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Original article

Straightforward palladium-mediated synthesis and biological evaluation of benzo[*j*]phenanthridine-7,12-diones as anti-tuberculosis agents

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ABSTRACT

In 1991, WHO recognized the resurgence of tuberculosis as a global health problem. Although modern chemotherapy is effective against the causative pathogen *Mycobacterium tuberculosis*, the current drug regimens have failed to eradicate the disease. The success of the pathogen, partially attributed to drug resistance, necessitates the development of novel anti-tuberculosis drugs. Benzo[*j*]phenanthridine-7,12-diones, tetracyclic derivatives of the natural product benz[*g*]isoquinoline-5,10-dione, were conveniently synthesized via palladium-catalyzed intramolecular cyclization of *N*-methanesulfonyl-3-bromo-2-(arylamino)methyl-1,4-naphthoquinones. Here we report on the bioactivity of eight benzo[*j*]phenanthridine-7,12-dione derivatives as candidate drug molecules against *M. tuberculosis* and on their cytotoxicity on C3A human hepatocytes. The strongest antimicrobial activity (as detected by growth inhibition of bacteria, using luminometry and BACTEC 460-TB) and lowest cytotoxicity was found for 3-methylbenzo[*j*]phenanthridine-7,12-dione **5e**, which was also effective in targeting intracellular *M. tuberculosis* (in murine J774 macrophages) and was not genotoxic for C3A hepatocytes.

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1. Introduction

Tuberculosis (TB) is an infectious pulmonary disease, spread by aerosolized droplets generated by a person with active disease. Caused by the weakly gram positive bacterium *Mycobacterium tuberculosis* (*M.tb.*) it presents a serious challenge to modern chemotherapy [1–3]. For starters, *M.tb.* has a thick, lipid rich cell wall, highly impermeable for antimicrobial agents, secondly, *M.tb.* has developed bacillary resistance against the current drug treatments, giving rise to Multi-Drug Resistant (MDR) and even Extensively Drug Resistant (XDR) *M.tb.* Moreover, this intracellular parasite has also developed mechanisms to persist in a non-replicating, dormant stage, leading to a so-called latent infection, estimated to affect about 2 billion of people worldwide. Currently known anti-mycobacterial agents are ineffective against these metabolically inactive, dormant bacteria [4]. The treatment of MDR and XDR *M.tb.* cases necessitates very expensive and often more toxic compounds, and it is clear that the development of novel anti-tuberculosis drugs is urgently needed [5]. The negative effects of the current drug regimens are further exacerbated by the global human immunodeficiency virus (HIV) pandemic. The anti-tuberculosis therapy is prone for drug–drug interactions and side effects when combined with anti-retroviral compounds. As a result, antibiotic treatment of people co-infected with both HIV and TB is even more problematic [6]. Although a few decades ago TB was announced to be eradicated before the end of the 20th century, at present, it is still the most severe infectious disease caused by a single pathogen [7]. According to the latest WHO reports, more than 9 million people developed TB in 2008 and 1.7 million people died of the disease. Thus, TB seems far from eliminated [8,9]. Poverty, imperfect diagnostic assays, poor access to health care, limited vaccine efficacy and lack of new drugs remain obstacles [10]. The development of new anti-tuberculosis drugs is far from a trivial matter and although some promising candidates are in the pipeline, not one new TB drug has been developed during the last

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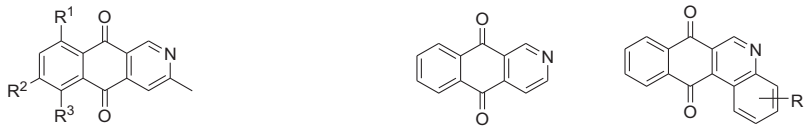
40 years [11]. Identification of new classes of possible drug candidates would therefore be extremely valuable in the battle against TB [12]. The naturally occurring 2-azaanthraquinones such as bostrycoidin **1**, 9-*O*-methylbostrycoidin **2**, tolypocladin **3** and benz[*g*]isoquinoline-5,10-dione **4** are of considerable interest in organic synthesis due to their important biological activities (Fig. 1) [13]. For instance, bostrycoidin **1** shows *in vitro* antibiotic activity against *M. tuberculosis*. 9-*O*-Methylbostrycoidin **2**, an antibiotic effective against Gram positive bacteria, has been isolated from cultures of *Fusarium moniliforme* [14]. Other members of the class of natural 2-azaanthraquinones are known as intercalating DNA binding agents or possess metal-chelating properties, e.g. tolypocladin **3** [15]. Benz[*g*]isoquinoline-5,10-dione **4**, isolated from a.o. *Psychotria camponutans* [16] and *Mitracarpus scaber Zucc.* [17] was found to be active against multi-drug resistant *Plasmodium falciparum* [16] and *Leishmania* [17]. In the course of our research on the synthesis of biologically active 2-azaanthraquinones [18], we became interested in benzo[*j*]phenanthridine-7,12-diones **5**, which are considered as tetracyclic derivatives of the natural product benz[*g*]isoquinoline-5,10-dione **4** and which are of considerable interest as potential antitumor agents. Here, we describe a new and convenient method for the synthesis of substituted benzo[*j*]phenanthridine-7,12-diones **5** and we report on the antimicrobial activity against *M.tb.* and the cytotoxicity on C3A hepatocytes of these derivatives.

In the literature, only three reports for the synthesis of benzo[*j*]phenanthridine-7,12-diones **5** have been described so far. The first method is based on the oxidative coupling of 1-(1,4-dihydroxynaphth-2-yl)ethanone with different anilines in the presence of sodium iodate [19]. However, under these reaction conditions a mixture of different coupling products was obtained, amongst which benzo[*j*]phenanthridine-7,12-diones **5**. In a second report, the phthalide annulation reaction of the carbanion derived from 3-cyano-1(3*H*)-isobenzofuranone and the corresponding heteroaryne derivative of 4-bromoquinoline to benzo[*j*]phenanthridine-7,12-dione **5a** was reported [20]. Finally, a more recent paper describes the heteroatom-directed tandem lithiation methodology for the synthesis of benzo[*j*]phenanthridine-7,12-dione **5a** [21]. Because of the fact that the above methods have disadvantages such as low yields and harsh reaction conditions, the development of a new reliable method for the synthesis of benzo[*j*]phenanthridine-7,12-diones **5** is needed. Furthermore, the synthesis of benzo[*j*]phenanthridine-7,12-diones **5** with a different substitution pattern is hampered in the above presented literature methods since different regioisomers would be formed. Therefore, it was decided to investigate a new synthesis of different benzo[*j*]phenanthridine-7,12-diones based on an intramolecular Heck reaction, which was studied earlier at our department for the synthesis of different heterocyclic quinones [22]. In this way, the synthesis started from 2-bromo-3-bromomethyl-1,4-dimethoxynaphthalene **7**, which was believed to give the key intermediates **6** after substitution of the benzylic bromide with a proper aniline, *N*-protection and subsequent oxidation of the 1,4-dimethoxynaphthalene moiety (Scheme 1).

2. Results and discussion

The total synthesis of benzo[*j*]phenanthridine-7,12-diones **5** started with 2-bromo-3-bromomethyl-1,4-dimethoxynaphthalene **7**, which was synthesized using the commercially available menadione [23]. Treatment of the functionalized naphthalene **7** with two equivalents of a suitable aniline in ethanol at room temperature for 16 h afforded 2-(arylamino)methyl-3-bromo-1,4-dimethoxynaphthalenes **8a**–**i** in 32–80% yield. Then, the amino group of 2-(arylamino)methyl-3-bromo-1,4-dimethoxynaphthalenes **8a**–**i** was protected by treatment with methanesulfonyl chloride (1.3 equiv.) in dichloromethane in the presence of pyridine at reflux for 12 h, resulting in the corresponding *N*-protected compounds **9a**–**i** in 44–91% yield. Subsequent oxidative demethylation of *N*-methanesulfonyl-2-(arylamino)methyl-3-bromo-1,4-dimethoxynaphthalenes **9a**–**i** by treatment with 2.3 equivalents of cerium(IV) ammonium nitrate in aqueous acetonitrile at room temperature for 30 min gave *N*-methanesulfonyl-2-(arylamino)methyl-3-bromo-1,4-naphthoquinones **6a**–**i** in 50–95% yield (Scheme 2 and Table 1).

Finally, the intramolecular cyclization of *N*-methanesulfonyl-2-(arylamino)methyl-3-bromo-1,4-naphthoquinones **6** using a palladium(0)-catalyzed reaction was investigated as a method to achieve the synthesis of tetracyclic benzo[*j*]phenanthridine-7,12-diones **5**. In our attempts to induce the palladium(0)-catalyzed ring closure of compounds **6a**–**i**, the aryl coupling to vinyl bromides using palladium(II) acetate in the presence of triphenylphosphine as a catalyst was applied (Scheme 2, Table 1). However, using these standard reaction conditions during the initial attempts, the formation of complex reaction mixtures was noticed, from which benzo[*j*]phenanthridine-7,12-diones **5** could not be isolated. Therefore, the Heck reaction was investigated further and it was found that degassing the solvent prior to use was important in order to remove residual air oxygen, which can reoxidize the generated Pd(0) to Pd(+II) and which can oxidize the triphenylphosphine making a Heck reaction impossible. The intramolecular cyclization could then be achieved with 20 mol% of palladium(II) acetate in the presence of 40 mol% of triphenylphosphine and 3 equivalents of potassium carbonate in boiling toluene for 1–3 h. Under the above mentioned reaction conditions, tetracyclic benzo[*j*]phenanthridine-7,12-diones **5a**–**h** were obtained in moderate to fair yield (27–62%). These moderate yields can possibly be ascribed to the presence of a naphthoquinone moiety in compounds **6**, which can reoxidize the necessary Pd(0) to Pd(+II) and thus limiting the overall efficiency of the Heck reaction. Furthermore, upon the presence of electron-donating substituents at the *ortho*-position of the arylaminogroup of the intermediates **6**, a lower isolated yield of the corresponding benzo[*j*]phenanthridine-7,12-diones, i.e. derivatives **5d** and **5g**, was witnessed. Moreover, the introduction of *N*-methanesulfonyl-3-bromo-2-(2,4-dimethoxyphenylamino)methyl-1,4-naphtho-quinone **6i**, which bears two methoxy substituents at the *ortho*- and *para*-position of the arylaminogroup, in the Heck reaction furnished a complex reaction mixture from which the pure benzo[*j*]phenanthridine-7,12-dione **5i**

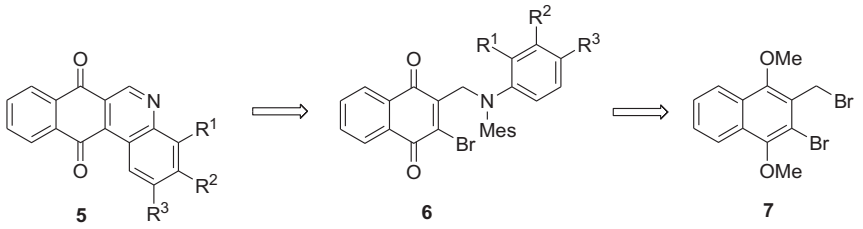


1 $R^1 = R^3 = OH$, $R^2 = OMe$ (Bostrycoidin)
2 $R^1 = R^2 = OMe$, $R^3 = OH$ (9-*O*-Methylbostrycoidin)
3 $R^1 = R^2 = R^3 = OH$ (Tolypocladin)

4 (Benz[*g*]isoquinoline-5,10-dione)

5

Fig. 1. Natural 2-azaanthraquinones, benz[*g*]isoquinoline-5,10-dione and its synthetic analogs.



Scheme 1. Retrosynthetic analysis of benzo[*j*]phenanthridine-7,12-diones **5** from 2-bromo-3-bromomethyl-1,4-dimethoxynaphthalene.

could not be obtained. These observations can most probably be ascribed to electronic as well as steric effects of the electron-donating substituents of the arylaminogroup in compounds **6**.

