium. The critical concentration at



Growth of MDR *M.tb* was monitored in BACTEC MGIT 960. Growth was compared to an untreated bacterial suspension and an untreated bacterial suspension diluted 100 times (C/100) to study the minimal inhibitory concentration MIC. Resistance was confirmed with isoniazid (INH). Mfx moxifloxacin was used as a positive control at 1.14 μM.

[2]Susceptibility of an MDR *M.tb* LAM-1 strain to the compounds **5a**, **5d**, and **5e**.

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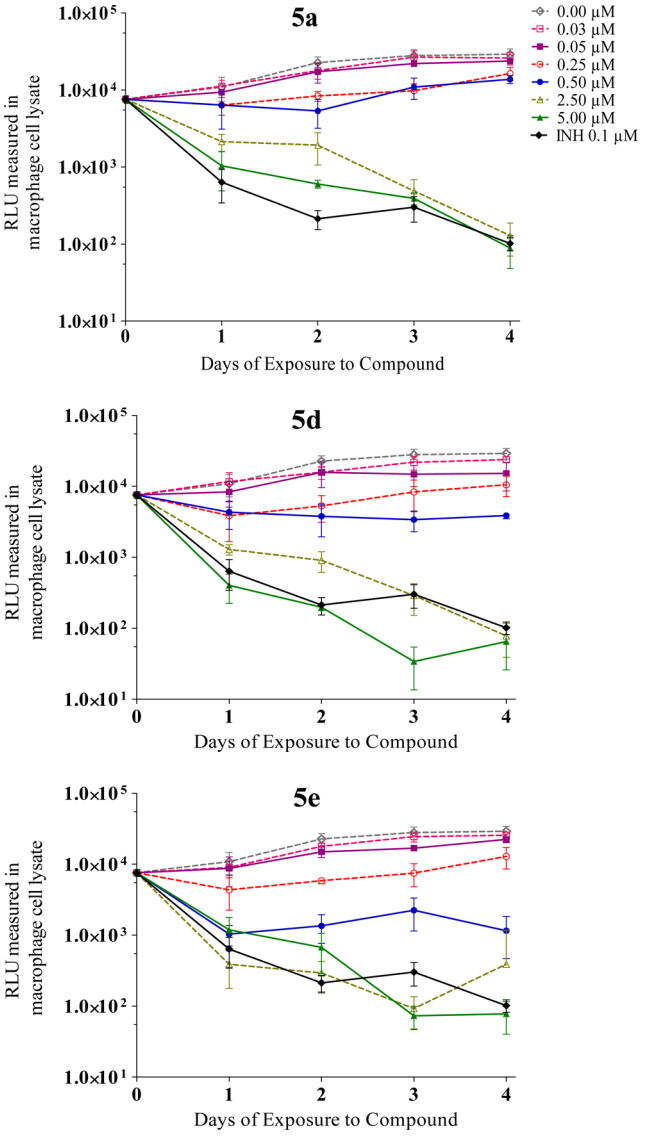
which the mycobacterial growth is reduced by 99% was determined with fluorometric BACTEC MGIT 960 Mycobacteria Growth Indicator Tubes.[34] The growth of a control culture, 100 times diluted upon inoculation (C/100), was measured simultaneously. By comparing the growth index (GI) of this culture with the cultures exposed to the compound, the critical concentration could be determined. At day 6, the C/100 had reached a GI of 890. For compound **5a**, the GI at 0.50 μM was 1982, whereas at 2.50 μM, the GI was 537, placing the critical concentration of **5a** between these two concentrations and confirming the susceptibility of the MDR strain for the derivative. Compound **5d** was more active with a GI of 1970 at 0.25 μM and a GI of 367 at 0.5 μM. The critical concentration of **5e** was slightly less active than **5d** with a GI of 2382 at 0.25 μM and a GI of 638 at 0.5 μM.

For most of its infectious cycle, *M.tb* resides within the early endosome of the macrophages where it replicates.[35] Therefore, it was important to investigate whether the selected compounds, **5a**, **5d**, and **5e** were able to reach the bacteria through the macrophage membrane. For this reason, J774 A.1 murine macrophage-like monocytes were infected with the *M.tb* H37Rv^{lux} strain. At 24 h post infection, the infected macrophage monolayers were treated with serial dilutions of the compounds. After 1 to 4 days, the monolayers were washed, and the number of luminescent bacteria was measured in the cell lysate.

As shown in Figure 3, at low concentrations of 0.03 and 0.05 μM, the selected compounds **5a**, **5d**, and **5e** had no effect. At 0.25 and 0.50 μM, the bacterial replication was reduced by, respectively, 43.83 ± 6.41% and 52.65 ± 7.37% for **5a** and by 63.88 ± 6.79 and 86.66 ± 0.79% for compound **5d**, whereas at the same concentrations, compound **5e** reduced the intracellular growth by, respectively, 55.8 ± 8.79 and 94.33 ± 8.03%. The MIC in this assay was reached at 2.48 μM for compound **5a**, and when dividing the IC_{50} by this value, a SI of 20.73 was calculated. Compound **5d** showed a similar MIC at 2.38 μM, but as a consequence of its lower IC_{50}, the calculated SI was 10.75. Finally, the potency of **5e** to inhibit intracellular *M.tb* replication was similar, with a MIC at 2.41 μM, resulting in a calculated SI of 79.31.

The possibility of genotoxicity is often overlooked in the initial phase of screening research and drug development. Nevertheless, for treatment of TB, which can take 6 months to 2 years, it is crucial that genotoxicity is ruled out. For economic reasons, genotoxic compounds are better discarded before more costly assays are envisaged in an early stage. The possibility of genotoxicity caused by the octahydrobenzophenanthridinediones **5a**, **5d**, and **5e** or their metabolites was studied in a VITOTOX assay from Gentaur, as shown in Figure 4.[36,37] Briefly, this model makes use of two recombinant *Salmonella typhimurium* reporter strains, the TA104 recN2-4 (Genox strain) and TA104 pr1 (Cytox strain). The Cytox strain expresses episomally encoded bacterial luciferase. A reduction in the signal/noise of the luminescent signal indicates cytotoxicity, whereas an increase indicates interference of the compound with the luminescent signal itself. The Genox strain carries an integrated lux operon under transcriptional control of the *recN* promoter. When a compound is genotoxic, transcription of the DNA repair mechanism will lead to an increase of the luminescent signal. Addition of rat liver S9 fraction is used to mimic the mammalian metabolic conditions so that the mutagenic potential of metabolites derived from a parent compound in the hepatic system can be assessed. The



Growth inhibition of *M.tb* H37Rv inside J774 A.1 macrophages. Results are presented as mean ± SEM RLU of triplicate cultures. Treatment of the infected cells started 24 h post infection and lasted for 3 days. The first line drug INH was used as a positive control at 0.1 μM.

[3]Inhibition of intracellular replication of *M.tb* inside macrophages by compounds **5a**, **5d**, and **5e**.

luminescence is measured for 4 h with a 5 min interval, and generally, when the signal-to-noise ratio in the Cytox strain model is reduced below 0.8, the compound is regarded as cytotoxic for *Salmonella typhimurium*, and the genotoxicity cannot be studied. On the other hand, when the signal-to-noise ratio in the Genox strain model is above 1.5, the DNA repair mechanism is activated by the cell as an early response to genotoxicity by the compound.

Serial dilutions of the compounds **5a**, **5e** from 0.25 μM to 50.00 μM were tested but for compound **5d** the serial dilution was limited form 0.25 μM to 5.00 μM to avoid exceeding the IC_{50} of the compound. The tested concentrations of the compounds did not reduce the signal-to-noise ratio of the treated Cytox cultures below 0.8. This was also the case after addition of the S9 liver extract in the TA104 pr1 strain, indicating lack of toxicity toward the *S. typhimurium* model and

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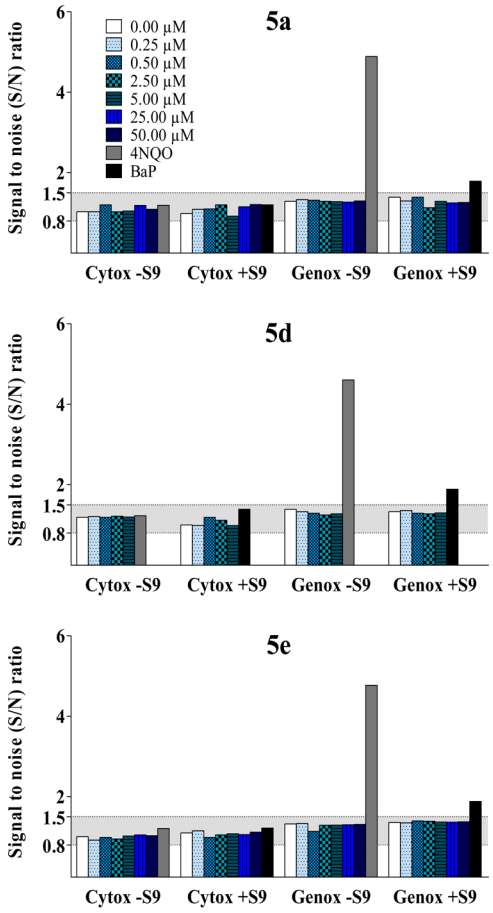
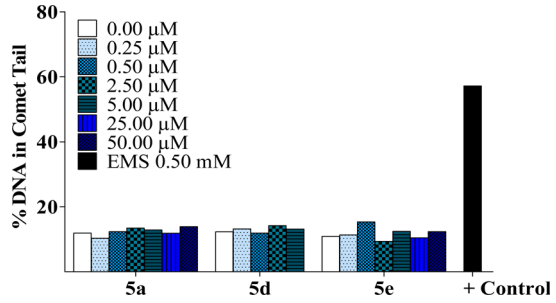


Maximum recorded S/N in a time span of 4 h by the Genox (recN 2–4) and Cytox (pr1) reporter strains. 4NQO; 4-nitroquinolone 1-oxide genotoxic positive control in samples without S9 liver fraction. Bap; benzo[*a*]pyrene, the positive control, only turns genotoxic after S9 metabolization.

