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ARTICLE

Synthesis and antitubercular activity of 1- and 3-substituted benzo[*g*]isoquinoline-5,10-diones

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In this study, a small library of twenty benzo[*g*]isoquinoline-5,10-diones were synthesized in a novel straightforward approach, starting from 2-methyl-1,4-naphthoquinone (vitamin K). An intramolecular Heck reaction of a *N*-vinylacetamide was a crucial step in the synthetic route, at which the combination of cesium carbonate and a bulky, electron rich trialkylphosphine (^{t}BuCy$_2$P.HBF$_4$) provided high 6-*endo-trig* selectivity. The anti-tubercular activity against *Mycobacterium tuberculosis* H37Ra and acute cytotoxicity against J774 A.1 macrophages were studied. From the structure activity relationship, it could be derived that in general the substitution of position 3 yielded analogs with a higher antitubercular potency. Among these, two analogs, **27a** and **27b**, showed remarkable activity with minimal inhibition concentrations of respectively 28.92 µM and 1.05 µM, and acute cytotoxic concentrations of > 128 µM and 34.85 µM. In addition, the analogs and their possible metabolites were evaluated using a Vitotox™ assay to study the possibility of genotoxicity. Results indicated that none of the evaluated analogs and their possible metabolites showed early signs of genotoxicity.

Introduction

Quinones form an exciting class of compounds with interesting properties such as antifungal, antibacterial and anticancer activity.[1-2] These activities might be attributed to the structural similarity with coenzyme Q, i.e. ubiquinone (**1**) (Figure 1), which has a major role in the mitochondrial electron transport system.[2] Compounds that are able to specifically target this electron transport system of pathogens are therefore of great interest. Atovaquone (**2**) (Figure 1), perhaps the most successful medicinal quinone to date, uses this aspect to target the cytochrome bc_1 complex of *Plasmodium falciparum* and *Plasmodium yoelii*, effectively disturbing this part of the parasite's mitochondrion.[3] Atovaquone is one of the two active ingredients of Malarone® (the other being proguanil), an antimalarial medicine released in 2000 by GlaxoSmithKline.

2-Azaanthraquinones have been of particular interest to our research group, and over the years these compounds have shown promising activities. Even the core structure, benzo[*g*]isoquinoline-5,10-dione (**5**) (Figure 2), has shown an antiplasmodial, antitrypanosomal, antibacterial and anti-mycobacterial activity.[4-5]

New octahydrobenzo[*j*]phenanthridinediones **3** (Figure 1) with high *in vitro* selectivity indexes (up to 191.38) towards *Mycobacterium tuberculosis* (*Mtb*) were synthesized by De Kimpe *et al.*[6]. It is believed that the "out of plane" nature of these compounds contribute to a lower cytotoxity than their corresponding unsaturated analogs[7]. In our last publication[8] we have explored the effects of incorporating an amidine functional group into the C-ring (Figure 1) of the benzo[*g*]isoquinoline-5,10-dione core on solubility in polar solvents and the activity against *Mtb*. Improved solubility and nano-molar anti-mycobacterial activity of these compounds **4** proves the merit of core structure **5** as a template towards new antibiotics, notably for antitubercular compounds.

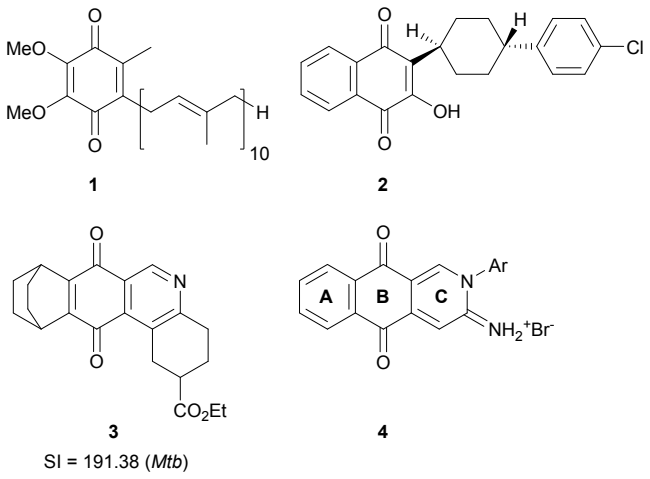

Figure 1 From the top left corner, clockwise: ubiquinone (**1**), atovaquone (**2**), *N*-

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arylbenzo[*g*]isoquinolin-5,10-dione-3-iminium bromides (**4**) and a 1,2,3,4,8,9,10,11-octahydrobenzo[*j*]phenanthridine-7,12-dione derivative **3**.

While tuberculosis may seem as a disease of the past, the latest numbers of the WHO's Global tuberculosis report[9] are very sobering: in 2016 an estimated 10.4 million people fell ill with TB, along with an estimated 1.7 million TB deaths. Moreover, it is the leading cause of death from a single infectious agent worldwide.[9] Along with emerging multi-drug resistance, the need for new antitubercular drugs is of utmost importance.

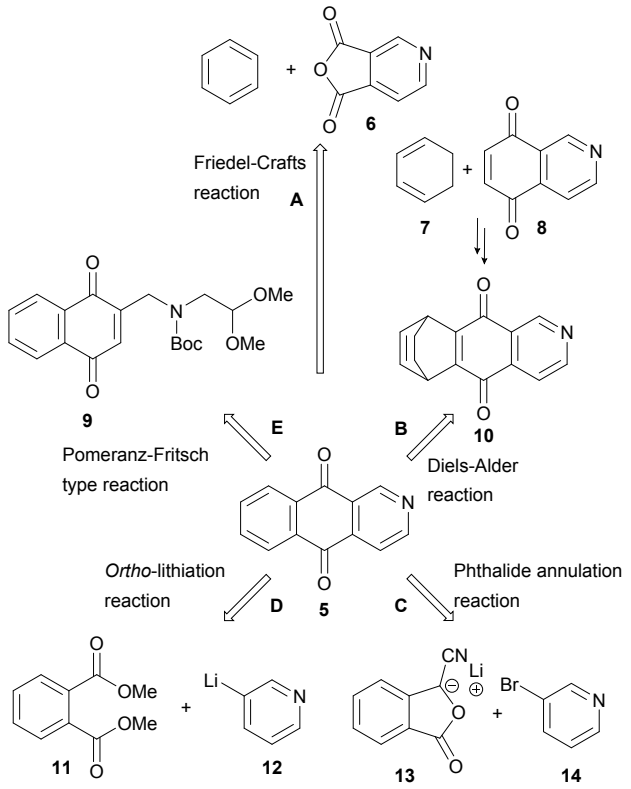

Figure 2 Previous approaches to benzo[*g*]isoquinoline-5,10-dione (**5**).

In this work we present a novel synthesis of the benzo[*g*]isoquinoline-5,10-dione (**5**) core and further explore the effects of functionalization of the C-ring, by using a chloropyridine intermediate. The compound library which was obtained was then evaluated for biological activity and toxicity.

Since the key goal of this research is to synthesize as expeditiously as possible a wide variety of functionalized compounds and to test them, we will use benzo[*g*]isoquinoline-5,10-dione (**5**) as the central building block. A summary of the different synthetic routes towards 2-azaanthraquinones has already been reported, partially by our group, and shows that **5** can be synthesized by a myriad of methods.[10] It is however important to mention that classic methods for preparing isoquinolines do not necessarily prove to be the methods of choice for preparing the benzo[*g*]isoquinoline-5,10-dione compounds. Since this report, no new methods towards the unfunctionalized benzo[*g*]isoquinoline-5,10-dione (**5**) have been published. The known syntheses towards **5** can be divided into five different categories: a) The oldest reported method is a Friedel-Crafts acylation of benzene with 3,4-pyridinedicarboxylic anhydride (**6**) (Figure 2, path A), followed by a ring closing reaction of the resulting nicotinic acid with oleum at elevated temperatures, providing **5** in a total yield of 26%.[11] b) A second approach uses a Diels-Alder reaction between 1,3-cyclohexadiene (**7**) and isoquinoline-5,8-dione (**8**) (Figure 2, path B), followed by oxidation of the resulting quinol tautomer (structure not depicted) with Ag_2O to the corresponding quinone **10** and subsequent thermal elimination of ethene, producing **5** in a total yield of 30% (excluding the synthesis of the starting material).[12-13] c) A third approach uses a phthalide annulation reaction[14] (Figure 2, path C) between 3-bromopyridine (**14**) and a cyanophthalide anion **13** at low temperature to produce benzo[*g*]isoquinoline-5,10-dione (**5**) in 65 % yield. However, we found this procedure impossible to reproduce, as the maximum yield of this procedure in our hands was only 35%. d) Another method using metallation is the reaction between 3-lithiopyridine (**12**) and dimethyl phthalate (**11**) (Figure 2, path D), which forms an intermediate ester that can be ring closed via an *ortho*-lithiation forming **5** in a total yield of 25% starting from 3-bromopyridine.[15-17] e) The final and arguably most elegant method to date for the preparation of benzo[*g*]isoquinoline-5,10-dione (**5**) is based on a Pomeranz-Fritsch type reaction for the ring closure of a suitable substrate **9** (Figure 2, path E).[18] While the procedure, originally designed to prepare 1,2-dihydrobenzo[*g*]isoquinoline-5,10-diones, was not always able to yield the target compounds, it was capable of producing **5** in a total yield of 28% after six steps starting from 1,4-dimethoxy-2-naphthaldehyde by use of *N*-Boc protected intermediates. The major benefit of this approach along with the Diels-Alder reaction (Figure 2, path B) is that it avoids the use of carbanions at very low temperatures using strong bases, which is laborious and unsafe on a larger scale.

Results and discussion

For the synthesis of **5** a novel approach was used, starting from 2-methyl-1,4-naphthoquinone (**15**) (vitamin K_3) (Scheme 1). Following a literature procedure, the starting material was first reduced with sodium thiosulfate. The resulting hydroquinone (not depicted) was methylated with dimethyl sulfate in acetone, to give the dimethoxy compound **16** in good yield.[19] Next, using an improved literature procedure[20], nucleophilic bromination with bromine in DCM provided the aryl brominated compound (not depicted) in excellent yield. This compound was subsequently brominated at the CH_3-position in a radical process. The usual solvent for this reaction[20], CCl_4, was successfully replaced with the less toxic EtOAc, and the dibrominated product **17** was obtained in a 93% yield over two steps. Since the dibrominated product **17** is instable on silica, purification was postponed until the benzyl bromide was substituted.

The pyridine ring was constructed using an enamide intermediate: first the bromomethylated compound underwent nucleophilic substitution by the anion of *N*-vinylformamide (**18a**). The latter was produced by adding **18a** to a sodium hydride suspension in DMSO.

Calcium hydride was also tested as a base in this reaction, but no substitution occurred under these conditions. The use of

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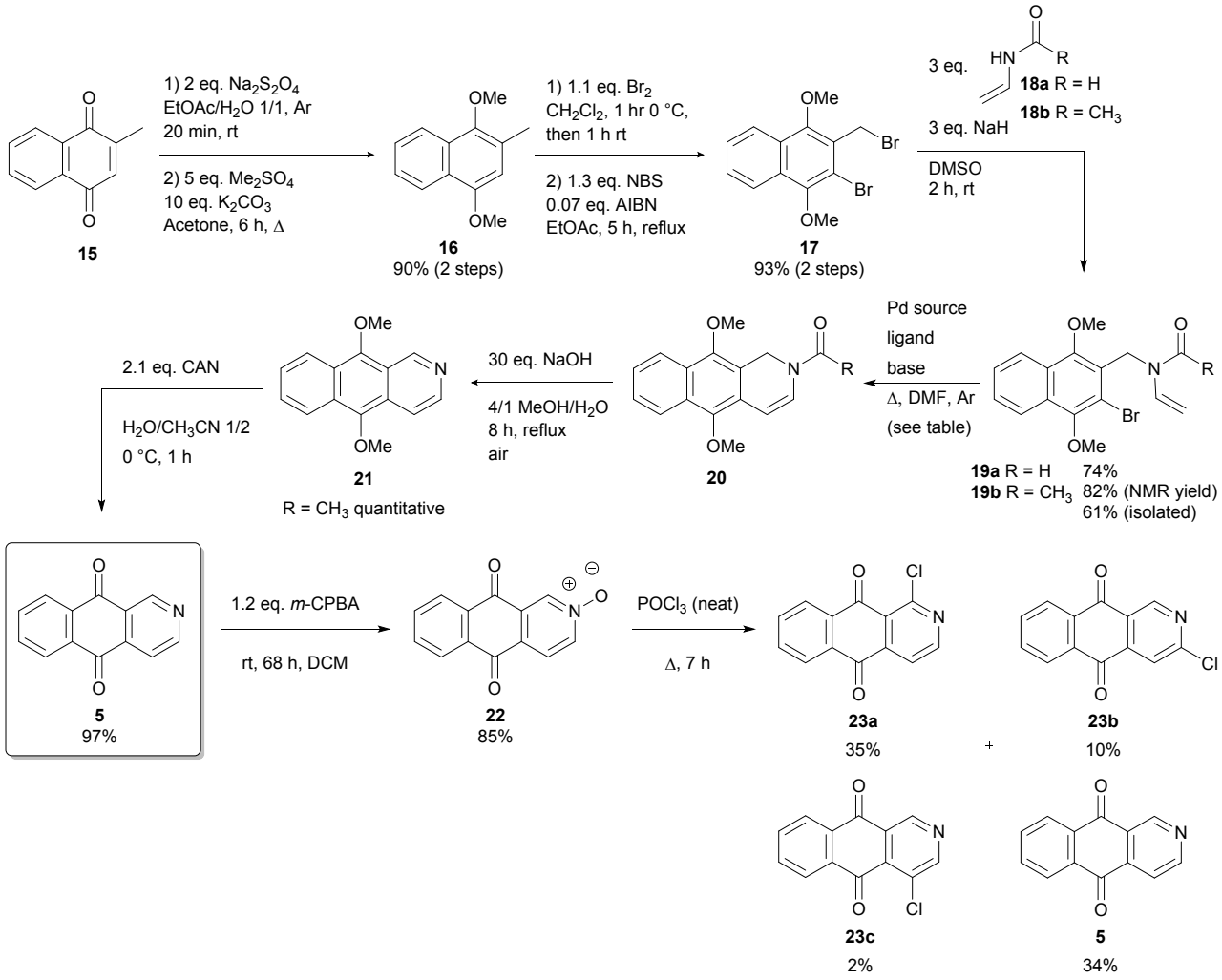
sodium hydride required little optimization, however, it is important to use three equivalents of *N*-vinylformamide and base to obtain a good yield (81%).