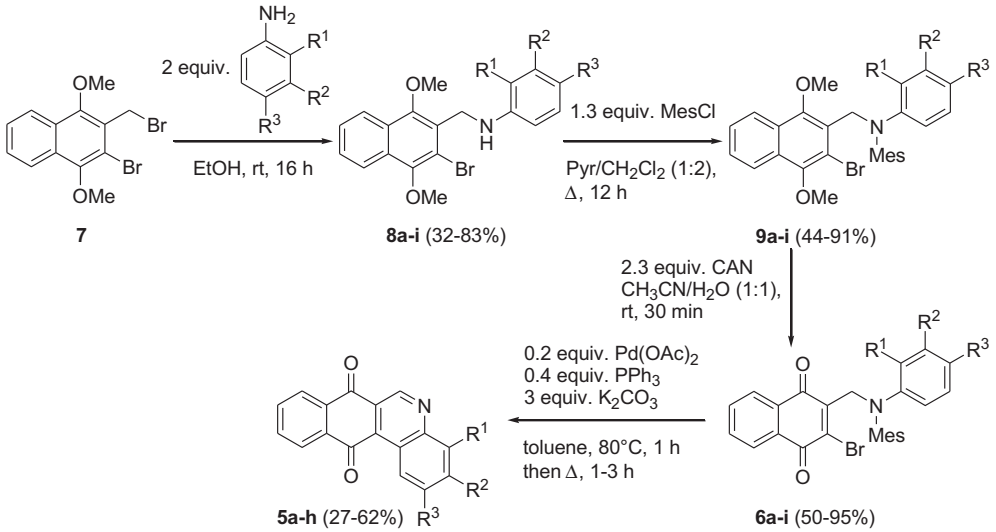
The next step was to test the benzo[*j*]phenanthridine-7,12-diones **5a**–**h** for cytotoxicity against C3A hepatocytes and antimicrobial activity against *M.tb.* Acute toxicity was analyzed using a neutral red dye uptake assay on a human cell line of C3A hepatocytes. Hepatocytes were chosen for this test, as drug toxicity can result in necrosis of liver tissue. Hepatotoxicity is a problem often encountered with the currently used second line drugs. The antituberculosis activity of the benzo[*j*]phenantridine-7,12-dione derivatives was evaluated *in vitro* by luminometry using a luminescent *M.tb.* H37Rv strain. This luminescent *M.tb.* H37Rv is transformed with pSMT1 plasmid encoding the *luxAB* genes from *V. harveyi* driven by the constitutive hsp60 promoter [24] and can be used for the rapid luminometric determination of the Minimal Inhibitory Concentration which reduces the bacterial growth by 50% (MIC50). This method allows a cheap and rapid screening of novel drug candidates and as shown below is as sensitive as the BACTEC 460-TB method, which is more cumbersome, more expensive and more hazardous for the environment, because of its use of the ^{14}C isotope [25].

The benzo[*j*]phenanthridine-7,12-diones **5a**–**h** all showed a high bioactivity against the *M.tb.* H37Rv and significantly inhibited the growth at micromolar to submicromolar levels (Table 2). The parental benzo[*j*]phenanthridine-7,12-dione **5a** showed a high potency (MIC50 = 1.20 μM), but its cytotoxicity on C3A hepatocytes (IC50 = 1.51 μM, detected in the neutral red dye exclusion assay [26]) was unacceptable when compared to its activity, resulting in a low Selectivity Index (SI) of only 1.26. On the other hand, the methylated analog **5e** with a methyl substitution at the 3rd position (R^2 = Me) showed a significant increase in potency (MIC50 = 0.21 μM) and a lower cytotoxicity (IC50 = 3.21 μM) resulting in a more than tenfold increase of SI to 15.29.

As shown in Table 2, the place and nature of the substituents profoundly affected the antimicrobial activity of the candidate drug molecules. Thus, in 3-methylbenzo[*j*]phenanthridine-7,12-dione **5e**, methylation at the R^2 *meta*-position was found to increase the antimicrobial potency and to decrease twofold the cytotoxicity (as compared to the parental compound **5a**), whereas R^1 *ortho*-methylation in derivative **5d** showed decreased antimicrobial activity albeit that cytotoxicity (IC50 = 3.96 μM) was also lower. On the other hand, methylation of the R^3 *para*-position of **5f** increased both potency (MIC50 = 0.88 μM) and cytotoxicity (IC50 = 1.48) resulting in a comparable selectivity index (SI = 1.69) as for the parental compound **5a**. The same trend was observed for benzo[*j*]phenanthridine-7,12-diones **5g** and **5h**. While R^1 substitution with OMe gave a decreased cytotoxicity (IC50 = 2.40 μM), it also decreased the potency (MIC50 = 1.66 μM) compared to **5a**. Derivative **5h** followed the same pattern as **5f** with a substitution of a OMe group at R^3 resulting in a lower MIC (MIC50 = 0.43 μM), but an increased cytotoxicity (IC50 = 0.95) affording only a slight rise in SI. Substitution with OMe seemed to be more potent than regular methylation, as indicated by results obtained for **5d** (MIC50 = 3.68; IC50 = 3.96; S = 1.08) and **5g** (MIC50 = 1.66; IC50 = 2.40; SI = 1.45) and those of **5f** (MIC50 = 0.88; IC50 = 1.48; SI = 1.69) and **5h** (MIC50 = 0.43; IC50 = 0.95; SI = 2.22), respectively.

For the various derivatives, the Polar Surface Area values (PSA) and the lipophility (MiLogP) were calculated using molinspirationplugin (Table 2).

The Minimal Inhibitory Concentration of 99% (MIC99) of *M.tb.* H37Rv was measured in a BACTEC 460-TB assay, which is considered to be the golden standard in susceptibility testing of *M.tb.* This tool was used to verify the data obtained in the luminescence assay



Scheme 2. Total synthesis of benzo[*j*]phenanthridine-7,12-diones **5**.

Table 1
Total synthesis of benzo[*j*]phenanthridine-7,12-diones **5** from 2-bromo-3-bromomethyl-1,4-dimethoxy-naphthalene.

Entry	R^1	R^2	R^3	Yield (%)			
				8	**9**	**6**	**5**
a	H	H	H	80	62	85	42
b	H	H	Cl	73	83	71	53
c	H	H	F	74	91	90	53
d	Me	H	H	34	44	50	27
e	H	Me	H	83	67	91	62
f	H	H	Me	57	76	95	59
g	OMe	H	H	44	84	87	28
h	H	H	OMe	47	84	94	39
i	OMe	H	OMe	32	82	70	/

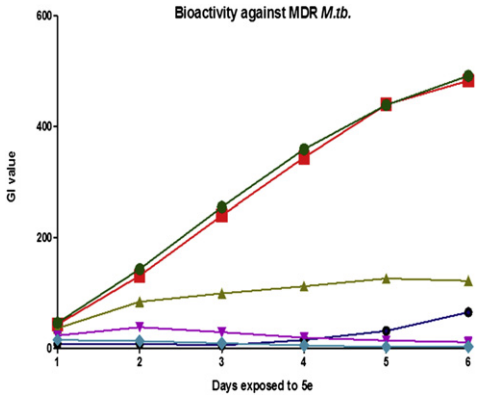


Fig. 2. 5e screened against a MDR *M. tuberculosis* LAM-1 strain. Results obtained for compound **T** represents the growth curve of the negative control and T/100 represents the growth curve of a 100 times diluted inoculum used as a reference to determine the MIC99 value = T/100 = T =(0.5 μM) =(1 μM) =(3 μM) = INH.

and served both as a control and a tool to accurately determine the MIC99. This system is a semi-automatic radiometric assay that relies on the unique ability of mycobacteria to metabolize ^{14}C-labeled palmitic acid [26]. Palmitic acid is converted to $^{14}CO_2$ which is expelled in the gaseous phase and can in turn be measured in a Beta counter. Results are expressed as Growth Index (GI) values and reduction in $^{14}CO_2$ (as compared to production by untreated control cultures) indicates growth inhibition. We used BACTEC 460-TB to determine at what concentration the derivatives were able to inhibit 99% growth of the *M.tb.* H37Rv culture by comparing the GI in cultures treated with compound dilutions, to untreated *M.tb.* H37Rv cultures which were 100 times diluted upon inoculation. All compounds, except for derivative **5h**, were tested. As shown in Table 2, results obtained with the BACTEC 460-TB were very similar to the luminometry results and showed the same trend. Thus, the most promising compound in luminometry, i.e. derivative **5e**, with the *meta* substitution, also showed the highest potency against the *M.tb.* strain H37Rv in BACTEC 460-TB with a submicromolar MIC99 concentration of 0.64 μM. From the most to the least active compounds, the trend of activity was the same in function of place and nature of substitution except for compound **5b** and **5c** in which the nature of the halogen substitution showed less importance in the BACTEC system. Again derivative **5d** with the methyl substitution in *ortho* was singled out as the least potent derivative followed by **5g** which has the same substitution place. This pointed out that substitution of this position makes the derivative less potent than the parental molecule **5a**. Substitutions in *para* **5f**, **5b** and **5c** showed increased activity as compared to **5a**, but not to the same extent as substitution in *meta*-position in the **5e** compound.

Next, compound **5e** was screened against a multi-drug resistant *M. tuberculosis* LAM-1 isolate which has been spolygotyped and shows resistance to isoniazid, rifampicin, rifabutin and prothionamid in a susceptibility assay. Susceptibility screening of the LAM-1 strain for compound **5e** was performed with the BACTEC 460-TB system.

As a negative control isoniazid was tested in parallel to ensure the bacillary resistance. The MIC99 of **5e** against this clinical isolate was determined and was found to be 0.71 μM, very close to the potency found against the H37Rv *M.tb.* laboratory strain. This finding indicates that the mechanism of action of compound **5e** differs from that of the four other drugs, suggesting that its target or the place on the target is different (Fig. 2).

New drug candidates cannot have cytotoxic and genotoxic effects at concentrations at which they have to be used to exert their antimicrobial effects. Possible mutagenicity of the various compounds was detected using the VITOTOX™ kit from Gentaur established by the Flemish institute for Technological Research. The model is regarded as rapid and highly sensitive for genotoxic screening of newly synthesized chemicals and is closely correlated to the Ames test [27]. The VITOTOX™ system makes use of two genetically engineered *Salmonella typhimurium* strains lacking the necessary oxidative enzymes. Addition of post-mitochondrial supernatant (S9), prepared from the liver of aroclor-treated rats enables the bacilli to metabolize foreign compounds to electrophilic metabolites. Genotoxicity is reported by the strain which carries a bacterial luciferase luxCDABE operon placed under transcriptional control of the RecN promoter. As shown in Table 3, none of the derivatives promoted transcription of the SOS DNA repair

Table 2
Bioactivity and drug-like characteristics of benzo[*j*]phenanthridine-7,12-diones **5a**–**h**.

Compd	R^1	R^2	R^3	MIC50 (μM)[a]	MIC99 (μM)[b]	Toxicity IC50 (μM)[c]	SI[d]	PSA (Å)[e]	MiLogP[f]
5a	H	H	H	1.20	1.90	1.51	1.26	47.03	4.10
5b	H	H	Cl	1.03	1.35	1.56	1.50	47.03	4.75
5c	H	H	F	0.74	1.38	1.09	1.46	47.03	3.27
5d	Me	H	H	3.68	4.30	3.96	1.08	47.03	4.50
5e	H	Me	H	0.21	0.64	3.21	15.29	47.03	4.52
5f	H	H	Me	0.88	1.53	1.48	1.69	47.03	4.52
5g	OMe	H	H	1.66	2.74	2.4	1.45	56.27	4.11
5h	H	H	OMe	0.43		0.95	2.22	56.27	4.13
INH				0.54		ND	ND	ND	ND
SDS				ND		0.222 nM	ND	ND	ND

[a] MIC50 minimal concentration that inhibits the growth by 50% of *M.tb.* H37Rv determined by luminescent assay.
[b] MIC99 minimal concentration that inhibits the growth by 99% of *M.tb.* H37Rv determined by BACTEC 460-TB.
[c] Concentration showing 50% neutral red uptake of C3A hepatocytes.
[d] SI, selectivity index: ratio of cytotoxicity to *in vitro* activity against H37Rv (IC50/*M.tb.* MIC50).
[e] Polar surface area (PSA) calculated using www.molinspiration.com.
[f] MiLogP calculated using www.molinspiration.com.

Table 3
VITOTOX™ results benzo[*j*]phenanthridine-7,12-diones **5a**–**h**.

Compd	Lowest concentration showing Genotoxicity[a]		Lowest concentration showing Cytotoxicity[a]	
	−S9[b]	+S9[c]	−S9[b]	+S9[c]
5a	–	–	–	1
5b	–	–	–	1
5c	–	–	–	1
5d	–	–	–	10
5e	–	–	–	10
5f	–	–	–	10
5g	–	–	–	10
5h	–	–	–	10

[a] Concentrations tested were 10 μM, 1 μM and 0.1 μM.
[b] Effects observed in absence of post-mitochondrial supernatant (S9).
[c] Effects observed after addition of post-mitochondrial supernatant (S9).

genes in the presence or absence of S9 in the *S. typhimurium* strain. It was interesting to observe that addition of the solution of compounds **5** in the absence of S9 did not cause mutagenic effects and only upon addition of S9, cytotoxicity of the compounds was observed in case of the *S. typhimurium* carrying an episomal lux operon. Concentrations at which toxicity occurs, shown in Table 3 are similar to those found in the neutral red uptake assay. It seems that only when the compounds are processed by the cell to reactive electrophilic metabolites they become active and enable to react with various targets.