[4]VITOTOX assay for the detection of early signs of genotoxicity caused by the investigated new compounds.

enabling to test for genotoxicity. The signal-to-noise ratio of the luminescence emitted by the Genox strain did not exceed 1.5 before and after addition of the S9 liver extract. At the tested concentrations, no activation of the SOS DNA repair mechanism or early signs of genotoxicity elicited by the compounds or their possible metabolites could be observed.

Intercalation or interaction of compounds with the genomic DNA can have serious mutagenic consequences for the cell, causing DNA breakage and fragmentation. The possibility of DNA breakage caused by compounds **5a**, **5d**, and **5e** was investigated in a Comet assay.[38] Briefly, JJ74 A.1 cells were exposed to serial dilutions of the octahydrobenzophenanthridinediones **5**. Possible DNA fragmentation caused by the compound was measured by submitting the cell nuclei to electrophoresis. Through pore exclusion of agarose, small fragments will migrate further (COMET TAIL) than larger fragments and unfragmented genomic DNA (COMET HEAD). The ratio DNA in TAIL to DNA in HEAD is a measure of DNA fragmentation. As shown in Figure 5, a serial dilution of compounds **5a** and **5e** was tested, ranging from 0.25 μM to 50.00 μM. Due to acute toxicity of compound **5d**, the serial dilutions of this compound for the Comet assay was limited from 0.25 μM to 5.00 μM. The test results were submitted to a Mann–Whitney U probability test, and no



Genomic DNA fragmentation after 24 h exposure to different concentrations of compounds **5a**, **5d**, and **5e**. The results show the mean % of genomic DNA detected in the comet tail of 200 stained nuclei. The Mann–Whitney U test was used to calculate statistical significance.

[5]Detection of DNA fragmentation by the Comet assay.

significant increase of genomic DNA in the TAIL was detected. It was concluded that there was no DNA fragmentation caused by compounds **5a**, **5d**, or **5e** observable with the comet assay.

CONCLUSION

The presented library of annellated 2-azaanthraquinone derivatives in this study represents a promising group of antitubercular compounds. Not only did the compounds show an increased potency compared to previous reported benzo[*j*]phenantridinediones, they also showed reduced acute toxicity. The best compounds reported in the study were **5a** (MIC = 0.59 μM, IC_{50} = 51.35 μM, SI = 87.03), **5d** ((MIC = 0.22 μM, IC_{50} = 8.49 μM, SI = 38.59), and **5e** (MIC = 0.22 μM, IC_{50} = 49.76 μM, SI = 191.38). The observed potency of these compounds against MDR *M.tb* and intracellular replicating *M.tb* encourage further study of this compound class. Combined with the lack of observable in vitro genotoxicity, this compound class would be suited for PK studies and a detailed in vivo dose–response study. To counter metabolic instability, the addition of protective groups to the scaffold is envisaged. The SI and absence of observable toxicity against the *Salmonella typhimurium* reporter strain in the VITOTOX suggest a certain degree of selectivity of the investigated compounds. The antimicrobial activity against other bacterial strains will be tested in a continuation of the project. Furthermore we will investigate the precise mechanism of action of the most potent compounds **5a**, **5d**, and **5e**.

EXPERIMENTAL SECTION

General Biology. *Materials.* 7H9 growth medium was purchased as a powder from BD Science (Franklin Lakes, NJ). DMEM, glutamax, nonessential amino acids, sodium pyruvate, gentamycin, 2-mercaptoethanol, and PBS were from GIBCO Invitrogen (Carlsbad, CA). OADC, mycobactin J, penicillin, fungizone, and hygromycin were from Roche (Basel, CH), and Triton X-100, glycerol, Tween 80, and *n*-decanal were purchased from Sigma Aldrich (St. Louis, MO)

Strains and Growth Conditions. Mycobacterium tuberculosis H37Rv (American Type Culture Collection 27294) is known to be sensitive to the five first line antituberculosis drugs (streptomycin, isoniazid, rifampin, ethambutol, and pyrazinamide). The LAM-1 strain is a clinical isolate from a patient diagnosed with TB. This strain has been spoligotyped for identification and characterized for antibiotic resistance by the national TB reference lab of the Institute of Public Health of Belgium. With the resistance to isoniazid, rifampin, rifabutin, and prothionamid, this strain was classified as a multidrug resistant (MDR) strain. Four other strains used for screening were *M. bovis* strain AN5, *M. avium* ssp. *avium* ATCC 15769, *M. avium* ssp.

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paratuberculosis ATCC19698, and *M. ulcerans* 1615. All strains were cultivated in 7H9 medium supplemented with 10% oleic acid−albumin−dextrose−catalase (OADC) and 0.2% glycerol. For *M. bovis*, the glycerol was replaced with 0.05% Tween 80. The growth medium of *M. avium* ssp. *paratuberculosis* was further supplemented with mycobactin J (2 μg/mL) required for optimal in vitro growth. Both *M. avium* strains and the *M. bovis* strain were grown at 39 °C,whereas *M. ulcerans* and *M.tb* were grown at 32 and 37 °C, respectively.

Transforming Mycobacteria. The general procedure for transformation of the different mycobacterial species is to cultivate until an O.D. between 0.8 and 1.0 was reached. The cultures were centrifuged at 6000 rpm for 15 min, and the pellet was dissolved in 20 mL of 10% glycerol. This step was repeated, but the pellet was dissolved in decreasing volumes of 10% glycerol until a volume of 5 mL. This 5 mL suspension was incubated together with the 10 ng/mL pSMT1 plasmid harboring the lux AB genes of *Vibrio harveyi* under control of a mycobacterial specific promoter for 1 h at 37 °C. Afterwards, the bacterial suspension was transferred to a cuvette and electroporated at 1000−1500 Ω and a time constant of 1.0 to 10.0 s. The cultures were left to recover for 2−3 h, after which they were plated on selective 7H11 agar plates. After 2−3 weeks of incubation, isolates were selected from the plate, grown, and tested for luminescene. After electroporation and selection, the *M.tb* Beijing strain was spoligotyped by the Belgian reference lab to confirm the strain.

Monitoring Mycobacterial Growth by Luminometry. The minimal inhibitory concentration (MIC) against mycobacteria of all synthesized compounds was evaluated by testing serial dilutions. The in vitro assay was based on a method in which luminescent mycobacteria transformed with pSMT1 luciferase reporter plasmid is used. The tested compounds were solubilized in DMSO (Sigma-Aldrich) at stock concentrations of 10 mM. Serial dilutions of each compound were made in liquid 7H9 medium [Middlebrook 7H9 broth based (Difco)] + 10% FCS (Gibco). Volumes of 20 μL of the serial dilutions were added in triplicate to 96-well, flat-bottomed micro-well plates. The bacterial suspension was made by thawing and dissolving a frozen mycobacteria pellet in 7H9−10% FCS. The dissolved pellet was passed through a 5.0 μM filter (Millipore) to eliminate clumps and left for 1 h to recover at 37 °C, 5% CO_2. Next, the bacterial suspension was diluted in 7H9−10% FCS to obtain 50 000 relative light units (RLU)/mL and a volume of 180 μL of bacteria was added to each well. A bacterial replication was analyzed by luminometry after 6 days of incubation. The bacterial suspension from each well was collected, and transferred to a black 96-well plate to evade cross luminescence between wells. The luminescent signal was evoked by addition of the substrate for the bacterial luciferase, 1% *n*-decanal in ethanol to each well by the multi plus reader from Promega and the light emission in each well was measured.

Activity Post Exposure to S9 Extract. S9 mixture from Sigma-Adrich is conditioned by addition of reagents A (NADPH) and B (G-6-P), as proposed by the manufacturer. The compounds (100 μM) were incubated with S9 mixture at 37 °C, 5% CO_2 for 15 and 30 min. In the next step, serial dilutions of both the S9 conditioned and S9 unconditioned compound solutions were made, and the MIC against *M.tb* H37Rv was investigated as outlined above.