Scheme 1 Synthesis of 2-azaanthraquinone (**5**) and subsequent *N*-oxidation and chlorination.



In the next step, ring closure of **19a** was attempted via a Heck approach. Due to a competitive 5-*exo*-*trig* pathway, selective 6-*endo*-*trig* Heck cyclization to produce dihydroisoquinoline derivatives is not an easy task.[21] The regiochemistry of a Heck reaction is dictated by a range of parameters, including (but not limited to) the electronic features of the alkene, steric effects, reaction conditions, type of halide present in the substrate, and with intramolecular Heck reactions the ring-size of the resulting cycle is an additional factor one has to keep in mind.[22] The intramolecular Heck reaction on *N*-vinylamides without any extra functionalities on the α- and β-position of the vinyl bond is not unprecedented. Domínguez *et al.*[23] have reported a tandem intramolecular Heck reaction on *N*-vinyl dihalobenzamides, where a first selective 5-*exo*-*trig* cyclization using a more reactive Ar-I bond leads to a second intramolecular cylization with a less reactive Ar-Br bond, which can then only undergo 6-*endo*-*trig* cyclization. Unfortunately, this elegant strategy cannot be used with the monohaloacetamide **19**.

In Table 1 select optimization results can be found (a more extensive table can be found in the supplementary information). Using $Pd(OAc)_2$ with mono- and bidentate ligands such as triphenylphosphine and *rac*-BINAP (Table 1, entries 1 & 2), in conjunction with K_2CO_3 and TBAB, did not exclusively yield 6-*endo* cyclization. In fact, the application of *rac*-BINAP (Table 1, entry 2) produced more 5-*exo* product **24a** than 6-*endo* product **20a**. Recent work of Wang *et al.*[21] on ring closing reactions of *N*-vinylacetamides shows that the used base has a major effect on selectivity: by using Cs_2CO_3 in combination with triphenylphosphine and $Pd(OAc)_2$ in DMF, they were able to get a high 6-*endo*-*trig* selectivity. Experience with other electron-rich dimethoxynaphthalene analogs in our lab shows that $^tBuCy_2PH.BF_4$ proves to be a superior ligand in intramolecular Heck reactions of these compounds. Therefore, these combined conditions were tested on **20a** (Table 1, entry 3), yielding only a meagre 10% of 6-*endo* Heck product **20a**. Side reactions included deprotection of the amide in 4% yield (**21**) and oxidation to 2-azaanthraquinone (**5**) in 11% yield. This led us to believe that the formyl protective group was not stable in the Heck reaction.

Therefore **19b** (Scheme 1) was made with an *N*-acyl protecting group. The introduction of the acyl protecting group had a drastic effect on the overall stability (Table 1, entries 4-30). Using the same conditions, a 39% increase in yield is observed

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with **19b** (Table 1, entries 3-4), yet deprotection of the acetamide (yielding **21**) still occurred in 4% yield. Besides these products, formation of the 5-*exo-trig* product **24b** in 6% was also observed. Decreasing the equivalents of Cs_2CO_3 (Table 1,

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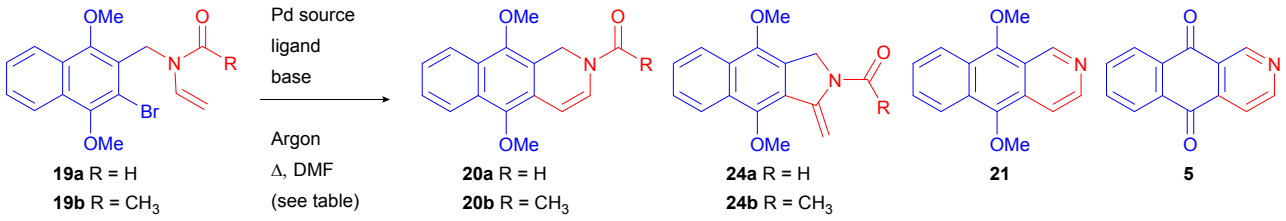
Table 1. Intramolecular Heck reaction of **19** to induce a 6-*endo-trig* ring closure[a]



19a R = H **19b** R = CH_3 **20a** R = H **20b** R = CH_3 **24a** R = H **24b** R = CH_3 **21** **5**

Entry	R	Pd source (eq.)	Ligand (eq.)	Base (eq.)	Additive (eq.)	T, t (°C, h)	**20** (%)[b]	**24** (%)[b]	**21** (%)[b]	**5** (%)[b]	**Rec. 19** (%)[b]
1	H	$Pd(OAc)_2$ (0.2)	TPP (0.3)	K_2CO_3 (2.0)	TBAB (2.0)	100, 6	15 [c]	<6 [c]	0 [c]	0 [c]	0 [c]
2	H	$Pd(OAc)_2$ (0.2)	*rac*-BINAP (0.3)	K_2CO_3 (2.0)	TBAB (2.0)	100, 16	15 [c]	<20 [c]	0 [c]	0 [c]	0 [c]
3	H	$Pd(OAc)_2$ (0.1)	$^tBuCy_2P.HBF_4$ (0.2)	Cs_2CO_3 (5.0)	/	130, 22.5	10 [c]	0 [c]	4 [c]	11 [c]	0 [c]
4	CH_3	$Pd(OAc)_2$ (0.1)	$^tBuCy_2P.HBF_4$ (0.2)	Cs_2CO_3 (5.0)	/	130, 17.5	49 [d]	6 [d]	4 [d]	1 [d]	0 [d]
5	CH_3	$Pd(OAc)_2$ (0.1)	$^tBuCy_2P.HBF_4$ (0.2)	Cs_2CO_3 (4.0)	/	130, 17.5	58	1	0	2	0
6	CH_3	$Pd(OAc)_2$ (0.1)	$^tBuCy_2P.HBF_4$ (0.2)	Cs_2CO_3 (3.0)	/	130, 16.5	57	trace	3	2	0
7	CH_3	$Pd(OAc)_2$ (0.1)	$^tBuCy_2P.HBF_4$ (0.2)	Cs_2CO_3 (2.0)	/	130, 16.5	63	4	2	1	0
8	CH_3	$Pd(OAc)_2$ (0.1)	$^tBuCy_2P.HBF_4$ (0.2)	Cs_2CO_3 (1.2)	/	130, 19	75	6	trace	trace	0
9	CH_3	$Pd(OAc)_2$ (0.1)	$^tBuCy_2P.HBF_4$ (0.2)	Cs_2CO_3 (1.2)	/	130, 16	71	11	trace	trace	0
10	CH_3	$Pd(OAc)_2$ (0.1)	$Cy_3P.HBF_4$ (0.2)	Cs_2CO_3 (1.2)	/	130, 16	74	8	trace	trace	0
11	CH_3	$Pd(OAc)_2$ (0.1)	$^tBu_3P.HBF_4$ (0.2)	Cs_2CO_3 (1.2)	/	130, 16	15	36	0	0	16
12	CH_3	$Pd(OAc)_2$ (0.1)	JohnPhos (0.2)	Cs_2CO_3 (1.2)	/	130, 16	29	38	0	0	0
13	CH_3	$Pd(OAc)_2$ (0.1)	TPP (0.2)	Cs_2CO_3 (1.2)	/	130, 16	48	29	0	0	0
14	CH_3	$Pd(OAc)_2$ (0.1)	DPPP (0.1)	Cs_2CO_3 (1.2)	/	130, 16	45	29	0	0	0
15	CH_3	$Pd(OAc)_2$ (0.1)	DPPF (0.1)	Cs_2CO_3 (1.2)	/	130, 16	49	31	0	0	0
16	CH_3	$Pd(OAc)_2$ (0.1)	DPPE (0.1)	Cs_2CO_3 (1.2)	/	130, 16	41	32	0	0	0
17	CH_3	$Pd(OAc)_2$ (0.1)	Xantphos (0.1)	Cs_2CO_3 (1.2)	/	130, 16	17	34	0	0	15
18	CH_3	$Pd(OAc)_2$ (0.1)	*rac*-BINAP (0.1)	Cs_2CO_3 (1.2)	/	130, 16	26	44	0	0	0
19	CH_3	Pd_2dba_3 (0.05)	*rac*-BINAP (0.1)	Cs_2CO_3 (1.2)	/	130, 16	36	44	0	0	0
20	CH_3	*rac*-BINAP-Pd-G3 Buchwald precat. (0.1)		Cs_2CO_3 (1.1)	/	130, 16	31	49	0	0	0
21	CH_3	*rac*-BINAP-Pd-G3 Buchwald precat. (0.1)		Et_3N (2.0)	/	130, 16	39	37	0	0	0
22 [e]	CH_3	*rac*-BINAP-Pd-G3 Buchwald precat. (0.1)		K_3PO_4 (1.1)	/	130, 16	23	27	0	0	8
23	CH_3	*rac*-BINAP-Pd-G3 Buchwald precat. (0.1)		Na_2CO_3 (1.1)	/	130, 16	31	42	0	0	0
24	**CH_3**	***rac*-BINAP-Pd-G3 Buchwald precat. (0.1)**		**K_2CO_3 (1.1)**	**/**	**130, 16**	**31**	**50**	**0**	**0**	**0**
25	CH_3	*rac*-BINAP-Pd-G3 Buchwald precat. (0.1)		K_2CO_3 (1.1)	TBAC (1.0)	130, 16	25	45	0	0	0
26 [f]	**CH_3**	**$Pd(OAc)_2$ (0.05)**	**$^tBuCy_2P.HBF_4$ (0.1)**	**Cs_2CO_3 (1.1)**	**/**	**130, 16.5**	**92** [c]	**0** [c]	**0** [c]	**0** [c]	**0** [c]
27 [g]	CH_3	$Pd(OAc)_2$ (0.1)	$Cy_3P.HBF_4$ (0.2)	Cs_2CO_3 (1.2)	/	130, 16	65 [c]	0 [c]	0 [c]	0 [c]	0 [c]
28	CH_3	$Pd(OAc)_2$ (0.1)	$^tBuCy_2P.HBF_4$ (0.2)	Et_3N (2.0)	/	130, 16	67	12	0	0	0
29	CH_3	$Pd(OAc)_2$ (0.1)	$^tBuCy_2P.HBF_4$ (0.2)	K_3PO_4 (1.2)	/	130, 16	61	15	0	0	0
30 [h]	CH_3	$Pd(OAc)_2$ (0.1)	$^tBuCy_2P.HBF_4$ (0.2)	Ag_2CO_3 (1.2)	/	130, 16	trace	0	0	0	0

[a]**19** (0.28 mmol), $Pd(OAc)_2$, ligand, base and additive were dissolved in DMF (2.8 mL), stirred and heated to the indicated temperature during the indicated time under Ar. [b]^1H NMR yield, calculated with dimethyl sulfon as internal standard. [c]Isolated yield after flash column chromatography. [d]^1H NMR yield, calculated with 1,4-diacetylbenzene as internal standard. [e]Among the other products, C-N bond cleavage of the vinyl functionality of the starting material by presumably hydrolysis was also observed in 4% (NMR yield) (structure not depicted). [f]Reaction performed on a 4.35 mmol scale. [g]Reaction performed on a 20.70 mmol scale. [h]C-N bond cleavage of the vinyl functionality of the starting material by presumably hydrolysis was observed in 60% (NMR yield) (structure not depicted).

entries 5-8) generally led to higher yields of **20b**, deprotection of the acetamide and subsequent oxidation to the quinone was only observed in trace amounts. Using only 1.2 equivalents of cesium carbonate (Tabel 1, entry 8) furnished the highest yield of 6-*endo* product (75%), along with 6% of **24b**. From entries 5 to 8, it seems that too much of cesium carbonate present in the Heck reaction leads to partial deprotection of **20b** in an inefficient manner, leading to degradation and lower overall yields. Repetition of the conditions in entry 8 for 16 hours instead of 19 hours (Table 1, entry 9) led to a similar conversion and ratio between 6-*endo* and 5-*exo*-product formation.

The effects of different ligands were then explored (Table 1, entries 10-20). Replacing ^{t}BuCy$_2$P.HBF$_4$ with the very similar, electron-rich and moderately bulky Cy$_3$P.HBF$_4$ (Table 1, entry 10) led to a very similar conversion and ratio of 6-*endo* versus 5-*exo*-product. Using the very bulky ^{t}Bu$_3$P.HBF$_4$ (Table 1, entry 11), however, severely hampered the reaction leading to incomplete conversion. Despite this incomplete conversion, a higher formation of **24b** could be observed. Replacing ^{t}Bu$_3$P.HBF$_4$ with JohnPhos (Table 1, entry 12), where one *tert*-butyl substituent is replaced with a biphenyl entity, led to complete conversion in favor of **24b**.

Using triphenylphosphine (Table 1, entry 13), the optimal conditions of Wang *et al.*[21] for 6-*endo-trig* cyclization on their substrate, significantly lowered the ratio of 6-*endo* versus 5-*exo*-product formation when compared to ^{t}BuCy$_2$P.HBF$_4$, but 6-ring formation was still favored.