Genomic DNA damage such as DNA breakage or fragmentation caused by the benzo[*j*]phenanthridine-7,12-diones could be ruled out by the Comet assay [28]. All compounds were tested at 3 μM, 1 μM, 0.3 μM and 0.1 μM, but none of the concentrations caused increased DNA fragmentation. The results obtained for compound **5e** are shown in Table 4. The percentage of DNA observed in the tail did not exceed 4% and showed no significant increase (Mann–Whitney *U* test, $P > 0.05$) as compared to the negative control. Significantly lower DNA damage than in the control sample was observed at 1 μM concentration, possibly as a result of DNA stabilization by intercalation of **5e** into the genome. This stabilization of the compounds would decrease the fragmentation and DNA migration under electrophoresis could be slowed down. Further elucidation of this effect is needed to confirm this hypothesis.

As *M.tb.* is an intracellular pathogen, we also examined whether the most promising derivative **5e** was able to reach and kill the bacilli residing inside J774 macrophages [29]. In this assay we infected a murine J774.A1 macrophage cell line with luminescent *M.tb.* H37Rv at a multiplicity of infection (MOI) of 0.1. After infection, the infected adherent cell layer was treated with a series of dilutions (10 μM, 3 μM, 1 μM, 0.3 μM and 0.1 μM) of the **5e** derivative. Streptomycin was used as positive control. After one, three

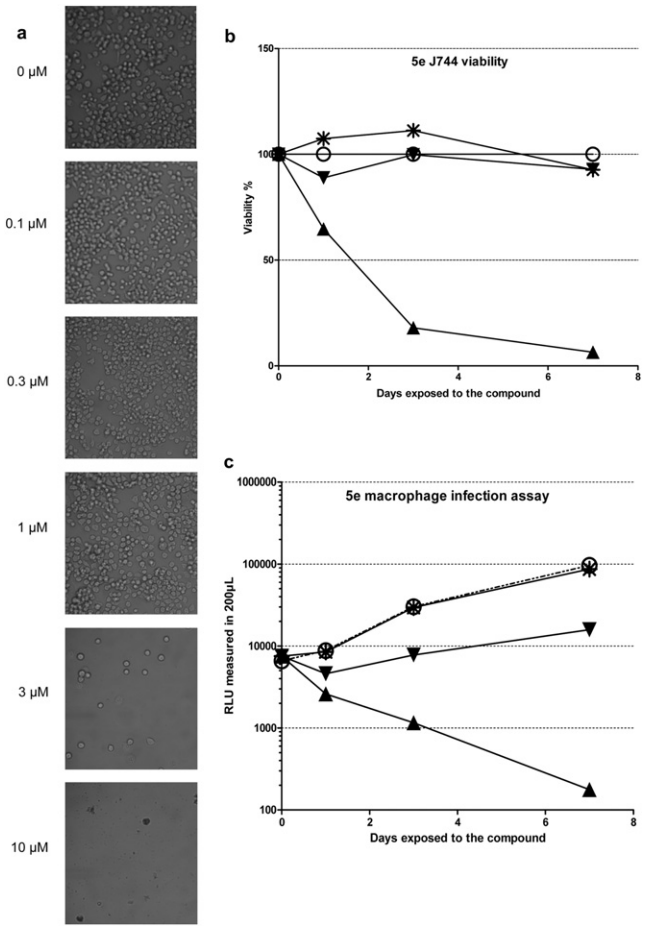
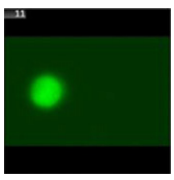
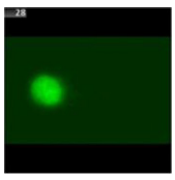
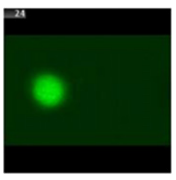
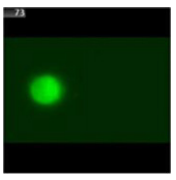
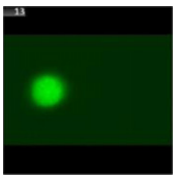
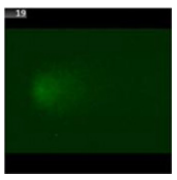


Fig. 3. Macrophage infection assay and neutral red uptake assay for compound **5e**. (a) J774 macrophage cell cultures after 7 days of exposure to the different concentrations of **5e**. (b) Neutral red uptake assay to test macrophage viability with -○- 0 μM, -▫- = 0.3 μM, -▼- 1 μM, -▲-3 μM. (c) Macrophage infection assay expressed in relative light units measured in the lysate of infected macrophages cultures at different concentrations of **5e**. The values obtained for 10 μM were regarded as unreliable due the high cytotoxicity and are not shown.

and seven days, the cell monolayer was lysed and the number of intracellular bacteria was determined by luminometry of the lysate. A neutral red dye uptake (RNU) assay was performed in parallel, to ascertain that the reduction in luminescent signal was related to the specific antimicrobial activity of **5e** against *M.tb.* and not to cytotoxicity. Uninfected J774 macrophages were treated with the compound and their viability was measured by the NRU assay at

Table 4
Comet assay using 3-methylbenzo[*j*]phenanthridine-7,12-dione **5e**.

	0 μM	0.1 μM	0.3 μM	1 μM	3 μM	EMS 0.7 mM
						
% DNA	3.69	3.47	2.52	2.15	3.31	62.34
# cells	104	73	31	147	62	49
SD	6.7	5.44	4.12	3.8	4.67	11.86
Median	1.12	0.23	0	0	0.98	65.83
P-Level		0.434663	0.16709	0.00509	0.744523	

Nucleus from lysed C3A cells, pre-incubated for 24 h in compound **5e**, immobilized and stained with gel red.

the same time points used for testing effects on bacterial replication. Compound **5e** could successfully inhibit intracellular mycobacterial growth (Fig. 3). At a concentration of 3 μM, the viability of the macrophages dropped to 6.5% on day 7, so the luminometric results obtained for 10 μM and 3 μM were regarded as unreliable. At 1 μM however, 92.9% of the macrophages were viable and at this concentration a 83.5% reduction in intracellular mycobacterial growth was detected. At the same concentration of 1 μM, the inhibition after 1 day and 3 days of exposure was respectively 47.6% and 74.2%. At a concentration of 0.3 μM of compound **5e** no intracellular growth inhibition could be measured.

Because the NRU assay relies on the uptake and exclusion of a neutral red dye, the NRU assay in parallel indicated not only that the J774 macrophages where viable but more importantly, that the cell membrane was fully intact and functional. By showing that the **5e** derivate could lower the bacterial burden inside the cells, it was shown that this compound can cross the natural barrier made up by the cell membrane while leaving it intact and reach the bacteria residing in the cytosol or vacuole of the cell. Because the reported toxicity of the compound class it is a favorable that the compound has a fast antimicrobial effect within the cells. This was shown for analog **5e** and activity was already noticeable after 24 h of treatment of the infected macrophages.

3. Conclusion

In conclusion, an efficient intramolecular palladium(0)-catalyzed cyclization of tetracyclic benzo[*j*]phenanthridine-7,12-diones **5a**–**h** from *N*-methanesulfonyl-2-(arylamino)methyl-3-bromo-1,4-naphthoquinones **6a**–**h** was achieved. All derivatives showed antimicrobial activities against *M.tb.* However, due to their high cytotoxicity for C3A hepatocytes, the resulting selectivity index of the tested derivatives was unacceptably low for most of them. Nevertheless, 3-methylbenzo[*j*]phenanthridine-7,12-dione **5e** showed a SI of 15.3 and a MIC50 value lower than the first line drug isoniazid. Although the **5e** SI is still too low for immediate application, our results showing the importance of the place and nature of substitution encourages the further exploration and synthesis of new derivatives with other side chains at the same position as **5e**. Although the calculated MiLogP value is 4.52 for the **5e** component, it may be possible to increase the efficiency of this compound by further increasing its lipophility. Because *M.tb.* is localized initially inside infected alveolar macrophages, it was important that these compounds would also be able to affect the intracellular growth of the bacilli, and this was confirmed for compound **5e**, which demonstrated a fast bacteriostatic effect against *M.tb.* growing in macrophages at a 1 μM concentration. Benzo[*j*]phenanthridine-7,12-diones are a new family of anti-tuberculosis candidates and their mechanism of action is novel and unknown. We showed the antimicrobial effect against a MDR clinical isolate of *M.tb.* LAM-1. The MIC99 against this MDR isolate was similar to the MIC99 against the susceptible *M. tuberculosis* H37Rv laboratory strain, confirming the hypothesis of a novel mechanism of action. Besides more rapid diagnosis and more effective vaccines, new drugs for anti-tuberculous therapy are urgently needed to control tuberculosis. Testing the activity of our molecules against dormant *M.tb.* as well as testing their potency against other mycobacterial species are the next steps taken by our research teams. Creation of bifunctional molecules, e.g., by fixing an isoniazid moiety as a sidechain on benzo[*j*]phenanthridine-7,12-diones might also increase their efficiency. Further optimization studies and development of new derivatives of benzo[*j*]phenanthridine-7,12-diones as well as investigating the mode of action are both ongoing projects.

4. Experimental section

4.1. General experimental methods

4.1.1. Biological data

4.1.1.1. Monitoring mycobacterial growth by luminometry. The minimal inhibitory concentration (MIC) against *M.tb.* H37Rv of all synthesized compounds was evaluated by testing serial dilutions. The *in vitro* assay was based on a method in which luminescent *M.tb.* H37Rv transformed with pSMT1 luciferase reporter plasmid is used [30]. The compounds were solubilized in DMSO (Sigma–Aldrich) at stock concentrations of 1 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 microwell plates. The bacterial suspension was made by thawing and diluting a frozen *M.tb.* H37Rv aliquot in 7H9-10% FCS. The diluted bacteria were 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 (corresponding to 5×10^4 CFU) and a volume of 180 μL of bacteria was added to each well. The final concentration of the compounds was 10 μM, 3 μM, 1 μM, 0.3 μM and 0.1 μM. Bacterial replication were analyzed by luminometry after 1, 3 and 7 days of culture. The bacterial suspension from each well was collected, and brought in a 2.5 ml Eppendorf tube. Each well was washed four times with 200 μL PBS (Difco). To measure the luminescence, 100 μL of 1% *n*-decanal in ethanol (Sigma–Aldrich) was added to the Eppendorf tube and light emission was measured over 10 s using a Turner Modulus Single Tube Luminometer from Biosystems.

4.1.1.2. Monitoring M.tb. growth by BACTEC 460-TB. Antimicrobial effects of the compounds were also monitored using BACTEC 460-TB cultures. The compounds were solubilized in DMSO (Sigma–Aldrich) at stock concentrations of 1 mM. Serial dilutions of each compound were made in 7H9 + 10% FCS, at 40 times the final concentrations. A bacterial pre-culture of *M.tb.* H37Rv was made in a 4 ml BACTEC vial containing 7H9-based medium until a Growth index (GI) of 300 was reached. When the pre-culture was fully grown, 100 μL of bacteria were inoculated into a new 4 ml BACTEC vial together with 100 μL of the serial dilutions of the compounds. Each day the GI was measured and compared to untreated inocula. Each measurement was repeated at least twice.

4.1.1.3. Inhibition of intracellular M.tb. growth. Inhibition of intracellular *M.tb.* growth was measured in a macrophage infection assay. The efficiency of the compounds was tested in the murine J774 cells infected with luminescent *M.tb.* H37Rv transformed with pSMT1 luciferase reporter plasmid. The J774 macrophages were grown at 37 °C 5% CO_2 in complete DMEM medium [DMEM (Gibco) + 10% FCS (Gibco) + 1% Non-essential amino acids (Gibco) + 1% Sodium Pyruvate (Gibco) + 1% Glutamax (Gibco) + 0.1% 2-mercaptoethanol 5×10^{-5} M (Gibco) + 1% gentamycin (Gibco)] until a semi confluent layer was formed. The macrophages were washed and seeded in a 96 well flat-bottomed microwell plate at a cell density of 100,000 cells per well. The cells were left to recover overnight and after recovery washed three times. *M.tb.* H37Rv was grown at 37 °C in 7H9 + 10% FCS + 0.2% hygromycin (Roche) to an O. D between 0.6 and 1.0. The fully grown bacterial suspension was measured and brought into complete DMEMPen/Fung [DMEM medium + 0.1% penicillin (Roche) + 0.8% Fungizone (Gibco) without gentamycin]. Compounds **5** were solubilized in DMSO at stock concentrations of 1 mM. Serial dilutions of each compound were made in DMEMPen/Fung in which 2 times

the final concentration of each compound was prepared. A volume of 100 μL of the bacterial suspension in DMEMPen/Fung containing 10,000 RLU (corresponding to 10^4 CFU) of bacteria (multiplicity of infection of 0.1.) and 100 μL of the serial compound dilutions was added to the macrophage cultures, resulting in final concentrations of 10 μM, 3 μM, 1 μM, 0.3 μM, 0.1 μM All tests were performed in triplicate.