Activity against Multidrug Resistant M.tb. Antimicrobial activity of the compounds was tested using the LAM-1 strain using the BACTEC MGIT 960 TB detection system. The compounds were solubilized in DMSO at stock concentrations of 1 mg/mL. Serial dilutions of each compound were made in 7H9 containing 10% OADC, at 83-fold the final concentrations. *M.tb* LAM-1 was precultured in a 4 mL BACTEC vial to a growth index (GI) of 300. Then, 100 μL of this preculture was inoculated into a new 4 mL BACTEC vial together with 100 μL of the serial dilutions of the compounds. As a positive control for the resistance, the MDR LAM-1 culture was inoculated with 0.1 μM isoniazid. The GI was measured each day. To determine the IC_{99}, the cultures treated with the compounds were compared with an untreated culture diluted 100 times upon inoculation.

Inhibition of Intracellular M.tb Growth. The compounds were tested on the murine J774 A.1 macrophage cell line infected with *M.tb* $H37Rv^{lux}$. The J774 macrophages were grown at 37 °C, 5% CO_2 in complete DMEM medium until a semi-confluent layer was formed. The macrophages were washed in fresh complete DMEM medium and seeded in a flat-bottomed 96-well microwell plate at a cell density of 40 000 cells per well. The cells were left to recover overnight and were washed three times in complete DMEM medium. *M.tb* $H37Rv^{lux}$ was grown at 37 °C in 7H9 containing 10% FCS and 0.2% hygromycin to an OD_{580} of 0.6−1.0. The fully grown bacterial suspension was measured and brought into complete DMEM-Pen/Fung [DMEM medium containing 0.1% penicillin and 0.8% fungizone but without gentamicin]. The synthesized compounds were solubilized in DMSO at stock concentrations of 10 mM. Serial dilutions of the peptides were made in DMEM-Pen/Fung at 2 times the concentration of each compound to be tested. A volume of 100 μL of the bacterial suspension in DMEM-Pen/Fung containing 4000 RLU of bacteria (multiplicity of infection of 0.1), and 100 μL of the serial compound dilutions were added to the macrophage cultures. To measure the effects of the compounds on intracellular growth of *M.tb*, the infected macrophages were washed three times on day 5 to remove all extracellular bacteria, incubated 1 h with 1% gentamicin to kill the residual extracellular bacteria, lysed with 200 μL of 1% Triton X-100 (Sigma), and the wells were washed four times with 200 μL of PBS. The lysate was transferred in a 2.5 mL tube together with the four PBS washings. One hundred microliters of 1% *n*-decanal in ethanol was added to the tube, and the luminescence was measured. RLU values shown were obtained from six replicate cultures. Cell viability of the macrophage culture was observed by Trypan blue with a microscope.

Assessment of Cytotoxicity. Inhibitory effects on the J774 A.1 murine macrophage cell line were determined for the derivatives by a neutral red uptake assay as described before. The J774 A.1 cells were grown in DMEM + 10% FCS until a semi-confluent layer of cells was obtained. The cells were trypsinized, washed, and 40 000 cells were seeded per well of a 96-well plate and left for recovery at 37 °C, 5% CO_2. The following days, the compounds were solubilized in DMSO to stock concentrations of 10 μM. A serial dilution of each compound was made in DMEM + 10% FCS. The J774 A.1 cells were washed and exposed to the derivatives by adding the serial dilutions of the compounds to the wells. The plates were left for incubation at 37 °C, 5% CO_2 for 24 h. After exposure, the cells were washed with 200 μL of PBS, and 200 μL of neutral red working solution (Sigma) was added per well. Subsequently, the plates were incubated for 3 h at 37 °C, 5% CO_2. The wells were washed with 200 μL of PBS and 200 μL of an ethanol/acetic acid (50%) mixture. The plates were left on the shaker until the color became homogeneous purple and the optical density was measured at 530 nm (NR max) and 620 nM (reference wavelength) with the Paradigm detection platform.

Vitotox Assay. Observations on genotoxicity were done with Vitotox (Gentaur, Kampenhout, BE), and the included protocol was followed. In brief, TA104 RecN2-4 (Genox) and TA104 (Cytox) *Salmonella typhimurium* bacteria were cultivated at 36 °C for 16 h in poor 869 medium. The bacterial culture was diluted 250 times, incubated for 1 h at 36 °C, and kept on ice. The bacterial culture was divided by 10 once more. S9 was added to the designated +S9 cultures to test the genotoxic effects of the metabolites of the compounds. The bacterial suspensions were then incubated, with shaking at 36 °C, and the luminescent signal was measured for 4 h with a 5 min interval.

Comet Assay. Possible DNA breakage effects of the derivatives on J774 A.1 cells were investigated by the alkaline comet assay. The J774 A.1 cells were grown in DMEM + 10% FCS until a semi-confluent layer of cells was obtained. The cells were trypsinized, washed, and seeded at 100 000 cells per well of a 24-well plate and left for recovery at 37 °C, 5% CO_2. The following day, the testing compounds were dissolved in DMSO as a stock concentration of 1 mM. Serial dilutions of each compound were made in DMEM + 10% FCS to obtain the final concentrations. The J774 A.1 cells were washed and exposed to the derivatives by adding 1 mL of the serial dilutions to each well. The plates were incubated at 37 °C, 5% CO_2 for 24 h. After incubation, the cells were trypsinized, washed with PBS, and 10 μL of cell suspension was dissolved in 300 μL of low-melting-point agarose. The dissolved cell suspension was then placed onto a frosted microscope slide and left on ice for 5 min. The slide was subsequently placed in a jar

containing lysing solution for 1 h. After lysis, the agarose cell suspension was subjected to electrophoresis for 20 min at 300 mA. The slides were washed with neutralization buffer for 5 min and dried in ice cold ethanol for 10 min. Staining of the DNA was done with gel red (Sigma-Aldrich). For the quantification of the DNA migration, a fluorescence microscope was used, and the percentage of DNA in the comet tail of the cells nuclei core was calculated in proportion to the total DNA present in the nuclei (comet head + tail) by appropriate imager software from Metasystems (Altlussheim, Germany).

General Chemistry. Column chromatography was carried out using a glass column with silica gel (Aldrich, particle size 0.035−0.070 mm, pore diameter ca. 6 nm). Solvent systems were determined via initial TLC analysis on silica gel (Merck, Kieselgel 60F_{254}, precoated 0.25 mm). Compounds were revealed by UV light or $KMnO_4$ oxidation. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded with a Jeol JNM-EX 300 NMR spectrometer. Peak assignments were performed with the aid of HSQC and HMBC techniques. The NMR samples were prepared with commercially available deuterated solvents with Me_4Si as an internal standard. Low-resolution mass spectra were recorded using an Agilent 1100 series VS (ESI, 4000 V) mass spectrometer via a direct inlet or via LC-MS coupling [Phenomenex luna column; 250 × 3 mm length, 5 μm particle size, 100 Å pore size with 5 mM NH_4OAc in H_2O and acetonitrile as eluents]. High-resolution mass spectra were recorded on a Finnigan MAT 95 XPAPI-GC-Trap tandem mass spectrometer or a tandem spectrometer Agilent 6220 TOF-LC/MS. Infrared spectra were recorded with a Perkin-Elmer BX FT-IR spectrometer. Melting points were recorded on a Buchi Melting point B-540 apparatus and are uncorrected. Final compounds were isolated with a purity higher than 95% (HPLC). Purity was assessed at 221, 255, and 281 nm. THF and CH_2Cl_2 were dried over CaH_2/benzophenone. All reagents were used without further purification, and all glassware was oven-dried prior to use. Stork enamines **7**, **13**, and **14** were prepared according to Stork et al.[39] and used immediately after high vacuum distillation.

*1,2,3,4-Tetrahydrobenzo[j]phenanthridine-7,12-diones **3** and **4**.* A stirred solution of 2-(1,3-dioxolan-2-yl)naphthoquinone **6a**[6] (1.5 mmol) or 2-acetylnaphthoquinone **6b** (1.5 mmol) in dry THF (10 mL) under a nitrogen atmosphere was cooled to 0 °C. Freshly distilled enamine **7** (1.65 mmol) was added dropwise in 2 mL of dry THF. Then, 7 M NH_3 in MeOH (1.4 mL, 10 equiv) was added dropwise, and the reaction was warmed to room temperature and stirred open to the air overnight. After careful evaporation of the solvents, the reaction mixture was redissolved in EtOAc (15 mL) and washed with brine (2 × 10 mL). Drying over $MgSO_4$ and evaporation of the solvent in vacuo gave a crude mixture, which was purified by means of preparative TLC (hexane/ethyl acetate) and subsequent recrystallization from EtOH to yield the desired 1,2,3,4-tetrahydrobenzo[*j*]-phenanthridine-7,12-diones **3** and **4**.