Bidentate ligands such as DPPP, DPPE and DPPF (Table 1, entries 14-16) gave similar yields as TPP: 6-*endo-trig* product formation was still favored, but these ligands were less selective than ^{t}BuCy$_2$P.HBF$_4$. Xantphos (Table 1, entry 17) behaved much like ^{t}Bu$_3$P.HBF$_4$ (Table 1, entry 11): incomplete conversion, with a preference towards 5-ring formation.

BINAP on the other hand (Table 1, entries 18-20) could uphold complete conversion of the starting material while maintaining a high 5-*exo* versus 6-*endo*-product formation, leading to an even higher yield of **24b** (44%) than JohnPhos (38%). Replacing a Pd(II)-source (Table 1, entry 18) with a Pd(0)-source (Table 1, entry 19) maintained an equal formation of **24b**, but increased the mass balance by producing more of the 6-*endo*-product (36%). Switching to a third generation Buchwald palladium precatalyst with *rac*-BINAP (Table 1, entry 20) led to the highest formation of **24b** (49%) yet, while **20b** was only formed in 31%.

The effect of various bases was then investigated when *rac*-BINAP-Pd-G3 Buchwald precatalyst was used (Table 1, entries 21-24). The organic base triethylamine (Table 1, entry 21) led to a lower selectivity towards **24b**. The inorganic base K$_3$PO$_4$ (Table 1, entry 22) hampered the reaction in such a way that incomplete conversion was obtained and even C-N bond cleavage of the vinyl functionality of **19b** occurred in 4% (structure not depicted) by presumably hydrolysis. Sodium carbonate (Table 1, entry 23) and potassium carbonate (Table 1, entry 24) gave similar results as cesium carbonate, with potassium carbonate yielding **24b** (50%) in a slightly improved percentage. Adding TBAC as an additive (Table 1, entry 25) to these latter conditions, however, caused a decrease in yields for both **20b** and **24b**.

Increasing the reaction scale of the optimized conditions (Table 1, entries 9 & 10) for 6-*endo-trig* cyclization led to some surprising results: decreasing the amount of Pd(OAc)$_2$ and ligand to respectively 5 mol% and 10 mol% at an increased reaction scale, furnished the 6-*endo-trig* product **20b** in an isolated yield of 92% (Table 1, entry 26) when ^{t}BuCy$_2$P.HBF$_4$ was used as the ligand. On the other hand, when Cy$_3$P.HBF$_4$ was used as the ligand (Table 1, entry 27), **20b** was only isolated in a yield of 65%. Presumably, the active catalytic species obtained by using ^{t}BuCy$_2$P.HBF$_4$ instead of Cy$_3$P.HBF$_4$ as the ligand is more efficient or has a longer lifespan during reactions with increased reaction scales, and therefore leads to less degradation.

Finally, a select few bases were screened on the catalytic system with ^{t}BuCy$_2$P.HBF$_4$ (Table 1, entries 28-30). The organic base triethylamine (Table 1, entry 28) was not as efficient in the Heck coupling, with a decrease of 5% in the formation of **20b** when compared to cesium carbonate (Table 1, entry 9). The inorganic base K$_3$PO$_4$ (Table 1, entry 29) led to a higher formation of the 5-*exo*-product, yet still favoring formation of **20b**. Silver carbonate (Table 1, entry 30) was completely detrimental to the reaction, with C-N bond cleavage of the vinyl functionality of the starting material as the major product in 60%.

Deprotection of **20b** to furnish 5,10-dimethoxybenzo[*g*]isoquinoline (**21**) (Scheme 1) went smoothly in quantitative yield, though it is important to note that performing this reaction on bigger reaction scales (>2 mmol) requires the introduction of extra air in the system to ensure full oxidation of the C-N bond after deprotection of the acetamide.

Subsequent oxidative demethylation by CAN oxidation of the two methoxy groups to the quinone core (Scheme 1) provided 2-azaanthraquinone (**5**) in 97% yield, resulting in an overall yield of 45% (starting from vitamin K$_3$ (**15**), Scheme 1). While this reaction sequence is certainly not the shortest in comparison with literature methods, each step can be easily and safely upscaled to prepare 2-azaanthraquinone (**5**) on a multiple-gram scale.

Having compound **5** in hands, a suitable method for functionalization of the pyridine ring was developed. A classical approach for functionalization of pyridines is *N*-oxidation followed by *cine* substitution with POCl$_3$. We found that treatment of benzo[*g*]isoquinoline-5,10-dione (**5**) with *m*-CPBA provided the *N*-oxide **22** in 85% yield (Scheme 1). Subsequent refluxing in POCl$_3$ led to a mixture of mainly 1- and 3-chlorobenzo[*g*]isoquinoline-5,10-diones (**23a**, **23b**), which were easily separated by flash chromatography. A small amount of the 4-chloro adduct **23c** was also isolated in 2% yield.

The chloro adducts were then functionalized using cross-coupling reactions. First we investigated the reactivity of **23a** in a Suzuki reaction with various arylboronic acids (Table 2). Electron rich arylboronic acids (phenyl- and *p*-ethoxyphenylboronic acid) were well tolerated, with respective yields of 71% (**25a**) and 78% (**25e**). Although *p*-tolylboronic acid was able to couple efficiently with **23a**, purification was tedious resulting in a low isolated yield of 34% for **25b**. Steric factors had a major effect on the reaction yields: *o*-tolylboronic acid and *o*-ethoxyphenylboronic acid only coupled in respective yields of 37% (**25c**) and 66% (**25f**), the latter emphasizing the beneficial effect of electron-rich

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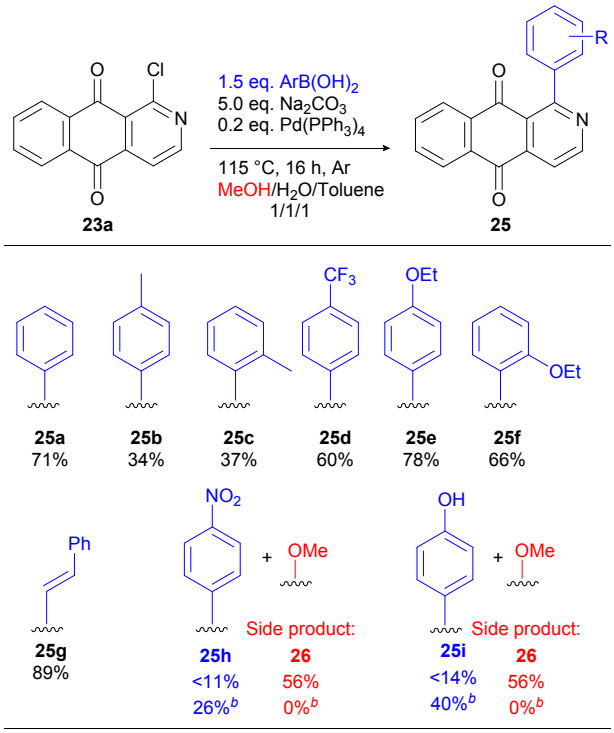
groups. The effect of electron-withdrawing groups starts to show with *p*-trifluoromethylphenylboronic acid, with this coupling partner a lower but still acceptable yield of 60% (**25d**) was achieved. Strong electron-withdrawing groups, such as in *p*-nitrophenylboronic acid, severely hampered the reaction and the product was only obtained in less than 11% (**25h**). Interestingly, in this case competition with one of our co-solvents, methanol, occurred producing the methoxylated compound **26** in 56%. The same situation occurred when using an unprotected, electron-rich boronic acid (*p*-hydroxyphenylboronic acid), producing the wanted product in less than 14% (**25i**) and 1-methoxybenzo[*g*]isoquinoline-5,10-dione (**26**) in 56%. In both cases, **25h** and **25i** could not be obtained fully pure after these conditions. The coupling of methanol with **23a** could be avoided in both cases by simply using toluene as the sole reaction solvent, producing **25h** and **25i** in modest isolated, respective yields of 26% and 40%. Steering away from phenylboronic acids towards β-Styreneboronic acid proved to be possible, yielding the product **25g** in an excellent 89%.

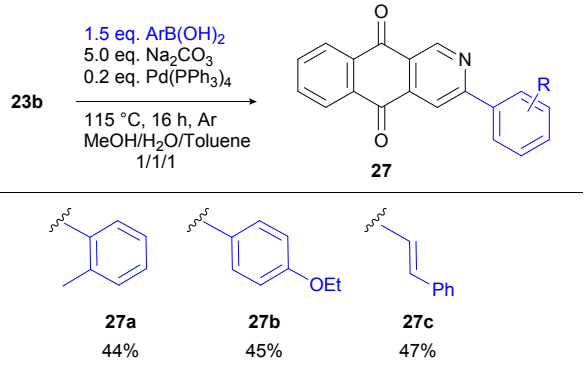
Table 2. Suzuki reactions of **23a** with various boronic acids[a]



[a]**23a** (0.2 mmol), boronic acid (0.3 mmol), Na_2CO_3 (1.0 mmol), $Pd(PPh_3)_4$ (0.04 mmol), MeOH (1 mL), H_2O (1 mL), toluene (1 mL), 115 °C, 16 h, Ar. All yields presented are isolated yields. [b]Isolated yield obtained by using the following conditions: **23a** (0.2 mmol), boronic acid (0.3 mmol), Na_2CO_3 (1.0 mmol), $Pd(PPh_3)_4$ (0.04 mmol), toluene (3 mL), 115 °C, 16 h, Ar.

Moving on to the Suzuki-coupling with the 3-chloro adduct (Table 3), only a select number of examples are shown here, as the chlorination of the N-oxide only yielded this product in 11%. The Suzuki-coupling with **23b** proved to be in general more troublesome than with the analog **23a**, with no clear reaction trends. Coupling with the electron-rich *p*-ethoxyphenylboronic acid was only moderate, yielding **27b** in 47% (opposed to 78% with **23a**). Coupling of the sterically hindered *o*-tolylboronic acid on the other hand was slightly smoother, but not in a significant way, producing **27a** in 44% (opposed to 37% with **23a**). While β-styreneboronic acid was a very successful coupling partner with **23a**, **27c** could only be obtained in a modest 47% from **23b**.

Table 3. Suzuki reactions of **23b** with various boronic acids[a]



[a]**23b** (0.2 mmol), boronic acid (0.3 mmol), Na_2CO_3 (1.0 mmol), $Pd(PPh_3)_4$ (0.04 mmol), MeOH (1 mL), H_2O (1 mL), toluene (1 mL), 115 °C, 16 h, Ar. All yields presented are isolated yields.

Next, we investigated the reactivity of **23a** in Buchwald-Hartwig aminations with various anilines (Table 4). Both electron-rich (*p*-anisidine) as electron-poor (*p*-nitroaniline) anilines resulted in high (74% for **28b**) to excellent (84% for **28e**) yields. *p*-Toluidine coupled very efficiently with **23a**, but purification was tedious leading to a poor 25% for **28a**. Mildly electron-withdrawing substituents, such as *p*-trifluoromethylaniline and *p*-chloroaniline, were less tolerated resulting in respective yields of 67% (**28c**) and 57% (**28d**).

Using the same conditions on 3-chlorobenzo[*g*]isoquinoline-5,10-dione (**23b**) with *p*-toluidine (Table 5, entry 1), however, did not furnish the expected product **29a**. Instead, diarylation of *p*-toluidine with **23b** occurred, yielding **30a** in an isolated yield of 42%. By increasing the steric hindrance of the aniline, using *o*-toluidine (Table 6, entry 2), still no monoarylation was observed. Instead, the diarylated aniline **30b** was obtained in an isolated yield of 77%.

Looking at the work of Hartwig *et al.*[24] on the use of bidentate ligands in aryl halide amination, one can find that the use of bidentate ligands with large bite angles, such as BINAP and DPPF, provides superior conditions for diarylation of primary amines.

Moreover, work of Buchwald *et al.*[25] shows that the use of monodentate ligands such as BrettPhos and XPhos can lead to selective monoarylation of primary amines with aryl chlorides.

Therefore we decided to test reaction conditions with BrettPhos as a ligand for the amination of **23b** in the presence of *p*-toluidine (Table 5, entry 3), in which case the diarylated amine **30a** was formed in an NMR yield of 80%. Using XPhos

(Table 5, entry 4) also resulted in the formation of **30a** in 78% yield. Clearly in the case of the reaction between 1-chlorobenzo[*g*]isoquinoline-5,10-dione (**23a**) and primary anilines, the steric hindrance of the adjacent carbonyl groups prevents the further reaction of the product **28** (a secondary aniline) with **23a**.

Biology

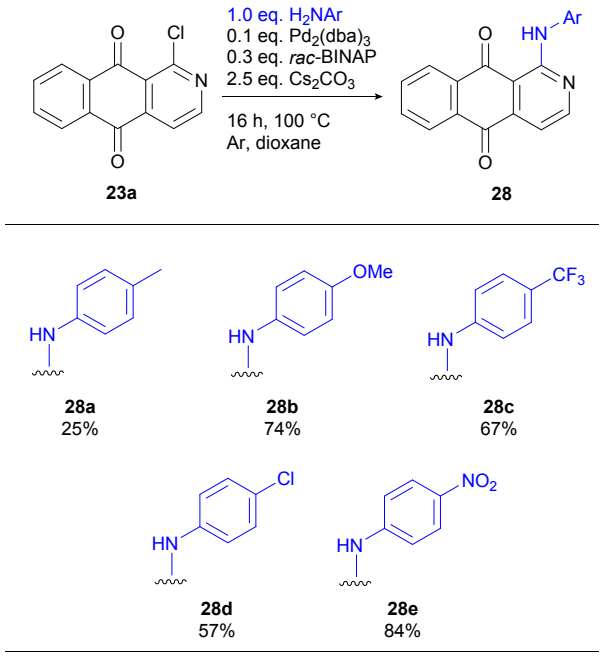
Upon synthesis, the series of benzo[*g*]isoquinoline-5,10-dione analogs were tested for their *in vitro* anti-mycobacterial activity against *Mtb* H37Ra using a luminometric assay based on a luminescent *Mtb* H37Ra reporter strain (H37Ra^{lux}). As reported previously in literature, this technique enables a sensitive and reducible tool to test the efficacy of potential novel antitubercular compounds, able to replace fastidious plating. *In vitro* anti-mycobacterial properties of the synthesized compounds were assessed by the reduction of luminescence emitted by a culture exposed to the compound, compared with a negative control culture. After seven days of exposure of *Mtb* H37Ra^{lux} to serial dilutions of the compounds, the potencies were calculated and reported as the minimal inhibitory concentration (MIC), i.e. the concentration at which the mycobacterial growth is reduced by 90% (Table 6).[6, 26-27] In parallel, the *in vitro* acute toxicity of the analogs against eukaryotic J774 A.1 cells, a murine macrophage like monocyte cell line, was studied using a neutral red uptake assay. The macrophage model was chosen since macrophages function as the most frequent host cell for *Mtb* in a tuberculosis infection. The neutral red uptake assay relies on the ability of viable cells to bind and incorporate the dye neutral red[7].