To measure the effect of the compounds on intracellular growth of *M.tb.*, the infected macrophages were washed to eliminate extracellular bacteria and lysed on day 1, day 3 or day 7 with 200 μL 1% Triton X-100 (Sigma–Aldrich) and the wells washed four times with 200 μL PBS. The lysate was transferred in a 2.5 ml Eppendorf tube together with the 4 PBS washings of 200 μL. A volume of 100 μL of 1% *n*-decanal in ethanol was added to the Eppendorf tube and emission of luminescence was measured over a time span of 10 s with a Turner Modulus Single Tube Luminometer from Biosystems. Mean RLU values obtained from triplicate cultures are shown. Cell viability of uninfected J774 cells cultured in the presence of the various compounds was examined by neutral red uptake assay.

4.1.1.4. Assessment of cytotoxicity. The 50% inhibitory concentration (IC50) toward C3A human hepatocytes was determined for the 8 derivatives **5** by a neutral red uptake assay as described before [26]. The C3A 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 (Sigma–Aldrich) to stock concentrations of 1 mM. A serial dilution of each compound was made in DMEM + 10% FCS to achieve the final concentrations (10 μM, 3 μM, 1 μM, 0.3 μM, 0.1 μM). The C3A 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 PBS and 200 μL 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 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.

4.1.1.5. DNA breakage. Possible DNA breakage effects of the derivatives on C3A cells were investigated by the alkaline comet assay [28]. The C3A 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, compounds **5** 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 (10 μM, 3 μM, 1 μM, 0.3 μM, 0.1 μM). The C3A 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 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 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.

4.1.2. Chemical data

Spectroscopic data were recorded as follows: ^{1}H NMR spectra were recorded at 300 MHz, ^{13}C NMR spectra were recorded at 75 MHz and ^{19}F NMR spectra were recorded at 282 MHz using a Jeol NMR spectrometer. Peak assignments were performed with the aid of 2D-COSY and HSQC spectra. Melting points (mp) were determined on a Büchi B540 Melting Point Apparatus with a temperature gradient of 1 °C/min. The reported melting points are not corrected. Infrared spectra were recorded with a Perkin Elmer Spectrum BX FT-IR apparatus using the attenuated total reflection (ATR) technology. Mass spectra were recorded using a direct inlet system (70 eV) with a VL detector (ES, 4000 V). Elemental analyses were analyzed with Perkin Elmer Series II CHNS/O Analyzer 2400. All tested compounds were found to possess a purity of at least 95%. Flash chromatography was carried out using a glass column with silica gel (particle size 0.035–0.07 mm, pore diameter ca. 6 nm). Solvent systems were determined via initial TLC analysis.

4.2. Synthesis of 2-(arylamino)methyl-3-bromo-1,4-dimethoxynaphthalenes **8**

4.2.1. General procedure

To a stirred solution of an aniline (4 mmol), which was distilled previously, in 10 ml of ethanol was added dropwise a solution of 2-bromo-3-bromomethyl-1,4-dimethoxynaphthalene **7** [23] (720 mg, 2 mmol) in 20 ml of ethanol. The reaction mixture was stirred overnight at room temperature. Most of the solvent was evaporated *in vacuo* and the residue was dissolved in dichloromethane (100 ml), washed twice with water, and dried ($MgSO_4$). Flash chromatography on silica gel using dichloromethane as eluent gave 2-(arylamino) methyl-3-bromo-1,4-dimethoxynaphthalenes **8**, which were further purified by crystallization in diethyl ether through dropwise addition of petroleum ether.

*4.2.1.1. 3-Bromo-1,4-dimethoxy-2-(phenylamino)methylnaphthalene **8a**.* Yield 80%, white crystals, mp 98.2–99.5 °C. ^{1}H NMR ($CDCl_3$): δ 3.98 (3H, s, OMe), 3.99 (3H, s, OMe), 4.63 (2H, s, CH_2), 4.01 (1H, br. s, NH), 6.66–6.78 (2H, m, 2×=CH), 6.82 (1H, d, J = 8.3 Hz, C-2′ or C-6′), 7.13–7.26 (2H, m, 2×=CH), 7.54–7.60 (2H, m, H-6 and H-7), 8.08–8.14 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 43.12 (CH_2), 61.47 (OMe), 63.68 (OMe), 113.37 (2×=CH), 115.12 (=C_{quat}), 117.85 (=CH), 122.56 and 122.97 (C-5 and C-8), 126.89 and 127.24 (C-6 and C-7), 127.41 (=C_{quat}), 127.94 (=C_{quat}), 128.80 (=C_{quat}), 129.26 (2×=CH), 147.99 (C-1′), 150.36 (=C–O), 151.90 (=C–O). IR (ATR): ν_{max} 3420, 1601, 1514, 1354, 1262, 1078, 1069, 1002 cm^{-1}. MS m/z (%): 372/374 (M + H$^+$, 100). Anal. Calcd for $C_{19}H_{18}BrNO_2$: C 61.30%, H 4.87%, N 3.76%. Found: C 61.69%, H 5.012%, N 3.55%.

*4.2.1.2. 3-Bromo-2-(4-chlorophenylamino)methyl-1,4-dimethoxynaphthalene **8b**.* Yield 73%, white crystals, mp 134.8–135.3 °C. ^{1}H NMR ($CDCl_3$): δ 3.96 (3H, s, OMe), 3.97 (3H, s, OMe), 4.20 (1H, br. s, NH), 4.59 (2H, s, CH_2), 6.73 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 7.13 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 7.53–7.60 (2H, m, H-6 and H-7), 8.07–8.12 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 43.15 (CH_2), 61.48 (OMe), 63.67 (OMe), 114.43 (C-3′ and C-5′), 115.55 (=C_{quat}), 122.39 (=C_{quat}), 122.60 and 122.97 (C-5 and C-8), 126.98 and 127.33 (C-6 and C-7), 126.98 (=C_{quat}), 127.88 (=C_{quat}), 128.88 (=C_{quat}), 129.04 (C-2′ and C-6′), 146.43 (C-1′), 150.45 (=C–O), 151.88 (=C–O). IR (ATR): ν_{max} 3397, 1599, 1577, 1570, 1497, 1352, 1080, 1002 cm^{-1}. MS m/z (%): 406/408/410 (M + H$^+$, 20), 279/281 (100). Anal. Calcd for $C_{19}H_{17}BrClNO_2$: C 56.11%, H 4.21% N 3.44%. Found: C 56.03%, H 4.30% N 3.42%.

4.2.1.3. 3-Bromo-1,4-dimethoxy-2-(4-fluorophenylamino)methylnaphthalene ***8c***. Yield 74%, white crystals, mp 135.7–136.5 °C. ^{1}H NMR ($CDCl_3$): δ 3.97 (3H, s, OMe), 3.98 (3H, s, OMe), 4.07 (1H, br. s, NH), 4.58 (2H, s, CH_2), 6.72–6.76 (2H, m, H-3′ and H-5′), 6.87–6.93 (2H, m, H-2′ and H-6′), 7.53–7.59 (2H, m, H-6 and H-7), 8.08–8.11 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 43.79 (CH_2), 61.47 (OMe), 63.65 (OMe), 114.28 and 114.37 (C-3′ and C-5′), 115.49 and 115.78 (C-2′ and C-6′), 115.64 (=C_{quat}), 122.59 and 122.97 (C-5 and C-8), 126.95 and 127.27 (C-6 and C-7), 127.27 (=C_{quat}), 127.91 (=C_{quat}), 128.83 (=C_{quat}), 144.31 (C-1′), 150.41 (=C–O), 151.89 (=C–O), 156.12 (d, J = 235.4 Hz, C-4′). ^{19}F NMR ($CDCl_3$): δ −127.42. IR (ATR): ν_{max} 3391, 1509, 1354, 1213, 1081, 1004 cm^{-1}. MS *m*/*z* (%): 390/392 (M + H$^+$, 60), 279/281 (100). Anal. Calcd for $C_{19}H_{17}BrFNO_2$: C 58.48%, H 4.39%, N 3.59%. Found: C 58.54%, H 4.52%, N 3.63%.

4.2.1.4. 3-Bromo-1,4-dimethoxy-2-(2-methylphenylamino)methylnaphthalene ***8d***. Yield 34%, white crystals, mp 98.0–99.0 °C. ^{1}H NMR ($CDCl_3$): δ 2.12 (3H, s, Me), 3.98 (3H, s, OMe), 3.99 (3H, s, OMe), 4.00 (1H, br. s, NH), 4.66 (2H, s, CH_2), 6.66–6.71 (1H, m, =CH), 6.97 (1H, d, J = 8.3 Hz, =CH), 7.05 (1H, d, J = 7.7 Hz, =CH), 7.16–7.21 (1H, m, =CH), 7.52–7.58 (2H, m, H-6 and H-7), 8.09–8.13 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 17.61 (Me), 43.32 (CH_2), 61.55 (OMe), 63.80 (OMe), 110.62 (=CH), 115.94 (=C_{quat}), 117.53 (=CH), 122.67 and 123.13 (C-5 and C-8), 122.67 (=C_{quat}), 126.99 (C-6 or C-7), 127.33 (=CH and C-6 or C-7), 127.66 (=C_{quat}), 128.14 (=C_{quat}), 128.98 (=C_{quat}), 130.17 (=CH), 146.22 (C-1′), 150.54 (=C–O), 152.16 (=C–O). IR (ATR): ν_{max} 3429, 1513, 1449, 1356, 1257, 1247, 1082 cm^{-1}. MS *m*/*z* (%): 386/388 (M + H$^+$, 100), 279/281 (10). Anal. Calcd for $C_{20}H_{20}BrNO_2$: C 62.19%, H 5.22%, N 3.63%. Found: C 62.48%, H 5.36%, N 3.57%.

4.2.1.5. 3-Bromo-1,4-dimethoxy-2-(3-methylphenylamino)methylnaphthalene ***8e***. Yield 83% white crystals, mp 104.5–105.5 °C. ^{1}H NMR ($CDCl_3$): δ 2.30 (3H, s, Me), 3.97 (3H, s, OMe), 3.98 (3H, s, OMe), 4.11 (1H, br. s, NH), 4.61 (2H, s, CH_2), 6.57 (1H, d, J = 7.7 Hz, =CH), 6.64–6.65 (2H, m, 2×=CH), 7.07–7.14 (1H, m, =CH), 7.53–7.59 (2H, m, H6 and H-7), 8.10 (2H, ddd, J = 1.7, 3.0, 6.9 Hz, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 21.63 (Me), 43.24 (CH_2), 61.44 (OMe), 63.64 (OMe), 110.50 (=CH), 114.27 (=CH), 115.82 (=C_{quat}), 118.87 (=CH), 122.55 and 122.97 (C-5 and C-8), 126.87 and 127.22 (C-6 and C-7), 127.48 (=C_{quat}), 128.81 (=C_{quat}), 129.13 (=CH and =C_{quat}), 139.00 (=C_{quat}), 148.01 (C-1′), 150.34 (=C–O), 151.95 (=C–O). IR (ATR): ν_{max} 3426, 1605, 1355, 1077, 1001 cm^{-1}. MS *m*/*z* (%):386/388 (M + H$^+$, 100), 279/281 (7). Anal. Calcd for $C_{20}H_{20}BrNO_2$: C 62.19%, H 5.22%, N 3.63%. Found: C 62.48%, H 5.55%, N 3.42%.

4.2.1.6. 3-Bromo-1,4-dimethoxy-2-(4-methylphenylamino)methylnaphthalene ***8f***. Yield 57%, white crystals, mp 128.2–129.8 °C. ^{1}H NMR ($CDCl_3$): δ 2.24 (3H, s, Me), 3.97 (3H, s, OMe), 3.98 (3H, s, OMe), 4.00 (1H, br. s, NH), 4.60 (2H, s, CH_2), 6.74 (2H, d, J = 8.6 Hz, H-3′ and H-5′), 7.02 (2H, d, J = 8.6 Hz, H-2′ and H-6′), 7.52–7.58 (2H, m, H-6 and H-7), 8.07–8.12 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 20.42 (Me), 43.50 (CH_2), 61.41 (OMe), 63.63 (OMe), 113.57 (C-3′ and C-5′), 115.81 (=C_{quat}), 122.53 and 122.96 (C-5 and C-8), 126.83 and 127.16 (C-6 and C-7), 127.06 (=C_{quat}), 127.60 (=C_{quat}), 127.95 (=C_{quat}), 128.76 (=C_{quat}), 129.72 (C-2′ and C-6′), 145.74 (C-1′), 150.31 (=C–O), 151.89 (=C–O). IR (ATR): ν_{max} 3419, 1611, 1522, 1354, 1253, 1071, 995 cm^{-1}. MS *m*/*z* (%): 386/388 (M + H$^+$, 100). Anal. Calcd for $C_{20}H_{20}BrNO_2$: C 62.19%, H 5.22%, N 3.63%. Found: C 61.98%, H 5.30%, N 3.66%.