*1,2,3,4-Tetrahydrobenzo[j]phenanthridine-7,12-dione **3a**.* Yield 43%, yellow needles, mp 149.2 °C. ^{1}H NMR ($CDCl_3$): δ 1.86−2.00 (4H, m, 2xCH_2), 3.14 (2H, t, J = 6.1 Hz, CH_2), 3.42 (2H, t, J = 6.1 Hz, CH_2), 7.79−7.85 (2H, m, CH-9 and 10), 8.21−8.29 (2H, m, CH-8 and 11), 9.37 (1H, s, CH-6). ^{13}C NMR ($CDCl_3$): δ 21.98 (CH_2), 22.68 (CH_2), 28.21 (CH_2), 34.73 (CH_2), 125.13 (C_{quat}), 126.58 and 127.33 (CH-8 and 11), 132.41 (C_{quat}), 133.19 (C_{quat}), 134.28 and 134.45 (CH-9 and 10), 133.91 (C_{quat}), 135.42 (C_{quat}), 146.86 (CH-6), 166.33 (C_{quat}), 183.20 (C═O), 185.47 (C═O). IR (ATR): ν 2941 (CH), 2867 (CH), 1674 (C═O), 1660 (C═O), 1557 (CH_{Ar}), 1297, 1288, 712 cm^{-1}. MS: m/z (%) 264 ([M + H]$^+$, 100).

*2-Methyl-1,2,3,4-tetrahydrobenzo[j]phenanthridine-7,12-dione **3b**.* Yield 28%, yellow crystals, mp 125.9 °C. ^{1}H NMR ($CDCl_3$): δ 1.20 (3H, d, J = 6.6 Hz, CHC<u>H</u>$_3$), 1.50−1.64 (1H, m, CH), 1.84−1.96 (1H, m, CH), 2.00−2.09 (1H, m, CH), 2.88 (1H, dd, J = 10.7, 19.0 Hz, CH), 3.06−3.27 (2H, m, CH_2), 3.66 (1H, ddd, J = 1.7, 4.8, 19.1 Hz, CH), 7.79−7.85 (2H, m, CH-9 and 10), 8.21−8.30 (2H, m, CH-8 and 11), 9.37 (1H, s, CH-6). ^{13}C NMR ($CDCl_3$): δ 21.94 (CH_3), 28.96 (CH_2), 30.06 (CH_2), 34.39 (CH_2), 36.53 (CH), 125.13 (C_{quat}), 126.63 and 127.33 (CH-8 and 11), 132.48 (C_{quat}), 132.75 (C_{quat}), 134.31 and 134.49 (CH-9 and 10), 135.42 (C_{quat}), 146.93 (CH-3), 166.16 (C_{quat}), 183.24 (C═O), 185.59 (C═O). IR (ATR): ν 2948 (CH), 2873 (CH), 1672(C═O), 1557 (CH_{Ar}), 1288, 716 cm^{-1}. MS: m/z (%) 278 ([M + H]$^+$, 100).

*2-tert-Butyl-1,2,3,4-tetrahydrobenzo[j]phenanthridine-7,12-dione **3c**.* Yield 38%, amber crystals, mp 194.1 °C. ^{1}H NMR ($CDCl_3$): δ 1.06 (9H, s, C(CH_3)$_3$), 1.45−1.54 (2H, m, CH_2), 2.11−2.19 (1H, m, CH), 2.92−3.13 (2H, m, CH_2), 3.25 (1H, dd, J = 3.2, 19.1 Hz, CH), 3.64 (1H, dd, J = 3.2, 19.1 Hz, CH), 7.79−7.84 (2H, m, CH-9 and 10), 8.21−8.29 (2H, m, CH-8 and 11), 9.34 (1H, CH-6). ^{13}C NMR ($CDCl_3$): δ 23.35 (CH_2), 27.40 (C(<u>C</u>H_3)$_3$), 29.98 (CH_2), 32.71 (<u>C</u>(CH_3)$_3$), 35.54 (CH_2), 44.44 (CH-2), 125.18 (C_{quat}), 126.63 and 127.35 (CH-8 and 11), 132.46 (C_{quat}), 133.61 (C_{quat}), 134.28 and 134.49 (CH-9 and 10), 134.55 (C_{quat}), 146.87 (CH-6), 158.22 (C_{quat}), 166.43 (C_{quat}), 183.27 (C═O), 185.58 (C═O). IR (ATR): ν 2948 (CH), 2868 (CH), 1675 (C═O), 1660 (C═O), 1595 (CH_{Ar}), 1298, 709 cm^{-1}. MS: m/z (%) 320 ([M + H]$^+$, 100).

*3-Methyl-1,2,3,4-tetrahydrobenzo[j]phenanthridine-7,12-dione **3d**.* Yield 24%, yellow solid, mp 168 °C. ^{1}H NMR ($CDCl_3$): δ 1.15 (3H, d, J = 6.1 Hz, CHC<u>H</u>$_3$), 1.36−1.50 (1H, m, CH), 1.94−2.01 (2H, m, CH_2), 2.71 (1H, dd, J = 10.3, 18.4 Hz), 3.17−3.32 (2H, m, CH_2), 3.58−3.66 (1H, m, CH), 7.79−7.84 (2H, m, CH-9 and 10), 8.20−8.28 (2H, m, CH-8 and 11), 9.36 (1H, s, CH-6). ^{13}C NMR ($CDCl_3$): δ 21.57 (CH_3), 27.86 (CH_2), 28.18 (CH_2), 30.82 (CH_2), 43.00 (CH), 125.24 (C_{quat}), 126.66 and 127.39 (CH-8 and 11), 132.51 (C_{quat}), 132.95 (C_{quat}), 132.75 (C_{quat}), 134.34 and 134.51 (CH-9 and 10), 135.41 (C_{quat}), 147.09 (CH-6), 166.26 (C_{quat}), 183.35 (C═O), 185.62 (C═O). IR (ATR): ν 2952 (CH), 2925 (CH), 1672 (C═O), 1559 (CH_{Ar}), 1299, 1277, 710 cm^{-1}. MS: m/z (%) 278 ([M + H]$^+$, 100). HRMS (ESI): calcd for [$C_{18}H_{16}NO_2$]$^+$, 278.1181; found, 278.1181.

*4-Methyl-1,2,3,4-tetrahydrobenzo[j]phenanthridine-7,12-dione **3e**.* Yield 16%, yellow solid, mp 123 °C. ^{1}H NMR ($CDCl_3$): δ 1.44 (3H, d, J = 7.2 Hz, CHC<u>H</u>$_3$), 1.67−2.13 (4H, m, 2xCH_2), 3.19 (1H, sextet, J = 6.6 Hz, CH-4), 3.35 (1H, td, J = 6.6, 19.3 Hz, CH), 3.46 (1H, td, J = 6.6, 19.3 Hz, CH), 7.79−7.84 (2H, m, CH-9 and 10), 8.21−8.29 (2H, m, CH-8 and 11), 9.41 (1H, s, CH-6). ^{13}C NMR ($CDCl_3$): δ 19.84 (CH_2), 21.57 (CH_3), 28.70 (CH_2), 29.94 (CH_2), 37.57 (CH-4), 124.92 (C_{quat}), 126.52 and 127.32 (CH-8 and 11), 132.41 (C_{quat}), 132.95 (C_{quat}), 134.29 and 134.41 (CH-9 and 10), 134.55 (C_{quat}), 135.45 (C_{quat}), 146.86 (CH-6), 170.17 (C_{quat}), 183.26 (C═O), 185.52 (C═O). IR (ATR): ν 2944 (CH), 2874 (CH), 1672 (C═O), 1588 (CH_{Ar}), 1559 (CH_{Ar}), 1298, 1281, 720 cm^{-1}. MS: m/z (%) 278 ([M + H]$^+$, 100).

*6-Methyl-1,2,3,4-tetrahydrobenzo[j]phenanthridine-7,12-dione **4a**.* Yield 15%, orange needles, mp 180.7 °C. ^{1}H NMR ($CDCl_3$): δ 1.83−1.98 (4H, m, 2xCH_2), 3.02 (3H, s, CH_3), 3.06 (2H, t, J = 6.3 Hz, CH_2), 3.34 (2H, t, J = 6.3 Hz, CH_2), 7.74−7.83 (2H, m, CH-9 and 10), 8.14−8.22 (2H, m, CH-8 and 11). ^{13}C NMR ($CDCl_3$): δ 22.13 (CH_2), 22.99 (CH_2), 26.81 (CH_2), 28.41 (CH_2), 34.73 (CH_3), 123.97 (C_{quat}), 126.66 (CH-8 and 11), 131.51 (C_{quat}), 133.73 (C_{quat}), 133.79 (CH-9 or 10), 133.94 (C_{quat}), 134.23 (CH-9 or 10), 135.42 (C_{quat}), 158.17 (C_{quat}), 164.45 (C_{quat}), 185.03 (C═O), 186.46 (C═O). IR (ATR): ν 2942 (CH), 2868 (CH), 1670 (C═O), 1589 (CH_{Ar}), 1537 (CH_{Ar}), 1288, 722, 713 cm^{-1}. MS: m/z (%) 278 ([M + H]$^+$, 100). HRMS (ESI): calcd for [$C_{18}H_{16}NO_2$]$^+$, 278.1181; found, 278.0819.