The acute cytotoxic concentration (CC_{50}) of a compound was defined as the concentration at which the uptake of the neutral red dye by the cells is reduced by 50% (Table 6). By dividing the CC_{50} with the MIC, the selectivity index (SI) could be derived.

As shown in Table 6, the 3-phenyl substituted benzo[*g*]isoquinoline-5,10-dione analogs **27a** and **27b** showed a higher antitubercular potency (MIC = 28.92 and 1.05 µM respectively) than their 1-phenyl substituted counterparts **25a-i** and **26** (MIC-values ranging from 2.87 to > 64 µM).

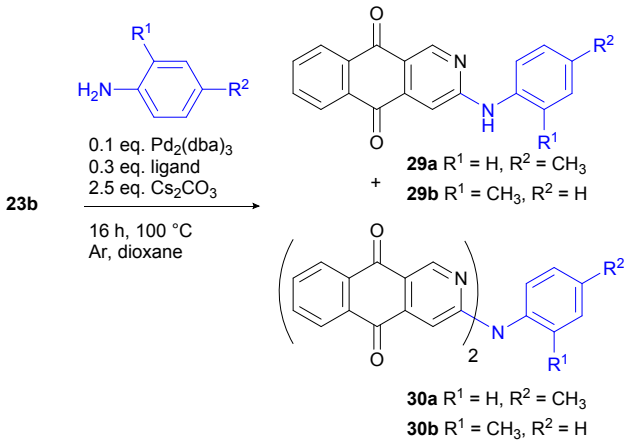
Considering the 3-phenyl substituted analogs **27a** and **27b**, substitution of the *o*-tolyl moiety of analog **27a** with a *p*-ethoxy moiety (**27b**) resulted only in an increase in antitubercular activity (MIC = 28.92 and 1.05 µM respectively). Though, acute cytotoxicity was influenced as well (CC_{50} = > 128 and 34.85 µM respectively), which in turn resulted in a SI of > 4.43 and 33.19 respectively). As for analog **27c**, the 3-styryl substitution resulted in a great decrease in acute cytotoxicity (CC_{50} = > 128 µM). However, antitubercular activity was annulled completely (MIC = > 128 µM). Considering the 1-phenyl substituted analogs **25a-i** and **26**, results showed that antitubercular activity abolished when the 1-phenyl group was left unsubstituted (**25a**; MIC > 64 µM). Likewise, substitution with a *p*-methyl (**25b**), *p*-NO_2 (**25h**) or *p*-hydroxy (**25i**) or the methoxylated side-product (**26**) resulted in an abolishment of antitubercular activity (MIC > 64 µM). Although substitution with a *p*-ethoxy (**25e**) or a styryl (**25g**) led to a decreased acute cytotoxicity (CC_{50} = 68.25 and 78.22 µM respectively), antitubercular activity was not influenced greatly (MIC = 4.01 and 9.09 µM respectively).

Table 4. Buchwald-Hartwig amination of **23a** with primary anilines[a]



[a]**23a** (0.2 mmol), $ArNH_2$ (0.2 mmol), Cs_2CO_3 (0.5 mmol), $Pd_2(dba)_3$ (0.02 mmol), *rac*-BINAP (0.06 mmol), dioxane (2.5 mL), 100 °C, 16 hr, Ar. All yields presented are isolated yields.

Table 5. Buchwald-Hartwig amination of **23b** with *p*- and *o*-toluidine[a]



Entry	R^1	R^2	Ligand	**29** (%) [b]	**30** (%) [b]
1	H	CH_3	BINAP	0	42
2	CH_3	H	BINAP	0	77
3	H	CH_3	BrettPhos	0[c]	80[c]
4	H	CH_3	XPhos	0[c]	78[c]

[a]**23b** (0.2 mmol), $ArNH_2$ (0.2 mmol), Cs_2CO_3 (0.5 mmol), $Pd_2(dba)_3$ (0.02 mmol), ligand (0.06 mmol), dioxane (2.5 mL), 100 °C, 16 h, Ar. [b]Isolated yield. [c]^{1}H NMR yield determined with 1,3,5-trimethoxybenzene as internal standard.

The amidine analogs **28a-e** showed to be less potent in comparison

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to the 1-phenyl substituted counterparts as well (SI ranging from 3.62 to 5.04). Furthermore, compounds **28a**,**d**,**e** exhibit lower

Table 6. *In vitro* anti-mycobacterial activity, acute cytotoxicity and genotoxic evaluation.

Compound	MIC (μM)[a]	CC_{50} (μM)[b]	SI[c]	Genox -S9[d]	Genox +S9[e]
25a	> 64	> 128	ND[f]	-	-
25b	> 64	> 128	ND	-	-
25c	3.44	18.12	5.27	-	-
25d	2.87	10.86	3.78	-	-
25e	4.01	68.25	17.02	-	-
25f	31.58	30.71	0.97	-	-
25g	9.09	78.22	8.61	-	-
25h	> 64	> 128	ND	-	-
25i	> 64	> 128	ND	-	-
26	> 64	> 128	ND	-	-
27a	28.92	> 128	> 4.43	-	-
27b	1.05	34.85	33.19	-	-
27c	> 64	> 128	ND	-	-
28a	9.62	35.41	3.68	-	-
28b	4.36	18.36	4.21	-	-
28c	> 64	> 128	ND	-	-
28d	3.34	16.82	5.04	-	-
28e	> 64	> 128	ND	-	-
30a	> 64	> 128	ND	-	-
30b	> 64	25.11	ND	-	-
Isoniazid	0.36	ND	ND	ND	ND
Tamoxifen	ND	12.01	ND	ND	ND
4NQO	ND	ND	ND	+	ND
Bap	ND	ND	ND	ND	+

[a]Minimal inhibitory concentration (MIC), i.e. the concentration at which 90% growth inhibition of *Mtb* H37Ra[lux] was observed as calculated from triplicate cultures (SD values < 10%). [b]Cytotoxic Concentration (CC_{50}), i.e. the concentration 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 CC_{50}/MIC. [d]Maximum recorded signal to noise ratio by the Genox strain in the absence of S9 fraction. [e]Maximum signal to noise ratio by the Genox strain in the presence of S9 fraction. [f]ND, not done. [g]Isoniazid was used as a positive control in anti-mycobacterial activity assays. [h]Tamoxifen was used as a positive control in acute cytotoxicity assays. [i]4-nitroquinolone-1-oxide (4NQO) was used as a positive control in samples without S9 fraction (Genox-S9), whereas [j] benzo[*a*]pyrene (Bap) was used in samples with S9 fraction (Genox-S9).

antitubercular activity in comparison to their structural analogs **4**.[8] In case of the diarylated analogs **30a** and **30b**, antitubercular activity again abolished completely (MIC > 64 µM).

Finally, possible genotoxicity of the analogs and their potential metabolites was investigated using a Vitotox™ assay.

In this bacterial reporter assay, two recombinant *Salmonella typhimurium* strains, i.e. the Genox strain TA104 (recN2-4) and Cytox *pr1* strain, are used. The former strain is used to detect early genotoxicity signs as the induction of the recN promotor, part of the regulatory SOS operon which is key to the repair of early cellular DNA damage, induces a luminescent signal. The latter strains is used as an internal control as the luminescent signal is under the transcriptional control of the strong constitutive *pr1* promotor. Based on the signal to noise ratio, being the luminescent signal produced by the bacterial suspension exposed to the analogs divided by the signal of the non-exposed suspension, genotoxicity can be assessed. Metabolites of the analogs were obtained by exposing the analogs to S9 liver extract, derived from aroclor-treated rats.[28] For all analogs, the maximum recorded signal to noise ratio by the Genox strain, in the presence and absence of S9 fraction, did not exceed threshold values and were therefore considered as negative (Table 6). As a result, it could be concluded none of the synthetized analogs or their possible metabolites showed early signs of genotoxicity.

Conclusion

In summary, a novel synthesis towards the unfunctionalized benzo[*g*]isoquinoline-5,10-dione has been developed, using eight steps starting from 2-methyl-1,4-naphthoquinone (Vitamin K) and producing 2-azaanthraquinone in a 45% overall yield. The electron rich and bulky trialkylphosphine $^{t}BuCy_2P.HBF_4$ combined with cesium carbonate proved to be essential in achieving a high 6-*endo-trig* selectivity in the intramolecular Heck cyclization, which was the crucial step in this synthetic route towards 2-azaanthraquinone. 2-AAQ was then further functionalized by N-oxidation and subsequent *cine* chlorination. Suzuki reactions and Buchwald-Hartwig aminations were then used to synthesize a small library containing 20 novel substituted benzo[*g*]isoquinoline-5,10-diones. Assessment of the *in vitro* antitubercular activity showed that compounds carrying a phenyl substituent on position 3, i.e. **27a** and **27b**, have an increased activity over the 1-phenyl-benzo[*g*]isoquinoline-5,10-diones **25a-i** and 1-methoxybenzo[*g*]isoquinoline-5,10-dione (**26**). Compounds containing an aniline functionality (**28a-e** and **30a-b**) exhibited a lower antitubercular activity as well. Finally, none of the analogs or their possible metabolites showed any signs of early genotoxicity.

Experimental

General Chemistry

^{1}H (^{13}C) NMR spectra were recorded at 400 (100) MHz on a Bruker Avance III HD spectrometer using $CDCl_3$ as solvent (unless stated otherwise) and TMS as internal standard. Assignments were determined using 2D (HSQC, HMBC and DEPT) spectra. The ^{13}C chemical shifts were referenced to residual solvent signals at δ_C 77.00 ($CDCl_3$). *J*-values are given in Hertz (Hz), chemical shifts are given in parts per million (ppm) and number of protons for each signal is also indicated. Melting points were determined on a Büchi melting point apparatus B-540 and are uncorrected.

TLC-analysis was performed on aluminium backed plates (Machery-Nagel) coated with 0.2 mm silica $60F_{254}$. Unless stated otherwise, products were purified on an automated column chromatography

device Biotage Isolera™ using Grace Resolv™ Silica Flash Cartridges (12, 40 or 80 g). Preparative TLC was performed on glass backed plates (Merck) coated with 0.5 mm silica $60F_{254}$. For high resolution mass spectrometric analysis, samples were dissolved in CH_3CN/H_2O (+0.1% formic acid) 50:50 and diluted to a concentration of approximately 10^{-5} mol/L and measured on a microTOF spectrometer equipped with orthogonal electrospray interface (ESI). The parent ions $[M+H]^+$ or $[M+Na]^+$ are quoted. UPLC analyses were obtained with an Acquity (Waters) system with a reversed phase C-18 column (Halo, 2.1 × 30 mm, 2.7 μM). Samples were prepared following the same procedure as for HRMS measurements described above. The mobile phase (water/acetonitrile) contained 0.1% formic acid. The standard gradient consisted of an 11.0 min run from 10% to 95% acetonitrile in 6.1 min, maintaining 95% acetonitrile for 0.4 min, and from 95% to 10% acetonitrile in 4.4 min, at a flow rate of 0.5 mL/min with diode array UV detection. This procedure was followed for every sample except **30a**, which was dissolved in CH_3CN/H_2O (without formic acid) 90:10 and diluted to a concentration of approximately 10^{-5} mol/L. The same UPLC gradient was applied with the exception that no formic acid was used during the gradient. Purity was determined by UPLC and was presented as an area percentage of the compound peak relative to the total area of all the peaks integrated.

Synthesis of 2-bromo-3-(2-bromomethyl)-1,4-dimethoxynaphthalene (17)

This compound was synthesized according to a modified literature procedure.[20]

To an oven-dried, argon filled 250 mL RBF equipped with a magnetic stirring bar, was added 1,4-dimethoxy-2-methylnaphthalene (**16**) (10.5 g, 52 mmol) in DCM (143 mL) under an argon atmosphere. The solution was cooled to 0°C before adding bromine (9.14 g, 57.2 mmol) dropwise while stirring vigorously. The mixture was stirred under argon atmosphere for 1 hour at 0°C, and for an additional 1 hour at room temperature. The reaction was then quenched with water (150 mL) and the organic layer was separated. The aqueous layer was extracted once more with DCM (150 mL). The combined organic layers were washed with brine (150 mL) and dried over anhydrous $MgSO_4$. After filtration over a glass filter and removal of the solvents *in vacuo*, 2-bromo-1,4-dimethoxy-3-methylnaphthalene was obtained as a purple solid (14.6 g, quant. yield) which was used as such in the next step.