4.2.1.7. 3-Bromo-1,4-dimethoxy-2-(2-methoxyphenylamino)methylnaphthalene ***8g***. Yield 44%, white crystals, mp 128.4–129.1 °C. ^{1}H NMR ($CDCl_3$): δ 3.79 (3H, s, OMe), 3.97 (3H, s, OMe), 3.98 (3H, s, OMe), 4.63 (2H, s, CH_2), 4.66 (1H, br.s, NH), 6.65–6.71 (1H, m, =CH), 6.75–6.77 (1H, m, =CH), 6.89–6.98 (1H, m, =CH), 7.51–7.56 (2H, m, H-6 and H-7), 8.07–8.12 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 42.96 (CH_2), 55.65 (OMe), 61.52 (OMe), 63.80 (OMe), 109.76 (=CH), 110.60 (=CH), 116.18 (=C_{quat}), 116.93 (=CH), 121.48 (=CH), 122.64 and 123.12 (C-5 and C-8), 126.89 and 127.25 (C-6 and C-7), 127.79 (=C_{quat}), 128.11 (=C_{quat}), 128.95 (=C_{quat}), 138.21 (=C_{quat}), 147.30 (C-1′), 150.46 (=C–O), 152.11 (=C–O). IR (ATR): ν_{max} 3416, 1600, 1510, 1356, 1220, 1083, 1024, 1000 cm^{-1}. MS *m*/*z* (%): 402/404 (M + H$^+$, 100). Anal. Calcd for $C_{20}H_{20}BrNO_3$: C 59.71%, H 5.01%, N 3.48%. Found: C 60.00%, H 5.27%, N 3.44%.

4.2.1.8. 3-Bromo-1,4-dimethoxy-2-(4-methoxyphenylamino)methylnaphthalene ***8h***. Yield 47%, white crystals, mp 139.5–140.1 °C. ^{1}H NMR ($CDCl_3$): δ 3.73 (3H, s, OMe), 3.91 (1H, br. s, NH), 3.97 (6H, s, 2 × OMe), 4.57 (2H, s, CH_2), 6.78–6.81 (4H, m, 4×=CH), 7.52–7.57 (2H, m, H-6 and H-7), 8.07–8.12 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 44.32 (CH_2), 55.88 (OMe), 61.55 (OMe), 63.74 (OMe), 114.96 (4×=CH), 115.91 (=C_{quat}), 122.66 and 123.07 (C-5 and C-8), 126.96 and 127.28 (C-6 and C-7), 127.77 (=C_{quat}), 128.06 (=C_{quat}), 128.88 (=C_{quat}), 142.37 (C-1′), 150.44 (=C_{quat}), 151.99 (=C_{quat}), 152.61 (=C_{quat}). IR (ATR): ν_{max} 3397, 1513, 1352, 1233, 1080, 1036, 1004 cm^{-1}. MS *m*/*z* (%): 402/404 (M + H$^+$, 100). Anal. Calcd for $C_{20}H_{20}BrNO_3$: C 59.71%, H 5.01%, N 3.48%. Found: C 60.09%, H 5.35%, N 3.38%.

4.2.1.9. 3-Bromo-1,4-dimethoxy-2-(2,4-dimethoxyphenylamino) methylnaphthalene ***8i***. Yield 32%, red crystals, mp 123.0–123.6 °C. ^{1}H NMR ($CDCl_3$): δ 3.75 (3H, s, OMe), 3.77 (3H, s, OMe), 3.97 (3H, s, OMe), 3.98 (3H, s, OMe), 4.58 (2H, s, CH_2), 6.45 (1H, s, H-3′), 6.44–6.49 (1H, m, H-5′), 6.88 (1H, d, J = 8.3 Hz, H-6′), 7.51–7.57 (2H, m, H-6 and H-7), 8.07–8.12 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 43.77 (CH_2), 55.66 (OMe), 55.89 (OMe), 61.49 (OMe), 63.77 (OMe), 99.45 (C-3′), 104.03 (C-5′), 111.14 (C-6′), 116.18 (=C_{quat}), 122.63 and 123.09 (C-5 and C-8), 126.84 and 127.19 (C-6 and C-7), 128.00 (=C_{quat}), 128.09 (=C_{quat}), 128.89 (=C_{quat}), 132.57 (=C_{quat}), 148.49 (=C_{quat}), 150.40 (=C_{quat}), 152.05 (=C_{quat}), 152.23 (=C_{quat}). IR (ATR): ν_{max} 3412, 1514, 1358, 1201, 1084, 1031, 999 cm^{-1}. MS *m*/*z* (%): 432/444 (M + H$^+$, 100). Anal. Calcd for $C_{21}H_{22}BrNO_4$: C 58.34%, H 5.13%, N 3.24%. Found: C 58.19%, H 5.32%, N 3.18%.

4.3. Synthesis of N-methanesulfonyl-2-(arylamino)methyl-3-bromo-1,4-dimethoxynaphthalenes ***9***

4.3.1. General procedure

To a stirred solution of a 2-(arylamino)methyl-3-bromo-1,4-dimethoxynaphthalene **8** (1 mmol) in 10 ml of pyridine was added dropwise a solution of methanesulfonyl chloride (1.3 mmol) in 10 ml of dichloromethane. The reaction mixture was boiled under reflux for 12 h and then poured in water. The reaction mixture was extracted with dichloromethane and the combined organic extracts were washed twice with water and dried ($MgSO_4$). Flash chromatography on silica gel using dichloromethane as eluent gave *N*-methanesulfonyl-2-(arylamino)methyl-3-bromo-1,4-dimethoxynaphthalenes **9**, which were further purified by recrystallization in from methanol. For some derivatives, the *N*-methanesulfonyl-2-(arylamino)methyl-3-bromo-1,4-dimethoxynaphthalenes **9** could be obtained pure directly from the reaction crude by recrystallization from methanol.

4.3.1.1. N-methanesulfonyl-3-bromo-1,4-dimethoxy-2-(phenylamino) methylnaphthalene ***9a***. Yield 62%, light-brown crystals, mp 156.0–157.4 °C. ^{1}H NMR ($CDCl_3$): δ 3.06 (3H, s, $MeSO_2$), 3.85 (3H, s, OMe), 3.95 (3H, s, OMe), 5.25 (2H, s, CH_2), 7.15–7.17 (5H, m, 5×=CH), 7.51–7.57 (2H, m, H-6 and H-7), 7.98–8.07 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 38.31 ($MeSO_2$), 48.66 (CH_2), 61.30 (OMe),

63.16 (OMe), 116.19 (=C_{quat}), 122.63 and 122.95 (C-5 and C-8), 124.33 (=C_{quat}), 126.80 and 127.49 (C-6 and C-7), 128.35 (=CH), 128.85 (2×=CH), 129.05 (=C_{quat}), 129.98 (2×=CH and =C_{quat}), 137.76 (C-1′), 150.16 (=C–O), 152.88 (=C–O). IR (ATR): ν_{max} 1360, 1347, 1330, 11316, 1302, 1148, 1086, 1076, 1002 cm^{-1}. MS *m*/*z* (%): 472/474 (M + Na, 2), 279/281 (M-[$N(SO_2Me)C_6H_5$], 100). Anal. Calcd for $C_{20}H_{20}BrNO_4S$: C 53.34%, H 4.48%, N 3.11%. Found: C 53.60%, H 4.67%, N 3.02%.

4.3.1.2. N-methanesulfonyl-3-bromo-2-(4-chlororophenylamino) methyl-1,4-dimethoxynaphthalene ***9b***. Yield 83%, yellow crystals, mp 176.6–178.4 °C. ^{1}H NMR ($CDCl_3$): δ 3.04 (3H, s, $MeSO_2$), 3.87 (3H, s, OMe), 3.95 (3H, s, OMe), 5.22 (2H, s, CH_2), 7.12 (4H, br. s, 4×=CH), 7.55 (2H, ddd, *J* = 3.7, 6.6, 6.6 Hz, H-6 and H-7), 7.98–8.08 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 38.27 ($MeSO_2$), 48.54 (CH_2), 61.33 (OMe), 63.21 (OMe), 115.82 (=C_{quat}), 122.69 and 122.93 (C-5 and C-8), 123.85 (=C_{quat}), 126.97 and 127.67 (C-6 and C-7), 127.49 (=C_{quat}), 129.05 (2×=CH), 129.15 (=C_{quat}), 131.11 (2×=CH), 134.22 (=C_{quat}), 136.34 (=C_{quat}), 150.34 (=C–O), 152.85 (=C–O). IR (ATR): ν_{max} 1359, 1337, 1159, 1150, 1080 cm^{-1}. MS *m*/*z* (%): 506/508 (M + Na, 9), 279/281 (M-[$N(SO_2Me)C_6H_4Cl$], 100). Anal. Calcd for $C_{20}H_{19}BrClNO_4S$: C 49.55%, H 3.95%, N 2.89%. Found: C 49.86%, H 4.23%, N 2.75%.

4.3.1.3. N-methanesulfonyl-3-bromo-1,4-dimethoxy-2-(4-fluorophenylamino)methylnaphthalene ***9c***. Yield 91%, white crystals, mp 193.0–193.4 °C. ^{1}H NMR ($CDCl_3$): δ 3.07 (3H, s, $MeSO_2$), 3.86 (3H, s, OMe), 3.95 (3H, s, OMe), 5.22 (2H, s, CH_2), 6.79–6.86 (2H, m, 2×=CH), 7.09–7.16 (2H, m, 2×=CH), 7.52–7.58 (2H, m, H-6 and H-7), 7.97–8.08 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 38.31 ($MeSO_2$), 48.69 (CH_2), 61.32 (OMe), 63.15 (OMe), 115.61 (=CH), 115.92 (=CH), 122.68 and 122.92 (C-5 and C-8), 124.03 (=C_{quat}), 126.94 and 127.64 (C-6 and C-7), 127.50 (=C_{quat}), 129.12 (=C_{quat}), 131.75 (=CH), 131.86 (=CH), 133.63 (=C_{quat}), 150.28 (=C–O), 152.86 (=C–O), 162.17 (d, *J* = 248.1 Hz, C-4′). ^{19}F NMR ($CDCl_3$): δ −112.42. IR (ATR): ν_{max} 1506, 1361, 1341, 1322, 1208, 1159, 1149, 1082, 1050 cm^{-1}. MS *m*/*z* (%): 490/492 (M + Na, 13), 279/281 (M-[$N(SO_2Me)C_6H_4F$], 100). Anal. Calcd for $C_{20}H_{19}BrFNO_4S$: C 51.29%, H 4.09%, N 2.99%. Found: C 50.99%, H 4.41%, N 2.90%.

4.3.1.4. N-methanesulfonyl-3-bromo-1,4-dimethoxy-2-(2-methylphenylamino)methylnaphthalene ***9d***. Yield 44%, light-yellow crystals, mp 147.0–148.4 °C. ^{1}H NMR ($CDCl_3$): δ 2.37 (3H, s, Me), 3.14 (3H, s, $MeSO_2$), 3.80 (3H, s, OMe), 3.97 (3H, s, OMe), 5.04 (1H, d, *J* = 13.8 Hz, $C\underline{H}_aH_bN$), 5.42 (1H, d, *J* = 13.8 Hz, $CH_a\underline{H}_bN$), 6.64 (1H, br. d, *J* = 7.7 Hz, H-3′ or H-6′), 6.85 (1H, ddd, *J* = 1.7, 7.4, 7.7 Hz, H-4′ or H-5′), 7.10 (1H, ddd, *J* = 1.1, 7.4, 7.7 Hz, H-4′ or H-5′), 7.15 (1H, dd, *J* = 1.7, 7.7 Hz, H-3′ or H-6′), 7.55 (2H, ddd, *J* = 3.5, 6.7, 6.7 Hz, H-6 and H-7), 8.01–8.09 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 18.36 (Me), 39.43 ($MeSO_2$), 48.53 (CH_2), 61.33 (OMe), 62.90 (OMe), 117.16 (=C_{quat}), 122.80 and 123.10 (C-5 and C-8), 124.99 (=C_{quat}), 126.11 (C-4′ or C-5′), 127.70 and 130.23 (C-6 and C-7), 127.74 (=C_{quat}), 128.84 (C-4′ or C-5′), 129.22 (=C_{quat}), 130.23 and 131.44 (C-3′ and C-6′), 136.36 (=C_{quat}), 140.67 (=C_{quat}), 150.14 (=C–O), 153.00 (=C–O). IR (ATR): ν_{max} 1513, 1504, 1450, 1358, 1323, 1142, 1083, 1064, 1000 cm^{-1}. MS *m*/*z* (%): 486/488 (M + Na, 10), 279/281 (M-[$N(SO_2Me)C_6H_4Me$], 100). Anal. Calcd for $C_{21}H_{22}BrNO_4S$: C 54.32%, H 4.78%, N 3.02%. Found: C 54.60%, H 4.97%, N 3.11%.