*2,6-Dimethyl-1,2,3,4-tetrahydrobenzo[j]phenanthridine-7,12-dione **4b**.* Yield 23%, orange crystals, mp 164.2 °C. ^{1}H NMR ($CDCl_3$): δ 1.18 (3H, d, J = 6.6 Hz, CHC<u>H</u>$_3$), 1.48−1.61 (1H, m, CH), 1.79−1.91 (1H, m, CH), 1.97−2.06 (1H, m, CH), 2.84 (1H, dd, J = 11.0, 18.7 Hz, CH), 3.00 (1H, s, CH_3), 3.04−3.12 (2H, m, CH_2), 3.50 (1H, ddd, J = 1.7, 4.5, 18.7 Hz, CH), 7.72−7.81 (2H, m, CH-9 and 10), 8.12−8.20 (2H, m, CH-8 and 11). ^{13}C NMR ($CDCl_3$): δ 21.97 (CH_3), 26.81 (CH_3), 29.20 (CH), 30.16 (CH_2), 34.39 (CH_2), 36.76 (CH_2), 123.88 (C_{quat}), 126.64 (CH-8 and 11), 131.01 (C_{quat}), 133.71 (C_{quat}), 133.80 (CH-9 or 10), 133.91 (C_{quat}), 134.23 (CH-9 or 10), 137.71 (C_{quat}), 158.22 (C_{quat}), 164.23 (C_{quat}), 184.97 (C═O), 188.48 (C═O). IR (ATR): ν 2933 (CH), 2869 (CH), 1667 (C═O), 1590 (CH_{Ar}), 1289, 724 cm^{-1}. MS: m/z (%) 292 ([M + H]$^+$, 100).

*3,6-Dimethyl-1,2,3,4-tetrahydrobenzo[j]phenanthridine-7,12-dione **4d**.* Yield 36%, yellow needles, mp 168 °C. ^{1}H NMR ($CDCl_3$): δ 1.14 (3H, d, J = 6.6 Hz, CHC<u>H</u>$_3$), 1.33−1.47 (1H, m, CH), 1.95−2.07 (2H, m, CH_2), 2.65 (1H, dd, J = 10.5, 18.2 Hz), 3.02 (3H, s,

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CH_3), 3.10−3.30 (2H, m, CH_2), 3.46−3.55 (1H, m, CH), 7.73−7.82 (2H, m, CH-9 and 10), 8.12−8.24 (2H, m, CH-8 and 11). ^{13}C NMR ($CDCl_3$): δ 21.58 (CH_3), 26.79 (CH_3), 27.95 (CH_2), 28.23 (CH_2), 31.05 (CH_2), 43.02 (CH), 123.88 (C_{quat}), 126.61 and 126.64 (CH-8 and 11), 130.92 (C_{quat}), 133.64 (C_{quat}), 133.74 (CH-9 or 10), 133.83 (C_{quat}), 134.19 (CH-9 or 10), 137.48 (C_{quat}), 158.31 (C_{quat}), 164.28 (C_{quat}), 184.86 (C═O), 186.30 (C═O). IR (ATR): ν 2948 (CH), 2928 (CH), 1667 (C═O), 1591 (CH_{Ar}), 1294, 1264, 724 cm^{-1}. MS: m/z (%) 292 ([M+H]$^+$, 100). HRMS (ESI): calcd for $[C_{19}H_{18}NO_2]^+$, 292.1338; found, 292.0978.

4,6-Dimethyl-1,2,3,4-tetrahydrobenzo[j]phenanthridine-7,12-dione ***4e***. Yield 18%, orange needles, mp 161.5 °C. ^{1}H NMR ($CDCl_3$): δ 1.42 (3H, d, J = 7.2 Hz, CHC$\underline{H}_3$), 1.64−1.81 (2H, m, CH_2), 1.87−1.99 (1H, m, CH), 2.02−2.11 (1H, m, CH), 3.01 (3H, s, CH_3), 3.11 (1H, sextet, J = 6.6 Hz, CH-4), 3.25 (1H, td, J = 6.6, 19.3 Hz, CH), 3.35 (1H, td, J = 6.6, 19.3 Hz, CH), 7.71−7.80 (2H, m, CH-9 and 10), 8.11−8.19 (2H, m, CH-8 and 11), 9.41 (1H, s, CH-6). ^{13}C NMR ($CDCl_3$): δ 20.19 (CH_3), 21.66 (CH_2), 26.90 (CH_3), 28.91 (CH_2), 30.10 (CH_2), 37.49 (CH-4), 123.71 (C_{quat}), 126.58 and 126.64 (CH-8 and 11), 131.12 (C_{quat}), 132.95 (C_{quat}), 133.73 (CH-9 or 10), 134.00 (C_{quat}), 134.14 (CH-9 or 10), 137.76 (C_{quat}), 158.11 (C_{quat}), 168.25 (C_{quat}), 185.09 (C═O), 185.52 (C═O). IR (ATR): ν 2981 (CH), 2935 (CH), 1666 (C═O), 1590 (CH_{Ar}), 1280, 725 cm^{-1}. MS: m/z (%) 292 ([M+H]$^+$, 100). HRMS (ESI): calcd for $[C_{19}H_{16}NO_2]^-$, 290.1181; found, 290.1336.

Synthesis of 6-(1,3-dioxolan-2-yl)-1,2,3,4-tetrahydronaphthalene-5,8-diones ***10a*** *and* ***10b***. A solution of 5,8-dimethoxy-1,2,3,4-tetrahydronaphthalene-6-carbaldehydes **8a** or **8b** or 3-bromo-2,5-dimethoxybenzaldehyde[40] (20 mmol), ethylene glycol (80 mmol), and p-TsOH.H_2O (0.2 mmol, 38 mg) in PhMe (40 mL) was equipped with a Dean−Stark apparatus and boiled under reflux for 4 h. Next, the reaction mixture was cooled to room temperature, diluted with EtOAc (40 mL), washed once with $NaHCO_{3,aq,\ sat}$ (40 mL) and 3 times with brine (40 mL). Drying over $MgSO_4$ and evaporation of the solvent yielded pure 2-(5,8-dimethoxy-1,2,3,4-tetrahydronaphthalen-6-yl)-1,3-dioxolanes **9a** or **9b** or 2-(3-bromo-2,5-dimethoxyphenyl)-1,3-dioxolane **11**. Due to the limited stability of dioxolanes **9a**, **9b**, and **11**, no MS or HRMS could be recorded.

2-(5,8-Dimethoxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-6-yl)-1,3-dioxolane ***9a***. Yield 82%, pale white solid, mp 106.4 °C. ^{1}H NMR ($CDCl_3$): δ 1.14−1.27 (2H, m, CH_2), 1.48 (1H, d, J = 8.8 Hz, C$\underline{H}_A$H$_B$), 1.70 (1H, d, J = 8.8 Hz, CH$_A\underline{H}_B$), 1.84−1.98 (2H, m, CH_2), 3.57 (1H, br s, CH-1 or CH-4), 3.61 (1H, br s, CH-1 or CH-4), 3.82 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 4.01−4.09 (2H, m, CH_2O), 4.10−4.21 (2H, m, CH_2O), 6.05 ($CH(OCH_2)_2$), 6.84 (1H, s, CH_{Ar}). ^{13}C NMR ($CDCl_3$): δ 26.42 (CH_2), 26.90 (CH_2), 39.84 (CH), 41.19 (CH), 49.14 (CH_2), 55.92 (OCH_3), 62.15 (OCH_3), 65.37 (OCH_2), 65.40 (OCH_2), 99.88 ($\underline{C}H(OCH_2)_2$), 106.93 (CH_{Ar}), 127.45 (C_{quat}), 138.45 (C_{quat}), 140.77 (C_{quat}), 146.71 (C_{quat}), 149.22 (C_{quat}). IR (ATR): ν 2951 (CH), 2868 (CH), 1489, 1458, 1387, 1218, 1110, 1054, 1021 cm^{-1}.

2-(5,8-Dimethoxy-1,2,3,4-tetrahydro-1,4-ethanonaphthalen-6-yl)-1,3-dioxolane ***9b***. Yield 89%, pale white crystals, mp 113 °C. ^{1}H NMR ($CDCl_3$): δ 1.26−1.38 (4H, m, 2xCH_2), 1.69−1.81 (4H, m, 2xCH_2), 3.36−3.37 (1H, m, CH), 3.45−3.46 (1H, m, CH), 3.78 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 4.03−4.11 (2H, m, CH_2O), 4.13−4.21 (2H, m, CH_2O), 6.11 (1H, s, $C\underline{H}(OCH_2)_2$), 6.92 (1H, s, CH_{Ar}). ^{13}C NMR ($CDCl_3$): δ 25.61 (2xCH_2), 25.74 (2xCH_2), 26.10 (CH), 27.46 (CH), 55.88 (OCH_3), 63.28 (OCH_3), 65.42 (OCH_2CH_2O), 99.92 ($\underline{C}H(OCH_2)_2$), 105.62 (CH_{Ar}), 127.09 (C_{quat}), 134.86 (C_{quat}), 137.97 (C_{quat}), 147.88 (C_{quat}), 154.54 (C_{quat}). IR (ATR): ν 2939 (CH), 1487, 1383, 1217, 1114 cm^{-1}.