To an oven-dried, argon filled 500 mL RBF equipped with a magnetic stirring bar, 2-bromo-1,4-dimethoxy-3-methylnaphthalene (14.6 g, 52.mmol) was dissolved in EtOAc (208 mL) under an argon atmosphere. AIBN (0.598 g, 3.6 mmol) and NBS (12.03 g, 67.6 mmol) were then added as solids to the reaction mixture. The RBF was equipped with a condenser and argon balloon, followed by heating the reaction mixture to reflux temperature while stirring vigorously. After 4 hours, the reaction mixture was concentrated *in vacuo*. The crude product was then diluted with heptane, and the resulting crystals were separated by filtration through a glass filter. The filtrate was then concentrated to obtain an off-white solid (18.7 g), mp 86-87 °C, which consisted of 2-bromo-3-(bromomethyl)-1,4-dimethoxynaphthalene (**17**) (17.4 g, 93%, purity of **17** in crude product: 92.5%) and 2,6-dibromo-3-(bromomethyl)-1,4-dimethoxynaphthalene or 3,6-dibromo-2-(bromomethyl)-1,4-dimethoxynaphthalene was identified as an impurity present in the crude product (1.3 g, 6%).

An analytically pure sample of **17** was obtained by preparative TLC with heptane/ethyl acetate 3/1 as a white solid, mp 88-91 °C (lit., [29]: 84-87 °C).

R_f = 0.62 (Hept/EtOAc: 3/1).

NMR data were in accordance with the literature.[20]

^{1}H NMR δ_H (400 MHz, $CDCl_3$) 3.99 (3H, s), 4.09 (3H, s), 4.93 (2H, s), 7.53-7.60 (2H, m), 8.04-8.13 (2H, m).

Synthesis of *N*-((3-bromo-1,4-dimethoxynaphtalen-2-yl)methyl)-*N*-vinylacetamide (19b)

To an oven-dried, argon filled 10 mL microwave vial equipped with a magnetic stirring bar, was added sodium hydride (20.0 mg, 0.83 mmol) in DMSO (2 mL). *N*-vinylacetamide (**18b**) (70.9 mg, 0.83 mmol) in DMSO (0.3 mL) was then added while stirring. The mixture was further stirred at room temperature until the solution was clear. After this time, a solution of 2-bromo-3-(bromomethyl)-1,4-dimethoxynaphthalene (**17**) (100.0 mg, 0.28 mmol) in DMSO (0.8 mL) was added dropwise over 1 hour, using an automated syringe pump. Another (2 × 0.3) mL of DMSO was used to ensure full transfer of 2-bromo-3-(bromomethyl)-1,4-dimethoxynaphthalene (**17**) to the reaction mixture. After stirring for 1 hour, the reaction mixture was poored in 10 mL of water. The aqueous phase was then extracted with EtOAc (3 × 10 mL). The combined organic fractions were washed with water (2 × 10 mL), brine (5 mL) and dried over anhydrous $MgSO_4$. After filtration over a glass filter and removal of the solvents *in vacuo*, a dark-red solid was obtained which was purified by an automated flash chromatography system over silica gel applying a heptane-ethyl acetate gradient, giving an off-white solid (62 mg, 61 %, purity 98.9%), mp 141 °C.

R_f = 0.37 (Hept/EtOAc: 6/4)

^{1}H NMR δ_H (400 MHz, $CDCl_3$) 2.29 (3H, br s, NCOC*H*$_3$), 3.89 (3H, s, OC*H*$_3$), 3.96 (3H, s, OC*H*$_3$), 4.26 (1H, d, *J* = 8.6 Hz NCH=C*H*), 4.58 (1H, d, *J* = 15.6 Hz, NCH=C*H*), 5.32 (2H, br s, ArC*H*$_2$N), 6.58 (1H, dd, *J* = 8.4, 12.6 Hz, NC*H*=CH$_2$), 7.50-7.58 (2H, m, arom. H), 7.99-8.14 (2H, m, arom. H). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 22.5 (NCO*C*H$_3$), 41.1 (N*C*H$_2$), 61.4 (BrCCO*C*H$_3$), 63.2 (NCH$_2$CCO*C*H$_3$), 98.3 (NCH*C*H$_2$), 115.1 (C$_{quat.}$), 122.7 (CH$_{arom.}$), 122.8 (CH$_{arom.}$), 124.8 (C$_{quat.}$), 126.8 (CH$_{arom.}$), 127.1 (CH$_{arom.}$), 127.8 (C$_{quat.}$), 128.6 (C$_{quat.}$), 132.5 (N*C*HCH$_2$), 150.4 (Br*C*COCH$_3$), 152.2 (NCH$_2$*C*COCH$_3$), 169.8 (N*C*OCH$_3$).

HRMS (ESI) *m/z* calculated for $[C_{17}H_{18}BrNO_3+H]^+$: 364.0543; found 364.**0551**.

Synthesis of *N*-((3-bromo-1,4-dimethoxynaphtalen-2-yl)methyl)-*N*-vinylformamide (19a)

The same procedure as described for the synthesis of *N*-((3-bromo-1,4-dimethoxynaphtalen-2-yl)methyl)-*N*-vinylacetamide (**19b**) was followed with 7.2 mmol of 2-bromo-3-(bromomethyl)-1,4-dimethoxynapthalene, 21.7 mmol of N-vinylformamide and 21.7 mmol sodium hydride in 80 mL DMSO. A red oil was obtained which was purified by an automated flash chromatography system over silica gel

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applying a heptane-ethyl acetate gradient, producing a brown oil (1.9 g, 74%).

R_f = 0.44 (Hept/EtOAc: 6/4)

NMR indicates that two atropoisomers are present in a 53/47 % ratio, probably due to the orientation of the *N*-formyl group, as is also observable in the proton NMR-spectrum of dimethylformamide. Because of this, side-peak formation in the proton NMR is observable and there are also 32 carbon signals present in the carbon NMR.

Major atropisomer (53%): ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 3.92 (3H, s), 3.97 (3H, s), 4.31 (1H, d, *J* = 9.1 Hz), 4.66 (1H, d, *J* = 15.6 Hz), 5.19 (2H, s), 6.36 (1H, dd, *J* = 9.1, 15.6 Hz), 7.54-7.58 (2H, m), 8.03-8.09 (2H, m), 8.47 (1H, s).

Minor atropisomer (47%): ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 3.93 (3H, s), 3.98 (3H, s), 4.52 (1H, d, *J* = 9.4 Hz), 4.91 (1H, d, *J* = 15.8 Hz), 4.93 (2H, s), 7.22 (1H, dd, *J* = 9.5, 16.3), 7.58-7.62 (2H, m), 8.09-8.15 (2H, m), 8.26 (1H, s).

^{13}C NMR δ_C (100 MHz, $CDCl_3$) 40.3, 45.2, 61.4, 61.6, 63.1, 63.2, 96.6, 97.5, 115.2, 115.3, 122.7, 122.7, 122.8, 122.9, 123.0, 123.7, 126.9, 127.2, 127.4, 127.7, 127.7, 127.8, 128.9, 129.3, 129.3, 132.7, 150.5, 150.8, 152.6, 152.9, 162.0, 162.3.

HRMS (ESI) *m/z* calculated for $[C_{16}H_{16}BrNO_3+H]^+$: 350.0386; found 350.0388.

Synthesis of 1-(5,10-dimethoxybenzo[*g*]isoquinolin-2(1*H*)-yl)ethanone (20b)

N-((3-bromo-1,4-dimethoxynaphthalen-2-yl)methyl)-*N*-vinylacetamide (**19b**) (1.58 g, 4.35 mmol) was loaded in a flame dried pressure tube, equipped with a magnetic stirring bar. Palladium (II) acetate (49 mg, 0.22 mmol) and *t*-butyldicyclohexylphosphonium tetrafluoroborate (149 mg, 0.44 mmol) were weighed each in an NMR vial and closed with a cap with a septum. Cesium carbonate (1.56 g, 4.78 mmol) was weighed in an NMR vial without cap and was oven-dried for at least one hour. The ligand was dissolved in dry DMF (3 mL), and the obtained solution was added to the catalyst by means of a syringe. The solution of the ligand and catalyst was then added to the enamide **19b** under argon atmosphere. The mixture was then diluted using 40.5 mL of DMF. The oven-dried cesium carbonate was, after cooling to room temperature, added to the mixture without argon atmosphere. The reaction mixture was then bubbled with argon for at least 10 minutes. The pressure tube was closed with a screw cap, and was then heated to 130 °C under an argon atmosphere for 16 hours. The solution was then poored onto 40 g of ice and extracted with EtOAc (3 × 100 mL). The combined layers were washed with water (2 × 200 mL), brine (200 mL) and dried over anhydrous $MgSO_4$. After filtration over a glass filter and removal of the solvents *in vacuo*, a dark-brown solid was obtained which was purified by an automated flash chromatography system over silica gel applying a heptane-ethyl acetate gradient, giving a brown solid (1.14 g, 92 %, purity 98.0%), mp 165 °C.

R_f = 0.45 (Hept/EtOAc: 4/6)

NMR indicates that two atropoisomers are present in a 77/23 % ratio, probably due to the orientation of the *N*-acyl group, as is also observable in the proton NMR-spectrum of dimethylformamide. Because of this, side-peak formation in the proton NMR is observable and there are also 21 carbon signals present in the carbon NMR (1 carbon peak of the major atropisomer at 39.3 ppm is hidden under the residual DMSO signal, as shown by HSQC and DEPT spectra). High-temperature NMR measurements to support this claim can be found in the ESI. Aromatic CH-signals and signals of both methoxy-groups are shared by the two atropisomers.

Major atropisomer (77%): ^{1}H NMR δ_H (400 MHz, DMSO-d_6) 2.24 (3H, s, NCOCH_3), 3.84 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.97 (2H, s, NCH_2), 6.15 (1H, d, *J* = 8.05 Hz, NCHC*H*), 7.15 (1H, d, *J* = 8.05 Hz, NC*H*), 7.48-7.57 (2H, m, $CH_{arom.}$), 7.94-8.02 (2H, m, $CH_{arom.}$). ^{13}C NMR δ_C (100 MHz, DMSO-d_6) 21.7 (COCH_3), 39.3 (NCH_2), 61.9 (OCH_3), 62.8 (OCH_3), 102.6 (NCH*C*H), 120.0 ($C_{quat.}$), 120.7 ($C_{quat.}$), 122.6 ($CH_{arom.}$), 122.6 ($CH_{arom.}$), 126.6 ($CH_{arom.}$), 127.0 ($CH_{arom.}$), 127.9 ($C_{quat.}$), 128.3 ($C_{quat.}$), 128.8 (N*C*H), 145.9 ($C_{quat.}$), 147.7 ($C_{quat.}$), 168.9 (N*C*O).

Minor atropisomer (23%): ^{1}H NMR δ_H (400 MHz, DMSO-d_6) 2.30 (3H, s, NCOCH_3), 3.85-3.90 (6H, m, 2 × OCH_3), 5.05 (2H, s, NCH_2), 6.19 (1H, d, *J* = 8.13 Hz, NCHC*H*), 7.33 (1H, d, *J* = 8.17 Hz, NC*H*), 7.48-7.57 (2H, m, $CH_{arom.}$), 7.94-8.02 (2H, m, $CH_{arom.}$). ^{13}C NMR δ_C (100 MHz, DMSO-d_6) 22.6 (COCH_3), 42.8 (NCH_2), 61.9 (OCH_3), 62.8 (OCH_3), 102.8 (NCH*C*H), 120.0 ($C_{quat.}$), 120.7 ($C_{quat.}$), 122.6 ($CH_{arom.}$), 122.6 ($CH_{arom.}$), 126.2 (N*C*H), 126.6 ($CH_{arom.}$), 127.0 ($CH_{arom.}$), 127.9 ($C_{quat.}$), 128.3 ($C_{quat.}$), 145.9 ($C_{quat.}$), 147.7 ($C_{quat.}$), 168.9 (N*C*O).

Dissolved in $CDCl_3$ the spectra of **20b** show two atropisomers in a 81/19 ratio. The major atropisomer accounts for only 16 signals in the ^{13}C NMR spectrum, as the multiplet at 7.95-8.07 ppm accounting for 2 CH protons in the ^{1}H NMR spectrum couples with only one CH-carbon signal at 122.4 ppm in the ^{13}C NMR spectrum, as shown in the HSQC spectrum. The minor atropisomer accounts for 17 signals in the ^{13}C NMR spectrum. The multiplet at 7.95-8.07 ppm couples with two ^{13}C signals at 122.2 and 122.5 ppm, and the multiplet at 7.41-7.50 ppm couples with two ^{13}C signals at 126.5 ppm and 126.5 ppm, one of which is also a signal of the major atropisomer. Aromatic CH-signals and signals of both methoxy-groups are shared by the two atropisomers.

Major atropisomer (81%): ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 2.25 (3H, s, NCOCH_3), 3.90 (6H, s, 2 × OCH_3), 5.08 (2H, s, NCH_2), 6.22 (1H, d, *J* = 8.0 Hz, NCHC*H*), 6.75 (1H, d, *J* = 8.0 Hz, NC*H*), 7.41-7.50 (2H, m, $CH_{arom.}$), 7.95-8.07 (2H, m, $CH_{arom.}$). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 21.5 (COCH_3), 39.7 (NCH_2), 61.7 OCH_3), 62.5 (OCH_3), 103.8 (NCH*C*H), 119.7 ($C_{quat.}$), 120.1 ($C_{quat.}$), 122.4 ($CH_{arom.}$), 126.0 ($CH_{arom.}$), 126.3 ($CH_{arom.}$), 126.5 ($CH_{arom.}$), 128.3 ($C_{quat.}$), 128.4 ($C_{quat.}$), 146.4 (CH_3OC), 148.0 (CH_3OC), 168.5 (N*C*O).