4.3.1.5. N-methanesulfonyl-3-bromo-1,4-dimethoxy-2-(3-methylphenylamino)methylnaphthalene ***9e***. Yield 67%, light-yellow crystals, mp 111.0–111.4 °C. ^{1}H NMR ($CDCl_3$): δ 2.16 (3H, s, Me), 3.05 (3H, s, $MeSO_2$), 3.84 (3H, s, OMe), 3.94 (3H, s, OMe), 5.23 (2H, s, CH_2), 6.91–7.04 (4H, m, 4×=CH), 7.52 (2H, ddd, *J* = 3.5, 6.7, 6.7 Hz, H-6 and H-7), 7.98–8.07 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 21.07 (Me), 38.24 ($MeSO_2$), 48.67 (CH_2), 61.25 (OMe), 63.13 (OMe), 116.19 (=C_{quat}), 122.60 and 125.95 (C-5 and C-8), 124.43 (=C_{quat}), 126.63 and 127.44 (H-6 and H-7), 126.76 (=CH), 127.57 (=C_{quat}), 128.48 (=CH), 129.05 (=CH and =C_{quat}), 130.56 (=CH), 137.72 (=C_{quat}), 138.67 (=C_{quat}), 150.16 (=C–O), 152.93 (=C–O). IR (ATR): ν_{max} 1578, 1569, 1491, 1452, 1358, 1326, 1284, 1258, 1168, 1148, 1086, 1070, 1003 cm^{-1}. MS *m*/*z* (%): 486/488 (M + Na, 3), 279/281 (M-[$N(SO_2Me)C_6H_4Me$], 100). Anal. Calcd for $C_{21}H_{22}BrNO_4S$: C 54.32%, H 4.78%, N 3.02%. Found: C 54.40%, H 4.81%, N 2.99%.

4.3.1.6. N-methanesulfonyl-3-bromo-1,4-dimethoxy-2-(4-methylphenylamino)methylnaphthalene ***9f***. Yield 76%, light-yellow crystals, mp 134.4–134.8 °C. ^{1}H NMR ($CDCl_3$): δ 2.19 (3H, s, Me), 3.04 (3H, s, $MeSO_2$), 3.86 (3H, s, OMe), 3.93 (3H, s, OMe), 5.23 (2H, s, CH_2), 6.94 (2H, br. d, *J* = 8.3 Hz, 2×=CH), 7.05 (2H, br. d, *J* = 8.3 Hz, 2×=CH), 7.53 (2H, ddd, *J* = 3.3, 6.6, 6.6 Hz, H-6 and H-7), 7.98–8.07 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 21.03 (Me), 38.18 ($MeSO_2$), 48.59 (CH_2), 61.26 (OMe), 63.13 (OMe), 116.24 (=C_{quat}), 122.64 and 123.01 (C-5 and C-8), 124.47 (=C_{quat}), 126.74 and 127.44 (C-6 and C-7), 127.56 (=C_{quat}), 129.05 (=C_{quat}), 129.51 (2×=CH), 129.64 (2×=CH), 135.12 (=C_{quat}), 138.24 (=C_{quat}), 150.17 (=C–O), 152.90 (=C–O). IR (ATR): ν_{max} 1508, 1451, 1358, 1334, 1158, 1149, 1079, 1048, 1007 cm^{-1}. MS *m*/*z* (%): 486/488 (M + Na, 1), 279/281 (M-[$N(SO_2Me)C_6H_4Me$], 100). Anal. Calcd for $C_{21}H_{22}BrNO_4S$: C 54.32%, H 4.78%, N 3.02%. Found: C 54.27%, H 4.86%, N 3.05%.

4.3.1.7. N-methanesulfonyl-3-bromo-1,4-dimethoxy-2-(2-methoxyphenylamino)methylnaphthalene ***9g***. Yield 84%, yellow crystals, mp 169.8–170.1 °C. ^{1}H NMR ($CDCl_3$): δ 3.11 (3H, s, $MeSO_2$), 3.72 (3H, s, OMe), 3.81 (3H, s, OMe), 3.94 (3H, s, OMe), 5.27 (2H, s, CH_2), 6.62 (1H, ddd, *J* = 1.7, 7.7, 7.7 Hz, =CH), 6.77 (1H, br. d, *J* = 8.3 Hz, =CH), 6.93 (1H, dd, *J* = 1.7, 7.7 Hz, =CH), 7.13 (1H, ddd, *J* = 1.7, 7.7, 8.3 Hz, =CH), 7.48–7.54 (2H, m, H-6 and H-7), 7.98–8.05 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 40.27 ($MeSO_2$), 47.86 (CH_2), 55.34 (OMe), 61.33 (OMe), 62.99 (OMe), 111.58 (=CH), 116.61 (=C_{quat}), 120.57 (=CH), 122.61 and 123.09 (C-5 and C-8), 125.09 (=C_{quat}), 125.93 (=C_{quat}), 126.70 and 127.41 (C-6 and C-7), 127.71 (=C_{quat}), 128.99 (=C_{quat}), 130.11 (=CH), 133.54 (=CH), 149.93 (=C_{quat}), 153.30 (=C–O), 157.47 (=C–O). IR (ATR): ν_{max} 1494, 1357, 1324, 1253, 1146, 1086, 1070, 1003 cm^{-1}. MS *m*/*z* (%): 502/504 (M + Na, 10), 279/281 (M-[$N(SO_2Me)C_6H_4OMe$], 100). Anal. Calcd for $C_{21}H_{22}BrNO_5S$: C 52.51%, H 4.62%, N 2.92%. Found: C 52.77%, H 4.81%, N 2.90%.

4.3.1.8. N-methanesulfonyl-3-bromo-1,4-dimethoxy-2-(4-methoxyphenylamino)methylnaphthalene ***9h***. Yield 84%, light-yellow crystals, mp 140.8–141.1 °C. ^{1}H NMR ($CDCl_3$): δ 3.04 (3H, s, $MeSO_2$), 3.66 (3H, s, OMe), 3.86 (3H, s, OMe), 3.93 (3H, s, OMe), 5.21 (2H, s, CH_2), 6.64 (2H, br. d, *J* = 8.8 Hz, 2×=CH), 7.06 (2H, br. d, *J* = 8.8 Hz, 2×=CH), 7.53 (2H, ddd, *J* = 3.3, 6.6, 6.6 Hz, H-6 and H-7), 7.98–8.07 (2H, m, H-5 and H-8). ^{13}NMR ($CDCl_3$): δ 38.18 ($MeSO_2$), 48.64 (CH_2), 55.27 (OMe), 61.30 (OMe), 63.12 (OMe), 114.05 (2×=CH), 114.05 (=C_{quat}), 122.65 and 123.00 (C-5 and C-8), 124.48 (=C_{quat}), 126.79 and 127.47 (C-6 and C-7), 127.58 (=C_{quat}), 129.08 (=C_{quat}), 130.24 (=C_{quat}), 131.17 (2×=CH), 150.19 (=C_{quat}), 152.91 (=C_{quat}), 159.32 (=C_{quat}). IR (ATR): ν_{max} 1508, 1357, 1329, 1247, 1148, 1077, 1047, 1035, 1006 cm^{-1}. MS *m*/*z* (%): 502/504 (M + Na, 2), 279/281 (M-[$N(SO_2Me)C_6H_4OMe$], 100). Anal. Calcd for $C_{21}H_{22}BrNO_5S$: C 52.51%, H 4.62%, N 2.92%. Found: C 52.29%, H 4.95%, N 2.84%.

4.3.1.9. N-methanesulfonyl-3-bromo-1,4-dimethoxy-2-(2,4-dimethoxyphenylamino)methylnaphthalene ***9i***. Yield 82%, orange crystals, mp 137.3–138.4 °C. ^{1}H NMR ($CDCl_3$): δ 3.01 (1H, s, $MeSO_2$), 3.58 (3H, s, OMe), 3.61 (3H, s, OMe), 3.76 (3H, s, OMe), 3.87 (3H, s, OMe), 5.16

(2H, s, CH_2), 6.06 (1H, dd, J = 2.5, 8.4 Hz, H-5′), 6.24 (1H, d, J = 2.5 Hz, H-3′), 6.76 (1H, d, J = 8.4 Hz, H-6′), 7.44 (2H, ddd, J = 3.3, 6.6, 6.6 Hz, H-6 and H-7), 7.91–7.99 (2H, m, H-5 and H-8). ^{13}NMR ($CDCl_3$): δ 40.06 ($MeSO_2$), 47.84 (CH_2), 55.34 (OMe), 55.43 (OMe), 61.34 (OMe), 62.97 (OMe), 99.19 (C-3′), 104.28 (C-5′), 116.70 (=C_{quat}), 118.83 (=C_{quat}), 122.60 and 123.15 (C-5 and C-8), 125.19 (=C_{quat}), 126.67 and 127.38 (C-6 and C-7), 127.74 (=C_{quat}), 128.98 (=C_{quat}), 134.00 (C-6′), 149.88 (=C_{quat}), 153.30 (=C_{quat}), 158.25 (=C_{quat}), 161.04 (=C_{quat}). IR (ATR): ν_{max} 1508, 1357, 1324, 1309, 1212, 1162, 1142, 1085, 1067, 1002 cm^{-1}. MS m/z (%): 431/433 ([M-methanesulfinate]+H$^+$, 100), 279/281 (M-[N(SO_2Me)C_6H_3(OMe)$_2$], 25). Anal. Calcd for $C_{22}H_{24}BrNO_6S$: C 51.77%, H 4.74%, N 2.74%. Found: C 51.93%, H 4.89%, N 2.77%.

4.4. Synthesis of N-methanesulfonyl-2-(arylamino)methyl-3-bromo-1,4-naphthoquinones **6**

4.4.1. General procedure

To a stirred solution of a *N*-methanesulfonyl-2-(arylamino) methyl-3-bromo-1,4-dimethoxy-naphthalene **9** (1 mmol) in 10 ml of acetonitrile was added dropwise a solution of cerium(IV) ammonium nitrate (CAN) (2.3 mmol) in 10 ml of water. The reaction mixture was stirred for 30 min at room temperature and then poured in water. The reaction mixture was extracted with three small portions of ethyl acetate. The combined organic extracts were washed with brine and dried ($MgSO_4$). Recrystallization from methanol afforded pure *N*-methanesulfonyl-2-(arylamino)methyl-3-bromo-1,4-naphthoquinones **6**.

4.4.1.1. N-methanesulfonyl-3-bromo-2-(phenylamino)methyl-1,4-naphthoquinone ***6a***. Yield 85%, yellow crystals, mp 176.8–179.1 °C. ^{1}H NMR ($CDCl_3$): δ 3.06 (3H, s, $MeSO_2$), 5.05 (2H, s, CH_2), 7.26–7.35 (5H, m, 5×=CH), 7.71–7.80 (2H, m, H-6 and H-7), 8.08–8.14 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 37.48 ($MeSO_2$), 49.48 (CH_2), 127.38 and 127.82 (C-5 and C-8), 128.80 (=CH), 128.92 (2×=CH), 129.53 (2×=CH), 130.96 (=C_{quat}), 131.27 (=C_{quat}), 134.32 and 134.63 (C-6 and C-7), 139.16 (=C_{quat}), 142.70 (=C_{quat}), 144.55 (=C_{quat}), 177.46 (C=O), 181.17 (C=O). IR (ATR): ν_{max} 1676, 1660, 1602, 1588, 1339, 1334, 1279, 1152, 1146, 1066 cm^{-1}. MS m/z (%): 420/422 (M + H$^+$, 100). Anal. Calcd for $C_{18}H_{14}BrNO_4S$: C 51.44%, H 3.36%, N 3.33%. Found: C 51.32%, H 3.43%, N 3.37%.