2-(3-Bromo-2,5-dimethoxyphenyl)-1,3-dioxolane ***11***. Yield 95%, colorless oil. ^{1}H NMR ($CDCl_3$): δ 3.77 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.01−4.09 (2H, m, CH_2O), 4.09−4.17 (2H, m, CH_2O, 6.06 ($CH(OCH_2)_2$), 7.03 (1H, d, J = 3.0 Hz, CH_{Ar}), 7.10 (1H, d, J = 3.0 Hz, CH_{Ar}). ^{13}C NMR ($CDCl_3$): δ 55.91 (OCH_3), 62.38 (OCH_3), 65.48 (OCH_2CH_2O), 99.44 ($\underline{C}H(OCH_2)_2$), 111.63 (CH_{Ar}), 117.62 (C_{quat}), 119.65 (CH_{Ar}), 133.36 (C_{quat}), 149.59 (C_{quat}), 156.26 (C_{quat}). IR (ATR): ν 2888 (CH), 1475 (CH_{Ar}), 1219, 1131 (C−O), 995 cm^{-1}. MS: m/z (%) 291/289 ([M + H]$^+$, 100/97).

A solution of freshly prepared LDA (2 equiv, 17.5 mmol) in dry THF (10 mL) was cooled to −78 °C, and furan (10 mL) was added dropwise. Next, 2-(3-bromo-2,5-dimethoxyphenyl)-1,3-dioxolane **11** (2.5 g, 8.65 mmol) dissolved in 2.5 mL THF was slowly added dropwise. The reaction mixture was stirred for 1 h at −78 °C and quenched with 10 mL of H_2O. The reaction mixture was diluted with 20 mL of EtOAc and washed twice with brine (10 mL). Drying over $MgSO_4$, evaporation of the solvent in vacuo, followed by recrystallization from Et_2O gave pure 2-(5,8-dimethoxy-1,4-dihydro-1,4-epoxynaphthalen-6-yl)-1,3-dioxolane **12**.

2-(5,8-Dimethoxy-1,4-dihydro-1,4-epoxynaphthalen-6-yl)-1,3-dioxolane ***12***. Yield 89%, pale white solid, mp 131 °C. ^{1}H NMR ($CDCl_3$): δ 3.81 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.98−4.07 (2H, m, CH_2O), 4.08−4.18 (2H, m, CH_2O), 5.90 (1H, d, J = 1.7 Hz, CH-1 or 8), 6.05 ($CH(OCH_2)_2$), 6.02 (1H, d, J = 1.7 Hz, CH-1 or 8), 6.83 (1H, s, CH-5), 7.03 (1H, dd, J = 1.7, 5.5 Hz, CH-9 or CH-10), 7.03 (1H, dd, J = 1.7, 5.5 Hz, CH-9 or CH-10). ^{13}C NMR ($CDCl_3$): δ 56.14 (OCH_3), 61.46 (OCH_3), 65.17 (OCH_2CH_2O), 79.76 and 81.30 (CH-1 and CH-8), 99.39 ($\underline{C}H(OCH_2)_2$), 109.54 (CH-5), 128.20 (C_{quat}), 138.03 (C_{quat}), 139.09 (C_{quat}), 142.29 and 142.97 (CH-9 and CH-10), 146.66 (C_{quat}), 148.36 (C_{quat}). IR (ATR): ν 2897 (CH), 1478, 1392, 1222, 1055 cm^{-1}. MS: m/z (%) 277 ([M + H]$^+$, 100). HRMS (ESI): calcd for $[C_{15}H_{17}O_5]^+$, 277.1076; found, 277.1072.

2-(5,8-Dimethoxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalen-6-yl)-1,3-dioxolane **9c** was prepared using the same procedure as 5,8-dimethoxy-1,2,3,4-tetrahydronaphthalene-6-carbaldehydes **8**.

2-(5,8-Dimethoxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalen-6-yl)-1,3-dioxolane ***9c***. Yield 99%, white crystals, mp 81−82.5 °C. ^{1}H NMR ($CDCl_3$): δ 1.35−1.51 (2H, m, CH_2), 1.99−2.13 (2H, m, CH_2), 3.82 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.01−4.07 (2H, m, CH_2O), 4.08−4.18 (2H, m, CH_2O), 5.56 (1H, d, J = 4.4 Hz, CH-1 or 8), 5.66 (1H, d, J = 4.4 Hz, CH-1 or 4), 6.04 ($CH(OCH_2)_2$), 6.93 (1H, s, CH-7). ^{13}C NMR ($CDCl_3$): δ 26.09 (CH_2), 26.71 (CH_2), 55.97 (OCH_3), 61.40 (OCH_3), 65.34 (OCH_2CH_2O), 76.47 and 77.72 (CH-1 and CH-8), 99.45 ($\underline{C}H(OCH_2)_2$), 108.89 (CH-5), 128.60 (C_{quat}), 136.02 (C_{quat}), 136.92 (C_{quat}), 145.16 (C_{quat}), 147.47 (C_{quat}). IR (ATR): ν 2954 (CH), 1482, 1394, 1069 cm^{-1}. MS: m/z (%) 279 ([M + H]$^+$, 100). HRMS (ESI): calcd for $[C_{15}H_{19}O_5]^+$, 279.1233; found, 279.1231.

To a stirred solution of 1,3-dioxolanes **9a**, **9b**, or **9c** (1.81 mmol) in CH_3CN (10 mL) was added a solution of CAN (5.43 mmol, 2.98 g) in H_2O (10 mL) in one portion at room temperature. After 3 min, the reaction mixture was diluted with EtOAc (20 mL) and washed twice with brine (10 mL). Drying over $MgSO_4$ and evaporation of the solvent in vacuo yielded the crude quinones **10**, which were used as such. All dioxolanylnaphthoquinones **10** were unstable and had to be used directly in the next step.

6-(1,3-Dioxolan-2-yl)-1,2,3,4-tetrahydro-1,4-methanonaphthalene-5,8-dione ***10a***. Yield 60%, orange solid, mp 68.4 °C. ^{1}H NMR ($CDCl_3$): δ 1.05−1.15 (2H, m, CH_2), 1.32−1.36 (1H, m, CH), 1.54−1.59 (1H, m, CH), 1.81−1.90 (2H, m, CH_2), 3.39 (1H, d, J = 1.4 Hz, CH-1 or CH-4), 3.42 (1H, d, J = 1.4 Hz, CH-1 or CH-4), 3.93−3.98 (4H, m, OCH_2CH_2O), 5.79 ($C\underline{H}(OCH_2)_2$), 6.60 (1H, s, CH-7). ^{13}C NMR ($CDCl_3$): δ 24.88 (CH_2), 24.93 (CH_2), 40.47 (CH), 40.53 (CH), 47.83 (CH_2), 65.31 (OCH_2), 65.45 (OCH_2), 98.00 ($\underline{C}H(OCH_2)_2$), 131.64 (CH_{Ar}), 142.26 (C_{quat}), 151.67 (C_{quat}), 151.93 (C_{quat}), 183.46 (C═O), 184.65 (C═O). IR (ATR): ν 2953 (CH), 2878 (CH), 1645 (C═O), 1337, 1088, 943 cm^{-1}.

6-(1,3-Dioxolan-2-yl)-1,2,3,4-tetrahydro-1,4-ethanonaphthalene-5,8-dione ***10b***. Yield 83%, red oil. ^{1}H NMR ($CDCl_3$): δ 1.29 (4H, br d, J = 8.0 Hz, 2xCH_2), 1.73 (4H, br d, J = 8.0 Hz, 2xCH_2), 3.33 (1H, s, CH), 3.37 (1H, m, CH), 4.05 (2H, s, CH_2O), 4.05 (2H, s, CH_2O), 5.93 (1H, s, $C\underline{H}(OCH_2)_2$), 6.80 (1H, s, CH_{Ar}). ^{13}C NMR ($CDCl_3$): δ 24.79 (4xCH_2), 26.00 (2xCH), 65.06 (OCH_2CH_2O), 97.75 ($\underline{C}H(OCH_2)_2$), 131.08 (CH_{Ar}), 141.82 (C_{quat}), 147.70 (C_{quat}), 147.90 (C_{quat}), 182.91 (C═O), 184.10 (C═O). IR (ATR): ν 2949 (CH), 2868 (CH), 1648 (C═O), 751 cm^{-1}.