Minor atropisomer (19%): ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 2.33 (3H, s, NCOCH_3), 3.90 (6H, s, 2 × OCH_3), 4.99 (2H, s, NCH_2), 6.32 (1H, d, *J* = 8.0 Hz, NCHC*H*), 7.38 (1H, d, *J* = 8.0 Hz, NC*H*), 7.41-7.50 (2H, m, CHC*H*C*H*CH), 7.95-8.07 (2H, m, C*H*CHCHC*H*). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 22.2 (COCH_3), 42.8 (NCH_2), 61.8 OCH_3), 62.5 (OCH_3), 104.3 (NCH*C*H), 119.2 (NCHCH*C*), 120.5 (NCH*C*), 122.2 (*C*HCHCH*C*H), 122.5 (*C*HCHCH*C*H), 125.6 (N*C*HCH), 126.5 (CH*C*H*C*HCH), 126.5 (CH*C*H*C*HCH), 127.9 ($C_{quat.}$), 128.7 ($C_{quat.}$), 146.5 (NCHCHC*C*), 147.6 (NCHC*C*), 168.4 (N*C*O).

HRMS (ESI) *m/z* calculated for $[C_{17}H_{17}NO_3+H]^+$: 284.1281; found 284.1285.

Synthesis of 1-(4,9-dimethoxy-1-methylene-1,3-dihydro-2*H*-benzo[*f*]isoindol-2-yl)ethan-1-one (24b)

The same procedure as described for the synthesis of 1-(5,10-dimethoxybenzo[*g*]isoquinolin-2(1*H*)-yl)ethanone (**20b**) was followed with *N*-((3-bromo-1,4-dimethoxynaphthalen-2-yl)methyl)-*N*-vinylacetamide (**19b**) (100 mg, 0.28 mmol), K_2CO_3 (42 mg, 0.30 mmol) and *rac*-BINAP-Pd-G3 (27 mg, 0.03 mmol) in 2.7 mL DMF. The crude product was obtained as a brown oil, from which the yield was determined with dimethyl sulfone as internal standard. 1-(5,10-dimethoxybenzo[*g*]isoquinolin-2(1H)-yl)ethanone (**20b**) was obtained in 31% NMR yield (24 mg) and 1-(4,9-dimethoxy-1-methylene-1,3-dihydro-2*H*-benzo[*f*]isoindol-2-yl)ethan-1-one (**24b**) was obtained in 50% NMR yield (39 mg). Compound **24b** proved to be unstable, as various attempts at isolation of this compound (normal phase flash chromatography, reversed phase flash chromatography and recrystallization in methanol) only led to degradation of this product. Therefore, no NMR and HRMS data could be recorded of this compound.

Synthesis of 5,10-dimethoxybenzo[*g*]isoquinoline-2(1*H*)-carbaldehyde (20a) and 4,9-dimethoxy-1-methylene-1,3-dihydro-2*H*-benzo[*f*]isoindole-2-carbaldehyde (24a)

The same procedure as described for the synthesis of 1-(5,10-dimethoxybenzo[*g*]isoquinolin-2(1*H*)-yl)ethanone (**20b**) was followed with *N*-((3-bromo-1,4-dimethoxynaphthalen-2-yl)methyl)-*N*-vinylformamide (**19a**) (50 mg, 0.14 mmol), K_2CO_3 (40 mg, 0.29 mmol), NBu_4Br (92 mg, 0.29 mmol), $Pd(OAc)_2$ (6 mg, 0.03 mmol), *rac*-BINAP (27 mg, 0.04 mmol) in 1.4 mL DMF. The crude product was purified by an automated flash chromatography system over silica gel applying a heptane-ethyl acetate gradient, giving 5,10-dimethoxybenzo[*g*]isoquinoline-2(1*H*)-carbaldehyde (**20a**) as a pale oil (6 mg, 15%).

R_f = 0.28 (Hept/EtOAc: 7/3)

NMR indicates that two atropoisomers are present in a 82/18 % ratio, probably due to the orientation of the *N*-formyl group, as is also observable in the proton NMR-spectrum of dimethylformamide. Because of this, side-peak formation in the proton NMR is observable and there are also 30 carbon signals present in the carbon NMR.

Major atropisomer (82%): ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 3.90 (6H, m), 5.07 (2H, s), 6.26 (1H, d, *J* = 8.0 Hz), 6.68 (1H, d, *J* = 8.0 Hz), 7.42-7.51 (2H, m), 7.96-8.08 (2H, m), 8.40 (1H, s).

Minor atropisomer (18%): ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 3.90 (6H, m), 4.94 (2H, s), 6.49 (1H, dd, *J* = 1.3, 8.0), 7.22 (1H, d, *J* = 8.0), 7.42-7.51 (2H, m), 7.96-8.08 (2H, m), 8.28 (1H, s).

^{13}C NMR δ_C (100 MHz, $CDCl_3$) 39.1, 42.1, 61.5, 61.9, 62.6, 62.7, 103.6, 107.2, 118.2, 118.7, 120.3, 120.9, 122.3, 122.5, 122.7, 123.5, 125.8, 126.2, 126.3, 126.5, 126.6, 128.3, 128.6, 128.7, 146.6, 147.0, 147.6, 148.3, 160.3, 161.5.

4,9-Dimethoxy-1-methylene-1,3-dihydro-2*H*-benzo[*f*]isoindole-2-carbaldehyde (24a) was also collected as a pale oil (8 mg, <20%). However, due to the limited stability no pure product could be isolated.

R_f = 0.24 (Hept/EtOAc: 7/3)

^{1}H NMR δ_H (400 MHz, $CDCl_3$) 3.96 (3H, s), 4.00 (3H, s), 5.02 (2H, s), 5.22 (1H, d, *J* = 2.2 Hz), 5.57 (1H, d, *J* = 2.2 Hz), 7.54-7.58 (2H, m), 8.12-8.18 (2H, m), 9.01 (1H, s). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 47.7, 60.8, 60.9, 88.9, 122.3, 122.6, 122.8, 123.7, 126.5, 126.9, 129.5, 142.3, 147.0, 148.6, 158.7.

Due to limited stability of 5,10-dimethoxybenzo[*g*]isoquinoline-2(1*H*)-carbaldehyde (**20a**) and 4,9-dimethoxy-1-methylene-1,3-dihydro-2*H*-benzo[*f*]isoindole-2-carbaldehyde (**24a**) no HRMS could be recorded.

Synthesis of 5,10-dimethoxybenzo[*g*]isoquinoline (21)

1-(5,10-Dimethoxybenzo[*g*]isoquinolin-2(1*H*)-yl)ethanone (**20b**) (3.51 g, 12.39 mmol) in a two-necked round-bottomed flask equipped with a magnetic stirring bar was dissolved in 300 mL methanol, and sodium hydroxide (74.3 mL of a 5 M aqueous solution, 372 mmol) was added while stirring. The mixture was heated at reflux temperature, while pressurized air was bubbled through the reaction mixture for 8 hours. After this time the mixture was neutralized with a concentrated aqueous HCl solution and the mixture was concentrated *in vacuo*. The crude mixture was then extracted with DCM (3 × 200 mL) and the combined organic layers were washed with water (200 mL), brine (200 mL) and dried over anhydrous $MgSO_4$. After filtration over a glass filter and removal of the solvents *in vacuo*, a dark-brown solid (2.96 g, 100%, purity 99.4%) was obtained which did not need further purification and was used as such in the next reactions, mp 121 °C.

^{1}H NMR δ_H (400 MHz, $CDCl_3$) 4.09 (3H, s, NCHCHCCOCH_3), 4.19 (3H, s, NCHCCOCH_3), 7.49-7.61 (2H, m, CCHC*H*C*H*CHC), 7.97 (1H, d, *J* = 6.2 Hz, NCHC*H*), 8.29 (1H, d, *J* = 8.5 Hz, NCHCHCCCC*H*), 8.33 (1H, d, *J* = 8.5 Hz, NCHCCCC*H*), 8.45 (1H, d, *J* = 6.2 Hz, NC*H*CH), 9.78 (1H, s, NC*H*C).

^{1}H NMR δ_H (400 MHz, DMSO-d6) 4.08 (3H, s), 4.18 (3H, s), 7.64-7.75 (2H, m), 8.02 (1H, dd, *J* = 1.1, 6.2 Hz), 8.28-8.33 (1H, m), 8.33-8.38 (1H, m), 8.48 (1H, d, *J* = 6.2 Hz), 9.72 (1H, s).

^{13}C NMR δ_C (100 MHz, $CDCl_3$) 63.3 (NCHCHCCO*C*H$_3$), 64.5 (NCHCCO*C*H$_3$), 114.4 (NCH*C*H), 119.7 (NCHCH*C*), 122.5 (NCHCHCCC*C*H), 123.1 (NCHCCC*C*H), 125.2 (NCH*C*), 125.8 (CCH*C*HCHC), 126.0 (NCHCHCC*C*), 127.4 (CCH*C*HCHC), 128.0 (NCHCC*C*), 140.7 (N*C*HCH), 147.5 (NCHCHC*C*), 149.9 (N*C*HC), 150.7 (NCHC*C*).

HRMS (ESI) *m/z* calculated for $[C_{15}H_{13}NO_2+H]^+$: 240.1019; found 240.1019.

Synthesis of benzo[*g*]isoquinoline-5,10-dione (5)

To a solution of 5,10-dimethoxybenzo[*g*]isoquinoline (**21**) (1.5 g, 6.4 mmol) in a mixture of ACN/H_2O (2:1, 40:20 mL) was added ceric ammonium nitrate (7.4 g, 13.5 mmol) portionwise over 5 minutes at 0 °C. The mixture was stirred at 0 °C for one hour. Next, the solution was quenched with water (275 mL), ACN was removed *in vacuo*, the mixture was then extracted with DCM (3 × 135 mL) and dried over anhydrous $MgSO_4$. After filtration through a pad of Celite over a glass filter and removal of the solvents *in vacuo*, a yellow solid (1.3 g, 97%, purity 99.6%) was obtained which did not need further purification, mp 178 °C (lit.[12]: 178-180 °C).

R_f = 0.62 (Hept/EtOAc: 4/6).

NMR data were in accordance with the literature.[12]

^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.81-7.90 (2H, m), 8.07 (1H, dd, *J*

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= 0.7, 4.9 Hz), 8.28-8.37 (2H, m), 9.10 (1H, d, *J* = 5.0 Hz), 9.55 (1H, d, *J* = 0.6 Hz).

Synthesis of 5,10-dioxo-5,10-dihydrobenzo[*g*]isoquinoline 2-oxide (22)

To a solution of benzo[*g*]isoquinoline-5,10-dione (**5**) (0.83 g, 4.0 mmol) in DCM (40 mL), *m*-chloroperbenzoic acid (1.10 g, 4.8 mmol) was added at room temperature while stirring. The reaction mixture was stirred at room temperature for 68 hours. Next, the solvent was removed *in vacuo*, after which a saturated aqueous $NaHCO_3$ solution (160 mL) and DCM (125 mL) was added, which was allowed to stir for 4 hours. The water layer was then separated and extracted with DCM (2 × 160 mL) and the combined organic layers were washed with brine (160 mL), and dried over anhydrous $MgSO_4$. After filtration over a glass filter and removal of the solvents *in vacuo*, a yellow solid was obtained which did not need further purification and was used as such in the next reactions (0.76 g, 85 %, purity 99.7%), mp 257 °C.

^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.84-7.92 (2H, m, $CH_{arom.}$), 8.14 (1H, d, *J* = 6.8 Hz, NOCHC*H*), 8.29-8.36 (2H, m, $CH_{arom.}$), 8.43 (1H, dd, *J* = 1.7, 6.8 Hz, NOC*H*CH), 8.89 (1H, d, *J* = 1.6 Hz, NOC*H*C). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 124.0 (NOCHC*H*), 127.6 ($CH_{arom.}$), 127.7 ($CH_{arom.}$), 128.0 (NOCHCH*C*), 131.1 (NOCH*C*), 132.9 ($C_{quat.}$), 133.1 ($C_{quat.}$), 134.8 ($CH_{arom.}$), 135.3 ($CH_{arom.}$), 137.4 (NO*C*HC), 143.8 (NO*C*HCH), 179.6 (NOCHCHC*C*O), 180.4 (NOCHC*C*O).

HRMS (ESI) *m/z* calculated for $[C_{13}H_7NO_3+H]^+$: 226.0499; found 226.0508.

Chlorination of 5,10-dioxo-5,10-dihydrobenzo[*g*]isoquinoline 2-oxide (22)

5,10-dioxo-5,10-dihydrobenzo[*g*]isoquinoline 2-oxide (**22**) (740 mg, 3.29 mmol) was divided into two equal portions in two RBFs equipped with a reflux condenser. Both portions were dissolved in 7.2 mL $POCl_3$ and were heated to reflux temperature for 7 hours while stirring. The reaction mixture was then cooled to room temperature and added very slowly to ice-water (100 mL) while stirring. The mixture was extracted with DCM (3 × 60 mL) and the combined organic layers were dried over anhydrous $MgSO_4$. After filtration over a glass filter and removal of the solvents *in vacuo*, a green-brown solid was obtained which was purified by an automated flash chromatography system over silica gel applying a heptane-ethyl acetate gradient, giving the respective 1-chloro- (**23a**) (277 mg, 35%, purity 99.3%), 3-chloro- (**23b**) (77 mg, 10%, purity 98.5%) and 4-chlorobenzo[*g*]isoquinoline-5,10-diones (**23c**) (19 mg, 2%, purity 99.9%) as yellow solids.

The above mentioned water layer was, after the chlorinated quinones were extracted, neutralized using an aqueous saturated $NaHCO_3$ solution and subsequently extracted with DCM (3 × 300 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and filtrated over a glass filter. After removal of the solvents, benzo[*g*]isoquinoline-5,10-dione (**5**) was obtained as a yellow solid (235 mg, 34%, purity 100%).