4.4.1.2. N-methanesulfonyl-3-bromo-2-(4-chlorophenylamino)methyl-1,4-naphthoquinone ***6b***. Yield 71%, yellow crystals, mp 170.8–172.5 °C. ^{1}H NMR ($CDCl_3$): δ 3.06 (3H, s, $MeSO_2$), 5.02 (2H, s, CH_2), 7.28–7.30 (4H, m, 4×=CH), 7.72–7.81 (2H, m, H-6 and H-7), 8.08–8.17 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 37.48 ($MeSO_2$), 49.30 (CH_2), 127.34 and 127.81 (C-5 and C-8), 129.66 (2×=CH), 130.05 (2×=CH), 130.88 (=C_{quat}), 131.11 (=C_{quat}), 134.35 and 134.62 (C-6 and C-7), 134.62 (=C_{quat}), 137.60 (=C_{quat}), 142.85 (=C_{quat}), 144.10 (=C_{quat}), 177.22 (C=O), 181.07 (C=O). IR (ATR): ν_{max} 1660, 1338, 1282, 1270, 1146 cm^{-1}. MS m/z (%): 454/456 (M + H$^+$, 100). Anal. Calcd for $C_{18}H_{13}BrClNO_4S$: C 47.54%, H 2.88%, N 3.08%. Found: C 47.63%, H 2.99%, N 3.02%.

4.4.1.3. N-methanesulfonyl-3-bromo-2-(4-fluorophenylamino)methyl-1,4-naphthoquinone ***6c***. Yield 90%, yellow needles, mp 133.2–133.9 °C. ^{1}H NMR ($CDCl_3$): δ 3.07 (3H, s, $MeSO_2$), 5.02 (2H, s, CH_2), 6.97–7.05 (2H, m, 2×=CH), 7.26–7.34 (2H, m, 2×=CH), 7.72–7.81 (2H, m, H-6 and H-7), 8.08–8.15 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 37.56 ($MeSO_2$), 49.48 (CH_2), 116.26 (=CH), 116.57 (=CH), 127.30 and 127.79 (C-5 and C-8), 130.78 (=CH), 130.86 (=C_{quat}), 130.91 (=CH), 131.09 (=C_{quat}), 134.34 and 134.62 (C-6 and C-7), 134.80 (=C_{quat}), 142.88 (=C_{quat}), 144.16 (=C_{quat}), 162.29 (d, J = 249.2 Hz, C-4′), 177.23 (C=O), 181.05 (C=O). IR (ATR): ν_{max} 1660, 1338, 1282, 1145 cm^{-1}. MS m/z (%): 438/440 (M + H$^+$, 100). Anal. Calcd for $C_{18}H_{13}BrFNO_4S$: C 49.33%, H 2.99%, N 3.20%. Found: C 49.20%, H 3.11%.

4.4.1.4. N-methanesulfonyl-3-bromo-2-(2-methylphenylamino)methyl-1,4-naphthoquinone ***6d***. Yield 50%, yellow-brown crystals, mp 135.9–136.8 °C. ^{1}H NMR ($CDCl_3$): δ 2.40 (3H, s, Me), 3.19 (3H, s, $MeSO_2$), 4.95 (1H, d, J = 13.2 Hz, C$\underline{H}_a$H$_b$N), 5.14 (1H, d, J = 13.2 Hz, CH$_a\underline{H}_b$N), 6.99–7.09 (2H, m, 2×=CH), 7.16–7.23 (2H, m, 2×=CH), 7.73–7.82 (2H, m, H-6 and H-7), 8.09–8.15 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 18.47 (Me), 39.07 ($MeSO_2$), 49.70 (CH_2), 126.63 (=CH), 127.24 and 127.82 (C-5 and C-8), 129.20 (=CH), 129.32 (=CH), 130.86 (=C_{quat}), 131.20 (=C_{quat}), 131.93 (=CH), 134.33 and 134.65 (C-6 and C-7), 136.89 (=C_{quat}), 139.92 (=C_{quat}), 143.18 (=C_{quat}), 144.18 (=C_{quat}), 177.26 (C=O), 181.35 (C=O). IR (ATR): ν_{max} 1677, 1657, 1335, 1278, 1148 cm^{-1}. MS m/z (%): 451/453 (M + NH_4^+, 100). Anal. Calcd for $C_{19}H_{16}BrNO_4S$: C 52.54%, H 3.71%, N 3.23%. Found: C 52.38%, H 3.80%, N 3.21%.

4.4.1.5. N-methanesulfonyl-3-bromo-2-(3-methylphenylamino)methyl-1,4-naphthoquinone ***6e***. Yield 91%, yellow crystals, mp 175.0–175.4 °C. ^{1}H NMR ($CDCl_3$): δ 2.29 (3H, s, Me), 3.06 (3H, s, $MeSO_2$), 5.03 (2H, s, CH_2), 7.05–7.22 (4H, m, 4×=CH), 7.70–7.79 (2H, m, H-6 and H-7), 8.07–8.14 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 21.25 (Me), 37.36 ($MeSO_2$), 49.41 (CH_2), 125.47 (=CH), 127.23 and 127.67 (C-5 and C-8), 129.08 (=CH), 129.43 (2×=CH), 130.86 (=C_{quat}), 131.25 (=C_{quat}), 134.16 and 134.50 (C-6 and C-7), 139.04 (=C_{quat}), 139.41 (=C_{quat}), 142.38 (=C_{quat}), 144.62 (=C_{quat}), 177.37 (C=O), 181.03 (C=O). IR (ATR): ν_{max} 1661, 1601, 1588, 1332, 1306, 1271, 1146, 1070 cm^{-1}. MS m/z (%): 434/436 (M + H$^+$, 100). Anal. Calcd for $C_{19}H_{16}BrNO_4S$: C 52.54%, H 3.71%, N 3.23%. Found: C 52.81%, H 3.89%, N 3.16%.

4.4.1.6. N-methanesulfonyl-3-bromo-2-(4-methylphenylamino)methyl-1,4-naphthoquinone ***6f***. Yield 95%, yellow needles, mp 189.3–190.0 °C. ^{1}H NMR ($CDCl_3$): δ 2.29 (3H, s, Me), 3.05 (3H, s, $MeSO_2$), 5.02 (2H, s, CH_2), 7.11 (2H, br. d, J = 8.0 Hz, 2×=CH), 7.20 (2H, br. d, J = 8.0 Hz, 2×=CH), 7.71–7.79 (2H, m, H-6 and H-7), 8.07–8.13 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 21.10 (Me), 37.28 ($MeSO_2$), 49.42 (CH_2), 127.26 and 127.67 (C-5 and C-8), 128.60 (2×=CH), 130.05 (2×=CH), 130.89 (=C_{quat}), 131.23 (=C_{quat}), 134.18 and 134.50 (C-6 and C-7), 136.34 (=C_{quat}), 138.74 (=C_{quat}), 142.53 (=C_{quat}), 144.57 (=C_{quat}), 177.37 (C=O), 181.05 (C=O). IR (ATR): ν_{max} 1660, 1600, 1588, 1331, 1270, 1144, 1065 cm^{-1}. MS m/z (%): 434/436 (M + H$^+$, 100), 355/357 (M-methanesulfinate, 80). Anal. Calcd for $C_{19}H_{16}BrNO_4S$: C 52.54%, H 3.71%, N 3.23%. Found: C 52.93%, H 3.95%, N 3.37%.

4.4.1.7. N-methanesulfonyl-3-bromo-2-(2-methoxyphenylamino) methyl-1,4-naphthoquinone ***6g***. Yield 87%, yellow crystals, mp 206.3–206.9 °C. ^{1}H NMR ($CDCl_3$): δ 3.10 (3H, s, $MeSO_2$), 3.63 (3H, s, OMe), 5.06 (2H, s, CH_2), 6.83 (1H, br. d, J = 8.3 Hz, =CH), 6.91 (1H, ddd, J = 1.1, 7.6, 7.6 Hz), 7.25–7.32 (2H, m, 2×=CH), 7.71–7.82 (2H, m, H-6 and H-7), 8.11–8.15 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 39.74 ($MeSO_2$), 49.47 (CH_2), 55.20 (OMe), 116.61 (=CH), 121.34 (=CH), 126.45 (=C_{quat}), 127.08 and 127.66 (C-5 and C-8), 130.60 (=CH), 130.80 (=C_{quat}), 131.46 (=C_{quat}), 133.64 (=CH), 134.07 and 134.50 (C-6 and C-7), 141.29 (=C_{quat}), 145.06 (=C_{quat}), 156.45 (=C_{quat}), 177.55 (C=O), 181.08 (C=O). IR (ATR): ν_{max} 1676, 1662, 1598, 1592, 1495, 1337, 1272, 1178, 1150 cm^{-1}. MS m/z (%): 472/474 (M + Na, 10), 450/452 (M + H$^+$, 10), 371/373 (M-methanesulfinate, 100). Anal. Calcd for $C_{19}H_{16}BrNO_5S$: C 50.68%, H 3.58%, N 3.11%. Found: C 50.46%, H 3.73%, N 3.19%.

4.4.1.8. N-methanesulfonyl-3-bromo-2-(4-methoxyphenylamino) methyl-1,4-naphthoquinone ***6h***. Yield 94%, yellow crystals, mp

120.8–122.6 °C. ^{1}H NMR ($CDCl_3$): δ 3.05 (3H, s, $MeSO_2$), 3.74 (3H, s, OMe), 5.01 (2H, s, CH_2), 6.80 (2H, dd, J = 2.2, 6.7 Hz, 2×=CH), 7.23 (2H, dd, J = 2.2, 6.7 Hz, 2×=CH), 7.70–7.80 (2H, m, H-6 and H-7), 8.07–8.13 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 37.57 ($MeSO_2$), 49.48 (CH_2), 55.41 (OMe), 114.56 (2×=CH), 127.26 and 127.69 (C-5 and C-8), 130.24 (2×=CH), 131.19 (=C_{quat}), 131.25 (=C_{quat}), 134.21 and 134.53 (C-6 and C-7), 142.68 (=C_{quat}), 144.48 (=C_{quat}), 159.55 (=C_{quat}), 177.34 (C=O), 181.03 (C=O). IR (ATR): ν_{max} 1681, 1658, 1508, 1330, 1273, 1251, 1145 cm^{-1}. MS m/z (%): 472/474 (M + Na, 18), 371/373 (M-methanesulfinate, 100). Anal. Calcd for $C_{19}H_{16}BrNO_5S$: C 50.68%, H 3.58%, N 3.11%. Found: C 50.77%, H 3.64%, N 3.08%.

4.4.1.9. N-methanesulfonyl-3-bromo-2-(2,4-dimethoxyphenylamino) methyl-1,4-naphthoquinone ***6i***. Yield 70%, yellow crystals, mp 233–235 °C. ^{1}H NMR ($CDCl_3$): δ 3.08 (3H, s, $MeSO_2$), 3.57 (3H, s, OMe), 3.75 (3H, s, OMe), 5.03 (2H, br. d, J = 12.7 Hz, CH_2), 6.34 (1H, d, J = 2.8 Hz, H-3′), 6.41 (1H, dd, J = 2.8, 8.3 Hz, H-5′), 7.21 (1H, d, J = 8.3 Hz, H-6′), 7.74 (1H, ddd, J = 1.7, 7.2, 7.2 Hz, H-6 or H-7), 7.79 (1H, ddd, J = 1.7, 7.2, 7.2 Hz, H-6 or H-7), 8.11–8.16 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 39.77 ($MeSO_2$), 49.60 (OMe), 55.25 (OMe), 55.57 (OMe), 99.31 (C-3′), 105.01 (C-5′), 119.34 (=C_{quat}), 127.16 and 127.73 (C-5 and C-8), 130.93 (=C_{quat}), 131.62 (=C_{quat}), 134.11 and 134.25 (C-6 and C-7), 134.54 (C-6′), 141.41 (=C_{quat}), 145.21 (=C_{quat}), 157.44 (=C_{quat}), 161.39 (=C_{quat}), 177.67 (C=O), 181.15 (C=O). IR (ATR): ν_{max} 1677, 1660, 1606, 1590, 1582, 1336, 1308, 1275, 1207, 1148, 1132 cm^{-1}. MS m/z (%): 502/504 (M + Na, 10), 401/403 (M-methanesulfinate, 100). Anal. Calcd for $C_{20}H_{18}BrNO_6S$: C 50.01%, H 3.78%, N 2.92%. Found: C 50.33%, H 3.95%, N 2.82%.

4.5. Synthesis of benzo[j]phenanthridine-7,12-diones ***5***

4.5.1. General procedure

A mixture of *N*-methanesulfonyl-2-(arylamino)methyl-3-bromo-1,4-naphthoquinone **6** (1 mmol) and palladium(II) acetate (0.2 mmol) (Acros Organics), triphenylphosphine (0.4 mmol) and potassium carbonate (3 mmol) in toluene (15 ml), which was previously degassed, was heated at 80 °C for 1 h under N_2 atmosphere, after which the reaction mixture was boiled under reflux for 1–3 h. The reaction mixture was poured in water, extracted with ethyl acetate, washed with water and dried ($MgSO_4$). Flash chromatography on silica gel with an appropriate mixture of petroleum ether/ethyl acetate, which was determined via initial TLC analysis, followed by recrystallization from methanol gave pure benzo[*j*]phentanthridine-7,12-diones **5**. Although the ^{13}C NMR of benzo[*j*]phenanthridine-7,12-dione **5a** is corresponding to data in the literature [21], a difference of more than 10 °C in melting point was witnessed, which could be ascribed to the use of a different solvent for the recrystallization, along with a different chemical shift for H-3 in the ^{1}H NMR in comparison with the first reported synthesis of benzo[*j*]phenanthridine-7,12-dione **5a** [20]. Therefore, all the spectroscopic data, which were recorded for benzo[*j*]phenanthridine-7,12-dione **5a**, are listed below.