6-(1,3-Dioxolan-2-yl)-1,2,3,4-tetrahydro-1,4-epoxynaphthalene-5,8-dione ***10c***. Yield 81%, red solid, mp 79 °C (decomp.). ^{1}H NMR ($CDCl_3$): δ 1.30−1.45 (2H, m, CH_2), 2.02−2.12 (2H, m, CH_2), 4.04 (4H, s, OCH_2CH_2O), 5.46 (2H, t, J = 5.0 Hz, CH-1 and CH-4), 5.87 ($C\underline{H}(OCH_2)_2$), 6.73 (1H, s, CH-5). ^{13}C NMR ($CDCl_3$): δ 23.77 (CH_2), 23.83 (CH_2), 64.93 (OCH_2), 65.11 (OCH_2), 76.27 and 76.34 (CH-1 and CH-4), 97.38 ($\underline{C}H(OCH_2)_2$), 131.30 (CH_{Ar}), 142.26 (C_{quat}), 149.78 (C_{quat}), 150.00 (C_{quat}), 181.90 (C═O), 182.92 (C═O). IR (ATR): ν 2980 (CH), 2885 (CH), 1655 (C═O), 1305, 1286, 875 cm^{-1}.

Synthesis of Octahydrobenzophenanthridinediones ***5a***−***5g***. *Method A.* A stirred solution of 2-(1,3-dioxolan-2-yl)naphthoquinones **10** (1.5 mmol) in dry THF (10 mL) under a nitrogen atmosphere was cooled to 0 °C, and $BF_3{\cdot}OEt_2$ was added dropwise (19 μL, 0.1 equiv). Next, freshly distilled enamine **7c**, **13**, or **14b** (1.65 mmol) was added dropwise in 2 mL of dry THF. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. Then, 7 M NH_3 in MeOH (1.4 mL, 10 equiv) was added dropwise, and the reaction was stirred open to the air for 15 h. After careful evaporation of the solvents, the reaction mixture was redissolved in EtOAc (15 mL) and washed with brine (2 × 10 mL). Drying over $MgSO_4$ and evaporation of the solvent in vacuo gave a crude mixture which was purified by means of preparative TLC (hexane/ethyl acetate). Recrystallization from EtOH yielded the desired 1,2,3,4-octahydrobenzo[j]-phenanthridine-7,12-diones **5**.

Method B. After the enamine addition, the reaction mixture was evaporated in vacuo and subsequently redissolved in 10 mL of HOAc. After the addition of NH_4OAc (15 mmol, 1.16 g), the reaction mixture was stirred at 60 °C for 1 h open to the air. Next, the reaction mixture was evaporated in vacuo, redissolved in 15 mL of EtOAc, and washed with both $NaHCO_{3,aq,sat}$ (2 × 10 mL) and brine (10 mL). Further purification proceeded as in method A.

2-tert-Butyl-1,2,3,4,8,9,10,11-octahydro-8,11-methanobenzo[j]-phenanthridine-7,12-dione ***5a***. Yield 48%, yellow solid, mp 140.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.02 (9H, s, $C(CH_3)_3$), 1.22−1.31 (2H, m, CH_2), 1.38−1.49 (3H, m, CH_2 and CH), 1.66−1.72 (1H, m, CH), 1.95−2.05 (2H, m, CH_2), 2.08−2.13 (1H, m, CH), 2.75 (1H, dd, J = 11.0, 18.7 Hz, CH), 2.91−3.06 (1H, m, CH), 3.15−3.22 (1H, m, CH), 3.61 (2H, s, 2xCH), 3.73 (1H, d, J = 18.7 Hz), 9.06 (1H, CH-6). ^{13}C NMR ($CDCl_3$): major isomer δ 23.28 (CH_2), 25.20 (CH_2), 25.26 (CH_2), 27.31 ($C(\underline{C}H_3)_3$), 29.04 (CH_2), 32.70 ($\underline{C}(CH_3)_3$), 35.51 (CH_2), 40.81 (CH), 41.19 (CH), 44.33 (CH_2), 47.72 (CH-2), 124.80 (C_{quat}), 133.09 (C_{quat}), 134.75 (C_{quat}), 145.33 (CH-6), 152.43 (C_{quat}), 155.33 (C_{quat}), 182.05 (C═O), 184.89 (C═O). Minor isomer δ 23.51 (CH_2), 25.26 (CH_2), 25.46 (CH_2), 27.31 ($C(\underline{C}H_3)_3$), 29.71 (CH_2), 32.64 ($\underline{C}(CH_3)_3$), 35.34 (CH_2), 40.91 (CH), 41.45 (CH), 44.33 (CH_2), 46.93 (CH-2), 124.86 (C_{quat}), 133.02 (C_{quat}), 135.32 (C_{quat}), 145.33 (CH-6), 152.51 (C_{quat}), 155.41 (C_{quat}), 182.16 (C═O), 185.07 (C═O). Major/minor 3.7/1. IR (ATR): ν 2954 (CH), 2872 (CH), 1656 (C═O), 1321 cm^{-1}. MS: m/z (%) 336 ([M + H]$^+$, 100). HRMS (ESI): calcd for $[C_{22}H_{26}NO_2]^+$, 336.1964; found, 336.1957.

2-tert-Butyl-1,2,3,4,8,9,10,11-octahydro-8,11-ethanobenzo[j]-phenanthridine-7,12-dione ***5b***. Yield 33%, yellow solid, mp 154.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.03 (9H, s, $C(CH_3)_3$), 1.23−1.34 (4H, m, 2xCH_2), 1.40−1.49 (2H, m, CH_2), 1.73−1.85 (4H, m, 2xCH_2), 2.09−2.16 (1H, m, CH), 2.86 (1H, dd, J = 11.0, 18.6 Hz, CH), 2.96−3.08 (1H, m, CH), 3.16−3.24 (1H, m, CH), 3.51 (2H, s, 2xCH), 3.60 (1H, dd, J = 18.6, 3.6 Hz), 9.14 (1H, CH-6). ^{13}C NMR ($CDCl_3$): δ 23.46 (CH_2), 25.20 (CH_2), 25.25 (CH_2), 25.40 (CH_2), 25.48 (CH_2), 26.52 (CH-8 or CH-11), 26.87 (CH-8 or CH-11), 27.39 ($C(\underline{C}H_3)_3$), 29.65 (CH_2), 32.72 ($\underline{C}(CH_3)_3$), 35.52 (CH_2), 44.47 (CH-2), 124.34 (C_{quat}), 133.10 (C_{quat}), 134.61 (C_{quat}), 145.71 (CH-6), 149.18 (C_{quat}), 151.84 (C_{quat}), 181.96 (C═O), 184.45 (C═O). IR (ATR): ν 2944 (CH), 2867 (CH), 1660 (C═O), 1618 (CH_{Ar}), 1566 (CH_{Ar}), 1297 cm^{-1}. MS: m/z (%) 350 ([M + H]$^+$, 100). HRMS (ESI): calcd for $[C_{23}H_{28}NO_2]^+$, 350.2120; found, 350.2119.

2-tert-Butyl-1,2,3,4,8,9,10,11-octahydro-8,11-epoxybenzo[j]-phenanthridine-7,12-dione ***5c***. Yield 14%, yellow solid, mp 128.5 °C. ^{1}H NMR ($CDCl_3$): major isomer δ 1.02 (9H, s, $C(CH_3)_3$), 1.37−1.51 (4H, m, 2xCH_2), 2.06−2.17 (3H, m, CH_2 and CH), 2.73 (1H, dd, J = 19.1, 10.9, CH), 3.02−3.08 (1H, m, CH), 3.16−3.19 (1H, m, CH), 3.72 (1H, dd, J = 3.9, 17.6 Hz, CH), 5.57 (2H, dd, J = 4.4, 11.0, CH-8 and CH-10), 9.08 (1H, CH-6). Minor isomer δ 1.02 (9H, s, $C(CH_3)_3$), 1.37−1.51 (4H, m, 2xCH_2), 2.06−2.17 (3H, m, CH_2 and CH), 2.87 (1H, dd, J = 19.1, 10.9, CH), 2.96−3.00 (1H, m, CH), 3.23−3.25 (1H, m, CH), 3.40 (1H, dd, J = 3.9, 17.6 Hz, CH), 5.57 (2H, dd, J = 4.4, 11.0, CH-8 and CH-10), 9.07 (1H, CH-6). Major/minor 3/1. ^{13}C NMR ($CDCl_3$): major isomer δ 23.19 (CH_2), 24.47 (CH_2), 24.61 (CH_2), 27.31 ($C(\underline{C}H_3)_3$), 29.94 (CH_2), 32.73 ($\underline{C}(CH_3)_3$), 35.54 (CH_2), 35.54 (2xCH), 44.27 (CH-2), 124.76 (C_{quat}), 133.61 (C_{quat}), 145.48 (CH-6), 150.66 (C_{quat}), 153.51 (C_{quat}), 167.19 (C_{quat}), 182.92 (C═O), 183.87 (C═O). Minor isomer δ 23.46 (CH_2), 24.55 (CH_2), 24.76 (CH_2), 27.31 ($C(\underline{C}H_3)_3$), 29.17 (CH_2), 32.65 ($\underline{C}(CH_3)_3$), 35.37 (2xCH), 42.76 (CH-2), 125.06 (C_{quat}), 134.54 (C_{quat}), 145.48 (CH-6), 158.00 (C_{quat}), 159.44 (C_{quat}), 167.07 (C_{quat}), 180.92 (C═O), 183.87 (C═O). IR (ATR): ν 2958 (CH), 2869 (CH), 1661 (C═O), 1561 (CH_{Ar}), 1320 cm^{-1}. MS: m/z (%) 338 ([M + H]$^+$, 100). HRMS (ESI): calcd for $[C_{21}H_{24}NO_3]^+$, 338.1756; found, 338.1755.