1-Chlorobenzo[*g*]isoquinoline-5,10-dione (23a)

R_f = 0.56 (Hept/EtOAc 6/4). mp 176 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.83 (1H, dd, *J* = 7.2, 8.2 Hz, NCHCHCCCCHC*H*), 7.89 (1H, dd, *J* = 7.3, 8.2 Hz, NCClCCCCHC*H*), 8.13 (1H, d, *J* = 4.8 Hz, NCHC*H*), 8.27 (1H, d, *J* = 7.5, NCHCHCCCC*H*), 8.33 (1H, d, *J* = 7.5 Hz, NCClCCCC*H*), 8.84 (1H, d, *J* = 4.8 Hz, NC*H*H). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 119.3 (NCH*C*H), 124.5 (NCHCH*C*), 127.0 (NCHCHCCOC*C*H); 127.8 (NCClCCOC*C*H), 131.9 (NCClCCO*C*), 134.0 (NCHCHCCO*C*), 134.3 (NCHCHCCOCCH*C*H), 135.5 (NCClCCOCCH*C*H), 142.5 (NCCl*C*), 151.5 (*C*Cl), 153.8 (N*C*H), 180.4 (NCClC*C*O), 181.3 (NCHCHC*C*O).

HRMS (ESI) *m/z* calculated for $[C_{13}H_6ClNO_2+H]^+$: 244.0160; found 244.0171.

3-Chlorobenzo[*g*]isoquinoline-5,10-dione (23b)

R_f = 0.64 (Hept/EtOAc 6/4). mp 175 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.83-7.93 (2H, m, NCClCHCCOCC*HCH*), 8.07 (1H, s, NCClC*H*), 8.26-8.35 (2H, m, NCHCCOCC*HCH*), 9.31 (1H, s, NC*H*). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 120.3 (NCCl*C*H), 125.3 (NCClCH*C*), 127.5 ($CH_{arom.}$), 127.6 ($CH_{arom.}$), 132.9 ($C_{quat.}$), 133.0 ($C_{quat.}$), 134.8 ($CH_{arom.}$), 135.4 ($CH_{arom.}$), 140.7 (NCH*C*), 150.6 (N*C*H), 157.7 (N*C*Cl), 181.3 (NCClCHC*C*O), 181.5 (NCHC*C*O).

HRMS (ESI) *m/z* calculated for $[C_{13}H_6ClNO_2+H]^+$: 244.0160; found 244.0169.

4-Chlorobenzo[*g*]isoquinoline-5,10-dione (23c)

R_f = 0.66 (Hept/EtOAc 6/4). mp 135-136 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.83-7.92 (2H, m, C*H*$_{arom.}$), 8.26-8.35 (2H, m, C*H*$_{arom.}$), 9.05 (1H, s, NC*H*CCl), 9.47 (1H, s, NC*H*C). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 127.0 ($CH_{arom.}$), 127.7 ($CH_{arom.}$), 129.9 ($C_{quat.}$), 130.0 (*C*Cl), 132.2 ($C_{quat.}$), 133.9 ($C_{quat.}$), 133.9 ($C_{quat.}$), 134.8 ($CH_{arom.}$), 135.1 ($CH_{arom.}$), 148.2 (N*C*HC), 157.3 (N*C*HCCl), 181.3 (NCHCClC*C*O), 181.8 (NCHC*C*O).

HRMS (ESI) *m/z* calculated for $[C_{13}H_6ClNO_2+H]^+$: 244.0160; found 244.0171.

Synthesis of 1- and 3-substituted benzo[*g*]isoquinoline-5,10-diones

General procedure – Suzuki reactions. To a 10 mL MW vial was added 50.0 mg (0.205 mmol) of **23a** or **23b**, the suitable boronic acid (0.308 mmol) and Na_2CO_3 (1.026 mmol). Then, $Pd(PPh_3)_4$ (0.041 mmol), prepared using a known method[30], was added under argon. Next, methanol (1 mL), water (1 mL) and toluene (1 mL) were added to the vial under argon while stirring and the reaction mixture was carefully bubbled with argon for at least 5 minutes before closing the MW vial. The reaction mixture was then heated at 115 °C for 16 hours. When the reaction was complete, the reaction mixture was cooled to room temperature, quenched with brine (7 mL), and extracted with (3 × 20 mL) DCM. The combined organic fractions were dried over anhydrous $MgSO_4$, filtrated over a glass filter and the solvents were removed *in vacuo*.

1-Phenylbenzo[*g*]isoquinoline-5,10-dione (25a)

Purified by flash chromatography over silica gel applying a heptane-ethyl acetate gradient. R_f = 0.31 (Hept/EtOAc 75/25). Yield 41 mg (71 %, purity 94.9%), yellow solid, mp 212 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.46-7.54 (5H, m), 7.76-7.86 (2H, m), 8.14 (1H, d, *J* = 4.9 Hz), 8.15-8.21 (1H, m), 8.26-8.33 (1H, m), 9.08 (1H, d, *J* = 4.9 Hz). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 118.6, 125.0, 126.9, 127.6, 128.1, 128.6, 128.8, 132.4, 134.1, 134.3, 135.1, 140.7, 140.8, 153.5, 161.8, 182.6, 182.7.

HRMS (ESI) *m/z* calculated for $[C_{19}H_{11}NO_2+H]^+$: 286.0863; found 286.0869.

1-*p*-Tolylbenzo[*g*]isoquinoline-5,10-dione (25b)

Purified by flash chromatography over silica gel applying a heptane-ethyl acetate gradient, subsequent recrystallization in heptane and subsequent purification by preparative TLC using DCM as eluent. R_f = 0.20 (Hept/EtOAc 80/20). Yield 21 mg (34 %, purity 99.7%), yellow solid, mp 209 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 2.45 (3H, s), 7.30 (2H, d, *J* = 7.9 Hz), 7.42 (2H, d, *J* = 8.0 Hz), 7.77-7.85 (2H, m), 8.10 (1H, d, *J* = 4.9 Hz), 8.15-8.21 (1H, m), 8.24-8.32 (1H, m), 9.05 (1H, d, *J* = 4.9 Hz). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 21.5, 118.3, 124.9, 126.9, 127.6, 128.7, 128.9, 132.4, 134.1, 134.3, 135.1, 137.7, 138.8, 140.8, 153.4, 161.8, 182.6, 182.8.

HRMS (ESI) *m/z* calculated for $[C_{19}H_{11}NO_2+H]^+$: 300.1019; found 300.1027.

1-*o*-Tolylbenzo[*g*]isoquinoline-5,10-dione (25c)

Purified by manual flash chromatography with silica gel using a heptane-ethyl acetate solvent system (75/25 Hept/EtOAc). R_f = 0.34 (Hept/EtOAc 75/25). Yield 22 mg (37 %, purity 94.9%), yellow solid, mp 163-165 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 2.08 (3H, s), 7.13-7.18 (1H, m), 7.29-7.36 (2H, m), 7.43-7.37 (1H, m), 7.78-7.83 (2H, m), 8.12-8.15 (1H, m), 8.18 (1H, d, *J* = 5.0 Hz), 8.26-8.32 (1H, m), 9.13 (1H, d, *J* = 5.0 Hz). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 19.6, 118.7, 125.1, 125.8, 126.9, 127.3, 127.6, 128.3, 129.9, 132.4, 133.9, 134.1, 134.7, 135.1, 140.2, 140.9, 154.0, 161.7, 182.2, 182.7.

HRMS (ESI) *m/z* calculated for $[C_{19}H_{11}NO_2+H]^+$: 300.1019; found 300.1022.

1-(*p*-(Trifluoromethyl)phenyl)benzo[*g*]isoquinoline-5,10-dione (25d)

Purified by manual flash chromatography with silica gel using a heptane-ethyl acetate solvent system (85/15 Hept/EtOAc). R_f = 0.15 (Hept/EtOAc 85/15). Yield 44 mg (60 %, purity 95.5%), pale yellow solid, mp 243 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.62 (2H, d, *J* = 8.0 Hz), 7.76 (2H, d, *J* = 8.0 Hz), 7.82-7.88 (2H, m), 8.16-8.19 (1H, m), 8.20 (1H, d, *J* = 4.9 Hz), 8.28-8.34 (1H, m), 9.10 (1H, d, *J* = 4.9 Hz). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 119.3, 122.8, 124.2 (q, *J* = 271.2 Hz), 125.1 (q, *J* = 3.8 Hz), 127.1, 127.6, 129.0, 130.6 (q, *J* = 32.05 Hz), 132.4, 133.9, 134.4, 135.3, 140.8, 144.3 (q, *J* = 1.2 Hz), 153.7, 160.2, 182.3, 182.5.

HRMS (ESI) *m/z* calculated for $[C_{20}H_{10}F_3NO_2+H]^+$: 354.0736; found 354.0734.

1-(*p*-Ethoxyphenyl)benzo[*g*]isoquinoline-5,10-dione (25e)

Purified by manual flash chromatography with silica gel using a heptane-ethyl acetate solvent system (75/25 Hept/EtOAc). R_f = 0.29 (Hept/EtOAc 75/25). Yield 53 mg (78 %, purity 97.4%), orange solid, mp 210 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 1.46 (3H, t, *J* = 7.0 Hz), 4.12 (2H, q, *J* = 7.0 Hz), 6.97-7.02 (2H, m), 7.46-7.51 (2H, m), 7.77-7.84 (2H, m), 8.06 (1H, d, *J* = 4.9 Hz), 8.16-8.21 (1H, m), 8.24-8.29 (1H, m), 9.03 (1H, d, *J* = 4.9 Hz). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 14.9, 63.5, 114.1, 117.9, 124.7, 126.8, 127.6, 130.5, 132.3, 132.6, 134.0, 134.4, 135.1, 140.9, 153.4, 159.7, 161.3, 182.7, 182.9.

HRMS (ESI) *m/z* calculated for $[C_{21}H_{15}NO_3+H]^+$: 330.1125; found 330.1141.

1-(*o*-Ethoxyphenyl)benzo[*g*]isoquinoline-5,10-dione (25f)

Purified by flash chromatography over silica gel applying a heptane-ethyl acetate gradient. R_f = 0.49 (Hept/EtOAc 6/4). Yield 45 mg (66 %, purity 98.7%), yellow solid, mp 140 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 0.95 (3H, t, *J* = 7.0 Hz), 3.91 (2H, q, *J* = 7.0 Hz), 6.94 (1H, d, *J* = 8.4 Hz), 7.14 (1H, t, *J* = 7.4 Hz), 7.44 (1H, td, *J* = 1.4, 7.8 Hz), 7.53 (1H, dd, *J* = 1.6, 7.4 Hz), 7.74-7.87 (2H, m), 8.10 (1H, d, *J* = 5.0 Hz), 8.12-8.20 (1H, m), 8.24-8.34 (1H, m), 9.09 (1H, d, *J* = 5.0 Hz). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 14.5, 63.5, 111.5, 118.1, 121.1, 126.9, 126.9, 127.2, 129.6, 130.3, 130.4, 132.5, 133.8, 134.5, 135.0, 139.5, 153.6, 155.6, 158.0, 182.8, 183.0.

HRMS (ESI) *m/z* calculated for $[C_{21}H_{15}NO_3+H]^+$: 330.1125; found 330.1130.

(*E*)-1-Styrylbenzo[*g*]isoquinoline-5,10-dione (25g)

Purified by flash chromatography over silica gel applying a heptane-ethyl acetate gradient. R_f = 0.43 (Hept/EtOAc 75/25). Yield 57 mg (89 %, purity 98.1%), yellow solid, mp 180-182 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.30-7.36 (1H, m), 7.37-7.44 (2H, m), 7.70 (2H, d, *J* = 7.4 Hz), 7.73-7.79 (1H, m), 7.79-7.86 (1H, m), 7.93-7.98 (1H, m), 8.05 (1H, dd, *J* = 1.9, 15.6 Hz), 8.22 (1H, dd, *J* = 1.2, 7.5 Hz), 8.28 (1H, dd, *J* = 1.0, 7.7 Hz), 8.68 (1H, dd, *J* = 1.9, 15.6 Hz), 8.97 (1H, dd, *J* = 1.7, 4.7 Hz).

^{13}C NMR δ_C (100 MHz, $CDCl_3$) 118.2, 122.7, 125.7, 126.8, 127.6, 128.0, 128.8, 129.0, 132.2, 133.9, 134.5, 135.0, 136.7, 138.8, 140.6, 154.0, 157.0, 182.8, 184.2.

HRMS (ESI) *m/z* calculated for $[C_{21}H_{13}NO_2+H]^+$: 312.1019; found 312.1033.

1-(*p*-Nitrophenyl)benzo[*g*]isoquinoline-5,10-dione (25h)

This compound was synthesized by using solely toluene (3 mL) as the reaction solvent in the general procedure described above. Purified by flash chromatography over silica gel applying a heptane-ethyl acetate gradient and recrystallized with heptane/ethyl acetate. R_f = 0.50 (Hept/EtOAc 4/6). Yield 18 mg (26 %, purity 98.0%), off-white solid, mp 257 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.65 (2H, d, *J* = 8.1 Hz), 7.83-7.89 (2H, m), 8.13-8.20 (1H, m), 8.24 (1H, d, *J* = 4.7 Hz), 8.30-8.35 (1H, m), 8.36 (2H, d, *J* = 8.2 Hz), 9.13 (1H, d, *J* = 4.8 Hz). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 119.7, 123.4, 125.3, 127.2, 127.7, 129.7, 132.4, 133.8, 134.6, 135.4, 140.8, 147.2, 147.9, 153.9, 159.3, 182.1, 182.5.

HRMS (ESI) *m/z* calculated for $[C_{19}H_{10}N_2O_4+H]^+$: 331.0713; found 331.0719.