4.5.1.1. Benzo[j]phenanthridine-7,12-dione ***5a***. Yield 42%, yellow needles, mp 188.3–188.9 °C (methanol), (Lit.: 200–202 °C, benzene [20]). ^{1}H NMR ($CDCl_3$): δ 7.78–7.93 (4H, m, H-2, H-3, H-9 and H-10), 8.23 (1H, dd, J = 1.1, 8.3 Hz, H-4), 8.27–8.34 (2H, m, H-8 and H-11), 9.60 (1H, dd, J = 1.1, 8.6 Hz, H-1), 9.84 (1H, s, H-6). ^{13}C NMR ($CDCl_3$): δ 122.68 (=C_{quat}), 124.59 (=C_{quat}), 126.63 and 127.35 (C-8 and C-11), 128.28 (C-1), 130.40 and 130.44 (C-2 and C-4), 131.92 (=C_{quat}), 132.11 (C-3), 133.82 (=C_{quat}), 134.33 (=C_{quat}), 134.50 and 134.66 (C-9 and C-10), 148.50 (C-6), 151.92 (=C_{quat}), 183.37 (C=O), 186.22 (C=O). IR (ATR): ν_{max} 1676, 1664, 1589, 1567, 1290, 1276 cm^{-1}. MS m/z (%): 260 (M + H$^+$, 100). Anal. Calcd for $C_{17}H_9NO_2$: C 78.76%, H 3.50%, N 5.40%. Found: C 79.01%, H 3.78%, N 5.53%.

4.5.1.2. 2-Chlorobenzo[j]phenanthridine-7,12-dione ***5b***. Yield 53%, yellow needles, mp 183.8–184.1 °C. ^{1}H NMR ($CDCl_3$): δ 7.80 (1H, dd, J = 2.2, 8.6 Hz, H-3), 7.84–7.89 (2H, m, H-9 and H-10), 8.13 (1H, d, J = 8.6 Hz, H-4), 8.24–8.31 (2H, m, H-8 and H-11), 9.61 (1H, d, J = 2.2 Hz, H-1), 9.77 (1H, s, H-6). ^{13}C NMR ($CDCl_3$): δ 123.20 (=C_{quat}), 124.91 (=C_{quat}), 126.70 and 127.09 (C-8 and C-11), 127.40 (C-1), 131.69 (C-4), 131.77 (=C_{quat}), 132.61 (=C_{quat}), 132.96 (C-3), 134.04 (=C_{quat}), 134.67 and 134.83 (C-9 and C-10), 136.94 (=C_{quat}), 148.63 (C-6), 150.22 (=C_{quat}), 182.96 (C=O), 185.63 (C=O). IR (ATR): ν_{max} 1668, 1586, 1567, 1336, 1284, 1006 cm^{-1}. MS m/z (%): 294/296 (M + H$^+$, 100). Anal. Calcd for $C_{17}H_8ClNO_2$: C 69.52%, H 2.75%, N 4.77%. Found: C 69.89%, H 3.14%, N 4.56%.

4.5.1.3. 2-Fluorobenzo[j]phenanthridine-7,12-dione ***5c***. Yield 53%, yellow needles, mp 182.4–182.8 °C. ^{1}H NMR ($CDCl_3$): δ 7.69 (1H, ddd, J = 2.8, 7.3, 9.3 Hz, H-3), 7.82–7.89 (2H, m, H-9 and H-10), 8.21 (1H, dd, J = 6.1, 9.3 Hz, H-4), 8.25–8.31 (2H, m, H-8 and H-11), 9.30 (1H, d, J = 2.8, 11.0 Hz, H-1), 9.76 (1H, s, H-6). ^{13}C NMR ($CDCl_3$): δ 112.11 (d, J = 26.5 Hz, C-1), 122.32 (d, J = 26.5 Hz, C-3), 123.69 (d, J = 11.7 Hz, =C_{quat}), 124.93 (=C_{quat}), 126.70 and 127.40 (C-8 and C-11), 131.84 (=C_{quat}), 132.74 (d, J = 9.2 Hz, C-4), 133.03 (d, J = 6.9 Hz, =C_{quat}), 134.08 (=C_{quat}), 134.62 and 134.80 (C-9 and C-10), 147.72 (d, J = 2.3 Hz, H-6), 149.19 (=C_{quat}), 163.26 (d, J = 251.5 Hz, C-2), 183.11 (C=O), 185.74 (C=O). IR (ATR): ν_{max} 1668, 1424, 1286, 1272, 1250, 1209, 1014 cm^{-1}. MS m/z (%): 278 (M + H$^+$, 100). Anal. Calcd for $C_{17}H_8FNO_2$: C 73.65%, H 2.91%, N 5.05%. Found: C 73.48%, H 3.04%, N 5.01%.

4.5.1.4. 4-Methylbenzo[j]phenanthridine-7,12-dione ***5d***. Yield 27%, yellow needles, mp 178.8–181.2 °C. ^{1}H NMR ($CDCl_3$): δ 2.87 (3H, s, Me), 7.67–7.76 (2H, m, H-2 and H-3), 7.82–7.89 (2H, m, H-9 and H-10), 8.27–8.34 (2H, m, H-8 and H-11), 9.44 (1H, d, J = 1.7, 8.3 Hz, H-1), 9.85 (1H, s, H-6). ^{13}C NMR ($CDCl_3$): δ 18.76 (Me), 122.83 (=C_{quat}), 124.31 (=C_{quat}), 126.15 (C-1), 126.61 and 127.45 (C-8 and C-11), 130.29 and 132.48 (C-2 and C-3), 132.08 (=C_{quat}), 134.08 (=C_{quat}), 134.46 and 134.67 (C-9 and C-10), 134.61 (=C_{quat}), 138.17 (=C_{quat}), 147.16 (C-6), 151.10 (=C_{quat}), 183.67 (C=O), 186.43 (C=O). IR (ATR): ν_{max} 1664, 1588, 1570, 1459, 1343, 1287, 1274 cm^{-1}. MS m/z (%): 274 (M + H$^+$, 100). Anal. Calcd for $C_{18}H_{11}NO_2$: C 79.11%, H 4.06%, N 5.13%. Found: C 78.83%, H 4.30%, N 5.01%.

4.5.1.5. 3-Methylbenzo[j]phenanthridine-7,12-dione ***5e***. Yield 62%, yellow needles, mp 186.1–187.3 °C. ^{1}H NMR ($CDCl_3$): δ 2.61 (3H, s, Me), 7.62 (1H, d, J = 8.8 Hz, H-2), 7.81–7.85 (2H, m, H-9 and H-10), 7.97 (1H, s, H-4), 8.25–8.30 (2H, m, H-8 and H-11), 9.44 (1H, d, J = 8.8 Hz, H-1), 9.76 (1H, s, H-6). ^{13}C NMR ($CDCl_3$): δ 21.96 (Me), 120.66 (=C_{quat}), 124.06 (=C_{quat}), 126.66 (C-1), 127.36 and 127.91 (C-8 and C-11), 129.51 (C-4), 132.06 (=C_{quat}), 132.86 (C-2), 133.71 (=C_{quat}), 134.40 (=C_{quat}), 134.51 and 134.61 (C-9 and C-10), 143.24 (=C_{quat}), 148.64 (C-6), 152.28 (=C_{quat}), 183.41 (C=O), 186.37 (C=O). IR (ATR): ν_{max} 1672, 1657, 1568, 1293, 1274 cm^{-1}. MS m/z (%): 274 (M + H$^+$, 100). Anal. Calcd for $C_{18}H_{11}NO_2$: C 79.11%, H 4.06%, N 5.13%. Found: C 78.78%, H 4.39%, N 4.99%.

4.5.1.6. 2-Methylbenzo[j]phenanthridine-7,12-dione ***5f***. Yield 59%, yellow needles, mp 174.1–174.8 °C. ^{1}H NMR ($CDCl_3$): δ 2.61 (3H, s, Me), 7.67 (1H, dd, J = 1.5, 8.7 Hz, H-3), 7.79–7.87 (2H, m, H-9 and H-10), 8.04 (1H, d, J = 8.7 Hz, H-4), 8.22–8.27 (2H, m, H-8 and H-11), 9.29 (1H, d, J = 1.5 Hz, H-1), 9.66 (1H, s, H-6). ^{13}C NMR ($CDCl_3$): δ 22.31 (Me), 122.63 (=C_{quat}), 124.50 (=C_{quat}), 126.51 (C-1), 126.91 and 127.20 (C-8 and C-11), 129.98 (C-4), 131.86 (=C_{quat}), 132.89 (=C_{quat}), 134.25 (C-3), 134.34 and 134.54 (C-9 and C-10, =C_{quat}), 141.00 (=C_{quat}), 147.40 (C-6), 150.62 (=C_{quat}), 183.35 (C=O), 186.20 (C=O). IR (ATR): ν_{max} 1665, 1589, 1565, 1358, 1287, 1275 cm^{-1}. MS m/z (%): 274 (M + H$^+$, 100). Anal. Calcd for $C_{18}H_{11}NO_2$: C 79.11%, H 4.06%, N 5.13%. Found: C 78.92%, H 4.33%, N 5.09%.

4.5.1.7. 4-Methoxybenzo[j]phenanthridine-7,12-dione ***5g***. Yield %, orange crystals, mp 209.9–210.7 °C. ^{1}H NMR ($CDCl_3$): δ 4.15 (3H, s, OMe), 7.25–7.29 (1H, m, H-3), 7.76 (1H, dd, J = 8.3, 8.8 Hz, H-2), 7.83–7.89 (2H, m, H-9 and H-10), 8.29–8.34 (2H, m, H-8 and H-11), 9.18 (1H, d, J = 8.8 Hz, H-1), 9.85 (1H, s, H-6). ^{13}C NMR ($CDCl_3$): δ 56.39 (OMe), 110.40 (C-3), 119.65 (C-1), 124.03 (=C_{quat}), 125.18 (=C_{quat}), 126.69 and 127.48 (C-8 and C-11), 128.41 (=C_{quat}), 128.55 (=C_{quat}), 130.98 (C-2), 129.67 (=C_{quat}), 134.54 and 134.78 (C-9 and C-10), 143.91 (=C_{quat}), 147.16 (C-6), 155.64 (=C_{quat}), 183.50 (C=O), 186.19 (C=O). IR (ATR): ν_{max} 1664, 1438, 1288, 1266 cm^{-1}. MS m/z (%): 290 (M + H$^+$, 100). Anal. Calcd for $C_{18}H_{11}NO_3$: C 74.73%, H 3.83%, N 4.84%. Found: C 75.00%, H 4.12%, N 4.59%.

4.5.1.8. 2-Methoxybenzo[j]phenanthridine-7,12-dione ***5h***. Yield 39%, orange needles, mp 185.9–186.7 °C. ^{1}H NMR ($CDCl_3$): δ 4.03 (3H, s, OMe), 7.47 (1H, dd, J = 2.8, 9.1 Hz, H-3), 7.78–7.85 (2H, m, H-9 and H-10), 8.04 (1H, d, J = 9.1 Hz, H-4), 8.22–8.28 (2H, m, H-8 and H-11), 8.96 (1H, d, J = 2.8 Hz, H-1), 9.61 (1H, s, H-6). ^{13}C NMR ($CDCl_3$): δ 55.89 (OMe), 105.25 (C-1), 124.43 (=C_{quat}), 124.84 (=C_{quat}), 125.16 (C-3), 126.61 and 127.22 (C-8 and C-11), 131.74 (C-4), 132.08 (=C_{quat}), 134.37 and 134.58 (C-9 and C-10, =C_{quat}), 134.41 (=C_{quat}), 145.70 (C-6), 148.86 (=C_{quat}), 161.35 (=C_{quat}), 183.53 (C=O), 186.39 (C=O). IR (ATR): ν_{max} 1665, 1288, 1275, 1230 cm^{-1}. MS m/z (%): 290 (M + H$^+$, 100). Anal. Calcd for $C_{18}H_{11}NO_3$: C 74.73%, H 3.83%, N 4.84%. Found: C 74.85%, H 3.92%, N 4.77%.

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