2-Ethoxycarbonyl-1,2,3,4,8,9,10,11-octahydro-8,11-methanobenzo[j]phenanthridine-7,12-dione ***5d***. Yield 18%, light brown solid, mp 61.5 °C. ^{1}H NMR ($CDCl_3$): Isomer I δ 1.10−1.14 (2H, m, CH_2), 1.18 (3H, t, J = 7.2 Hz, $C\underline{H}_3CH_2$), 1.37 (1H, d, J = 1.5 Hz, $C\underline{H}_AH_B$), 1.58−1.60 (1H, m, $CH_A\underline{H}_B$), 1.85−1.90 (2H, m, CH_2), 1.95−1.97 (1H, m, $C\underline{H}_CH_D$), 2.07−2.13 (1H, m, $CH_C\underline{H}_D$), 2.61−2.71 (1H, m, CH-2), 2.91−3.00 (2H, m, CH_2), 3.29 (1H, dd, J = 8.8, 19.1 Hz, $C\underline{H}_EH_F$), 3.47 (1H, dd, J = 5.0, 19.1 Hz, $CH_E\underline{H}_F$), 3.52 (2H, br. s, 2xCH), 4.09 (2H, q, J = 7.2 Hz, $C\underline{H}_2CH_3$), 8.93 (1H, CH-6). Isomer II δ 1.13−1.72 (2H, m, CH_2), 1.22 (3H, t, J = 7.2 Hz, $C\underline{H}_3CH_2$), 1.40 (1H, d, J = 1.5 Hz, $C\underline{H}_AH_B$), 1.60−1.63 (1H, m, $CH_A\underline{H}_B$), 1.90−1.92 (2H, m, CH_2), 1.98−2.07(1H, m, $C\underline{H}_CH_D$), 2.13−2.22 (1H, m, $CH_C\underline{H}_D$), 2.72−2.81 (1H, m, CH-2), 3.00−3.12 (2H, m, CH_2), 3.33 (1H, dd, J = 10.7, 19.1 Hz, $C\underline{H}_EH_F$), 3.52 (2H, br. s, 2xCH), 3.62 (1H, dd, J = 5.8, 19.1 Hz, $CH_E\underline{H}_F$), 4.12 (2H, q, J = 7.2 Hz, CH_2CH_3), 8.93 (1H, CH-6). Isomer I/II 1/1. ^{13}C NMR ($CDCl_3$): isomer I δ 14.26 (CH_3), 24.27 (CH_2), 25.16 (CH_2), 25.25 (CH_2), 29.27 (CH_2), 32.46 (CH_2), 38.82 (CH-2), 40.79 (CH), 41.25 (CH), 47.10 (CH_2), 124.93 (C_{quat}), 130.31 (C_{quat}), 134.98 (C_{quat}), 145.53 (CH-6), 152.49 (C_{quat}), 155.33 (C_{quat}), 164.42 (C_{quat}), 174.54 (C═O), 181.71 (C═O), 184.37 (C═O). Isomer II δ 14.26 (CH_3), 24.55 (CH_2), 25.16 (CH_2), 25.30 (CH_2), 29.92 (CH_2), 32.91 (CH_2), 39.34 (CH-2), 40.84 (CH), 41.33 (CH), 47.37 (CH_2), 124.93 (C_{quat}), 130.37 (C_{quat}), 135.20 (C_{quat}), 145.61 (CH-6), 152.55 (C_{quat}), 155.36 (C_{quat}), 164.42 (C_{quat}), 174.59 (C═O), 181.72 (C═O), 184.43 (C═O). IR (ATR): ν 2956 (CH), 2875 (CH), 1728 (C═O), 1655 (C═O), 1566 (CH_{Ar}), 1179 cm^{-1}. MS: m/z (%) 352 ([M + H]$^+$, 100). HRMS (ESI): calcd for $[C_{21}H_{22}NO_4]^+$, 352.1549; found, 352.1527.

2-Ethoxycarbonyl-1,2,3,4,8,9,10,11-octahydro-8,11-ethanobenzo[j]phenanthridine-7,12-dione ***5e***. Yield 10%, light brown solid, mp 118−120 °C. ^{1}H NMR ($CDCl_3$): δ 1.30 (3H, t, J = 7.2 Hz, $C\underline{H}_3CH_2$), 1.35 (4H, br d, J = 8.3 Hz, 2xCH_2), 1.79 (4H, m, br d, J = 8.3 Hz, 2xCH_2), 2.02−2.15 (1H, m, CH), 2.22−2.32 (1H, m, CH), 2.77−2.87 (1H, m, CH), 3.04−3.21 (2H, m, CH_2), 3.45 (1H, dd, J = 18.6, 8.1 Hz, CH), 3.51 (2H, br. S, CH_2), 3.70 (1H, dd, J = 18.6, 5.5 Hz), 4.20 (2H, q, J = 7.2 Hz, $OC\underline{H}_2CH_3$), 9.17 (1H, CH-6). ^{13}C NMR ($CDCl_3$): δ 14.35 ($\underline{C}H_3CH_2$), 24.56 (CH_2), 25.22 (CH_2), 25.25 (CH_2), 25.29 (2xCH_2), 25.34 (CH_2), 26.53 and 27.06 (CH-8 and CH-11), 29.89 (CH_2), 32.91 (CH_2), 39.29 (CH-2), 124.48 (C_{quat}), 130.37 (C_{quat}), 134.64 (C_{quat}), 146.11 (CH-6), 149.29 (C_{quat}), 151.87 (C_{quat}), 164.34 (C_{quat}), 174.69 (C═O), 181.68 (C═O), 184.02 (C═O). IR (ATR): ν 2980 (CH), 1725 (C═O), 1654 (C═O), 1301, 1240, 1180 cm^{-1}. MS: m/z (%) 366 ([M + H]$^+$, 100). HRMS (ESI): calcd for $[C_{22}H_{24}NO_4]^+$, 366.1705; found, 366.1692.

2-tert-Butoxycarbonyl-1,2,3,4,8,9,10,11-octahydro-2-aza-8,11-methanobenzo[j]phenanthridine-7,12-dione ***5g***. Yield 17%, orange solid, mp 98 °C. ^{1}H NMR ($CDCl_3$): ^{1}H NMR ($CDCl_3$): δ 1.23−1.26 (2H, m, CH_2), 1.02 (10H, s+m, $C(CH_3)_3$ and $C\underline{H}_AH_B$), 1.69−1.72 (1H, m, $CH_A\underline{H}_B$), 2.00 (2H, dd, J = 2.5, 6.9, CH_2), 3.15 (2H, t, J = 6.1 Hz,CH_2-4), 3.63 (2H, s, CH-8 and CH-11), 3.73 (1H, dt, J = 12.9, 6.1

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Hz, C$\underline{H}_A$H$_B$-3), 3.87 (1H, dt, J = 12.9, 6.1 Hz, C$\underline{H}_A$H$_B$-3), 9.15 (1H, CH-6). ^{13}C NMR ($CDCl_3$): δ 25.23 (CH_2), 25.28 (CH_2), 28.40 (C($\underline{C}H_3$)$_3$), 33.77 (CH_2-4), 40.89 (CH_2-3), 41.31 (CH-8 and CH-9), 45.11 (CH_2-1), 47.45 (CH_2-13), 80.52 ($\underline{C}(CH_3)_3$), 124.86 (C_{quat}), 124.86 (C_{quat}), 129.19 (C_{quat}), 134.11 (C_{quat}), 146.25 (CH-6), 152.98 (C_{quat}), 154.66 (C_{quat}), 155.21 (C_{quat}), 162.84 (C=O), 181.58 (2xC=O). IR (ATR): ν 2980 (CH), 1697 (C=O), 1659 (C=O), 1160, 1150, cm^{-1}. MS: m/z (%) 381 ([M + H]$^+$, 70). HRMS (ESI): calcd for $[C_{22}H_{25}N_2O_4]^+$, 381.1814; found, 381.1806. Synthesis of enamine adduct 15c.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental info and ^{1}H and ^{13}C NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AIDS,acquired immune deficiency syndrome; HIV,human immunodeficiency virus; IC_{50},neutral red uptake inhibition of 50%; MIC,concentration at which the growth is reduced by 99%; *M. tb*,*Mycobacterium tuberculosis*; *M. avium*,*Mycobacterium avium*; *M. bovis*,*Mycobacterium bovis*; *M. ulcerans*,*Mycobacterium ulcerans*; Spp,subspecies; TB,tuberculosis; WHO,World Health Organization

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