1-(*p*-Hydroxyphenyl)benzo[*g*]isoquinoline-5,10-dione (25i)

This compound was synthesized by using solely toluene (3 mL) as the reaction solvent in the general procedure described above. After following the general work-up procedure, the aqueous layer of the extraction was neutralized using an aqueous 1M HCl solution. The aqueous layer was then extracted with (5 × 20 mL) DCM. The combined organic fractions were dried over anhydrous $MgSO_4$, filtrated over a glass filter and the solvents were removed *in vacuo* to furnish **25i**, which did not need any further purification.

R_f = 0.26 (Hept/EtOAc 6/4). Yield 24 mg (40 %, purity 96.5%), yellow solid, mp 260 °C. ^{1}H NMR δ_H (400 MHz, DMSO-D6) 6.81 (2H, d, *J* = 8.3 Hz, OHCC*H*), 7.41 (2H, d, *J* = 8.3 Hz, OHCCHC*H*), 7.88-7.96 (2H, m, NCCCOCCHC*H*C*H*), 7.96 (1H, d, *J* = 4.7 Hz, NCHC*H*), 8.06 (1H, d, *J* = 7.1 Hz, NCCCOCC*H*), 8.18 (1H, d, *J* = 6.9 Hz, NCHCHCCOCC*H*), 9.02 (1H, d, *J* = 4.7 Hz, NC*H*), 9.68 (1H, s, O*H*). ^{13}C NMR δ_C (100 MHz, DMSO-D6) 115.0 (OHCC*H*), 117.7 (NCH*C*H), 125.3 (NCHCH*C*), 126.7 (NCHCHCCOC*C*H), 127.4 (NCCCOC*C*H), 131.4 (OHCCH*C*H), 131.7 (OHCCHCH*C*), 132.6 (NCCCO*C*), 134.5 ($CH_{arom.}$), 134.9 (NC*C*CO), 135.6 ($CH_{arom.}$), 141.3 (NCHCHCCO*C*), 153.5 (N*C*H), 158.6 (OH*C*), 160.7 (N*C*), 182.9 (NCHCHC*C*O), 183.5 (NCC*C*O)

HRMS (ESI) *m/z* calculated for $[C_{19}H_{11}NO_3+H]^+$: 302.0812; found 302.0801.

1-Methoxybenzo[*g*]isoquinoline-5,10-dione (26)

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Purified by flash chromatography over silica gel applying a heptane-ethyl acetate gradient. R_f = 0.79 (Hept/EtOAc 6/4). Yield 28 mg (56 %, purity 95%), yellow solid, mp 205 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 4.19 (3H, s), 7.73 (1H, d, *J* = 4.9 Hz), 7.77 (1H, dd, *J* = 7.4, 8.3 Hz), 7.83 (1H, d, *J* = 7.4, 8.2 Hz), 8.22 (1H, d, *J* = 7.5 Hz), 8.28 (1H, d, *J* = 7.5 Hz), 8.61 (1H, d, *J* = 5.0 Hz). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 55.0, 113.2, 114.7, 126.7, 127.4, 132.1, 133.6, 134.3, 135.0, 142.8, 153.1, 163.2, 181.4, 182.8.

HRMS (ESI) *m/z* calculated for $[C_{14}H_9NO_3+Na]^+$: 262.0475; found 262.0474.

3-(*o*-Tolyl)benzo[*g*]isoquinoline-5,10-dione (27a)

Purified by flash chromatography over silica gel applying a heptane-ethyl acetate gradient, subsequent recrystallization in heptane and subsequent purification by preparative TLC using DCM as eluent. R_f = 0.20 (Hept/EtOAc 9/1). Yield 27 mg (44 %, purity 95.9%), pale yellow solid, mp 179 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 2.46 (3H, s), 7.31-7.41 (3H, m), 7.51-7.56 (1H, m), 7.83-7.92 (2H, m), 8.21 (1H, d, *J* = 0.8 Hz), 8.30-8.39 (2H, m), 9.63 (1H, d, *J* = 0.8 Hz). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 20.5, 119.3, 124.2, 126.2, 127.3, 127.4, 129.5, 129.9, 131.3, 133.2, 133.3, 134.5, 135.0, 136.2, 138.7, 138.9, 149.4, 166.1, 182.5, 182.8. **HRMS** (ESI) *m/z* calculated for $[C_{20}H_{13}NO_2+H]^+$: 300.1019; found 300.1027.

3-(*p*-Ethoxyphenyl)benzo[*g*]isoquinoline-5,10-dione (27b)

Purified by flash chromatography over silica gel applying a heptane-ethyl acetate gradient. R_f = 0.42 (Hept/EtOAc 3/1). Yield 15 mg (45 %, purity 97.3%), yellow solid, mp 217-227 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 1.47 (3H, t, *J* = 7.0 Hz), 4.13 (2H, q, *J* = 7.0 Hz), 7.02-7.07 (2H, m), 7.82-7.91 (2H, m), 8.15-8.21 (2H, m), 8.31-8.38 (2H, m), 8.43 (1H, d, *J* = 0.8 Hz), 9.56 (1H, d, *J* = 0.7 Hz). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 14.8, 63.7, 114.3, 115.0, 123.9, 127.3, 127.4, 129.2, 130.1, 133.3, 133.4, 134.3, 135.0, 139.2, 150.0, 161.4, 162.5, 182.3, 183.0.

HRMS (ESI) *m/z* calculated for $[C_{21}H_{15}NO_3+H]^+$: 330.1125; found 330.1127.

(*E*)-3-Styrylbenzo[*g*]isoquinoline-5,10-dione (27c)

Purified by flash chromatography over silica gel applying a heptane-ethyl acetate gradient, subsequent recrystallization in heptane and subsequent purification by preparative TLC using DCM as eluent. R_f = 0.18 (Hept/EtOAc 9/1). Yield 30 mg (47 %, purity 98.5%), yellow solid, mp 199 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.26-7.46 (4H, m), 7.63 (2H, d, *J* = 7.3 Hz), 7.76-7.87 (2H, m), 7.90 (1H, d, *J* = 16.3 Hz), 8.07 (1H, s), 8.26-8.38 (2H, m), 9.50 (1H, s). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 117.0, 124.4, 126.8, 127.3, 127.4, 127.7, 128.9, 129.5, 133.2, 133.4, 134.3, 135.0, 135.8, 137.2, 139.1, 150.1, 161.5, 182.1, 182.8.

HRMS (ESI) *m/z* calculated for $[C_{21}H_{13}NO_2+H]^+$: 312.1019; found 312.1030.

General procedure – Buchwald-Hartwig aminations. To a 10 mL MW vial was added 50.0 mg (0.205 mmol) of 1-Cl-2-AAQ or 3-Cl-2-AAQ. Then, the corresponding aniline (0.205 mmol) was added to the vial and the contents were dissolved in 1,4-dioxane (2.5 mL). Then, $Pd_2(dba)_3$ (18.8 mg, 0.021 mmol), Cs_2CO_3 (167.0 mg, 0.513 mmol) and *rac*-BINAP (38.3 mg, 0.062 mmol) were added while stirring and the reaction mixture was carefully bubbled with argon for at least 5 minutes before closing the MW vial. The reaction mixture was then heated at 100 °C for 16 hours. When the reaction was complete, the reaction mixture was cooled to room temperature, quenched with water (10 mL), and extracted with DCM (3 × 10 mL). The combined organic fractions were dried over anhydrous $MgSO_4$, filtrated over a glass filter and the solvents were removed *in vacuo*.

1-(*p*-Tolylamino)benzo[*g*]isoquinoline-5,10-dione (28a)

Purified three times by preparative TLC, first eluent was heptane/ethyl acetate 8/1, second and third eluent was DCM. R_f = 0.67 (DCM). Yield 16 mg (25 %, purity 98.5%), blue-purple solid, mp 188 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 2.35 (3H, s, C*H*$_3$), 7.19 (2H, d, *J* = 8.2 Hz, NHCCHC*H*), 7.43 (1H, d, *J* = 4.8 Hz, NCHCHCCCC*H*), 7.61 (2H, d, *J* = 8.2 Hz, NHCC*H*), 7.77 (1H, dd, *J* = 7.5, 8.2 Hz, NCHCHCCCCHC*H*), 7.82 (1H, dd, *J* = 7.5, 8.1 Hz, NCCCCCHC*H*), 8.23 (1H, d, *J* = 7.6 Hz, NCHCHCCCC*H*), 8.30 (1H, d, *J* = 7.6 Hz, NCCCCC*H*), 8.66 (1H, d, *J* = 4.8 Hz, NC*H*), 11.52 (1H, s, N*H*). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 20.9 (*C*H$_3$), 108.5 (NHC*C*), 109.9 (NCH*C*H), 122.3 (CH$_3$CCH*C*H), 126.9 (NCHCHCCC*C*H), 127.0 (NCCCC*C*H), 129.5 (CH$_3$*C*CH), 132.5 (NCHCHC*C*), 133.7 (NCHCHCCCCHC*C*H), 133.8 ($C_{quat.}$), 134.0 ($C_{quat.}$), 134.9 (NCCCCH*C*H), 136.2 (CH$_3$*C*), 141.1 (NH*C*N), 156.2 (NCHCH*C*), 156.3 (N*C*H), 183.2 (NCHCHC*C*), 184.7 (NCC*C*).

HRMS (ESI) *m/z* calculated for $[C_{20}H_{14}N_2O_2+H]^+$: 315.1128; found 315.1141.

1-((*p*-Methoxyphenyl)amino)benzo[*g*]isoquinoline-5,10-dione (28b)

Purified by flash chromatography over silica gel applying a DCM-ethyl acetate. R_f = 0.16 (DCM). Yield 51 mg (74 %, purity 98.7%), royal blue solid, mp 172 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 3.82 (3H, s), 6.93 (2H, d, *J* = 8.9 Hz), 7.39 (1H, d, *J* = 4.8 Hz), 7.60 (2H, d, *J* = 8.9 Hz), 7.75 (1H, dd, *J* = 7.5, 8.3 Hz), 7.82 (1H, dd, *J* = 7.5, 8.3 Hz), 8.22 (1H, d, *J* = 7.5 Hz), 8.28 (1H, d, *J* = 7.5 Hz), 8.62 (1H, d, *J* = 4.7 Hz), 11.43 (1H, s). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 55.5, 108.3, 109.7, 114.2, 123.9, 126.9, 127.0, 131.9, 132.4, 133.7, 134.0, 134.8, 141.0, 156.2, 156.3, 156.5, 183.1, 184.5.

HRMS (ESI) *m/z* calculated for $[C_{20}H_{14}N_2O_3+H]^+$: 331.1077; found 331.1074.

1-((*p*-(Trifluoromethyl)phenyl)amino)benzo[*g*]isoquinoline-5,10-dione (28c)

Purified by flash chromatography over silica gel applying a heptane-ethyl acetate. R_f = 0.58 (Hept/EtOAc 75/25). Yield 51 mg (67 %, purity 99.3%), red solid, mp 192 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.53 (1H, d, *J* = 4.8 Hz), 7.61 (2H, d, *J* = 8.5 Hz), 7.78 (1H, dd, *J* = 7.4, 8.3 Hz), 7.84 (1H, dd, *J* = 7.4, 8.3 Hz), 7.92 (2H, d, *J* = 8.5 Hz), 8.23 (1H, dd, *J* = 0.8, 7.5 Hz), 8.29 (1H, dd, *J* = 0.8, 7.6 Hz), 8.69 (1H, d, *J* = 4.8 Hz), 11.79 (1H, s). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 109.3, 111.1, 120.8, 124.3 (q, *J* = 271.8 Hz), 125.1 (q, *J* = 32.6 Hz) 126.2 (q, *J* = 3.8 Hz), 127.1, 127.1, 132.4, 133.6, 134.1, 135.0, 141.0, 142.2 (q, *J* = 1.0 Hz), 155.3, 155.8, 182.6, 185.0.

HRMS (ESI) *m/z* calculated for $[C_{20}H_{11}F_3N_2O_2+H]^+$: 369.0845; found 369.0843.

1-((*p*-Chlorophenyl)amino)benzo[*g*]isoquinoline-5,10-dione (28d)

Purified by flash chromatography over silica gel applying a DCM-ethyl acetate gradient. R_f = 0.68 (DCM). Yield 40 mg (57 %, purity 98.8%), dark red solid, mp 226 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.34 (2H, d, *J* = 8.7 Hz), 7.51 (1H, d, *J* = 4.7 Hz), 7.74 (2H, d, *J* = 8.6 Hz), 7.79 (1H, dd, *J* = 7.4, 8.4 Hz), 7.86 (1H, dd, *J* = 7.6, 8.2 Hz), 8.26 (1H, d, *J* = 7.4 Hz), 8.32 (1H, d, *J* = 7.5 Hz), 8.69 (1H, d, *J* = 4.7 Hz), 11.64 (1H, s). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 108.9, 110.6, 123.0, 127.1, 127.1, 128.8, 129.0, 132.5, 133.8, 134.0, 135.0, 137.6, 141.1, 155.7, 156.0, 182.9, 184.9.

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HRMS (ESI) *m/z* calculated for $[C_{19}H_{11}ClN_2O_2+H]^+$: 335.0582; found 335.0587.

1-((*p*-Nitrophenyl)amino)benzo[*g*]isoquinoline-5,10-dione (28e)

Purified by applying a suspension of the crude product in DCM on a preparative TLC plate. The pure product solidified on top of the silica (and did not impregnate the silica), which was then carefully collected from the plate without scraping off the silica. The solid was then filtrated over a glass filter and the solvents were removed *in vacuo*. R_f = 0.74 (Hept/EtOAc: 4/6). Yield 59 mg (84 %, purity 98.5%), red-purple solid, mp 274-277 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 7.67 (1H, d, *J* = 4.8 Hz), 7.80-7.92 (2H, m), 8.05 (2H, d, *J* = 9.1 Hz), 8.22-8.32 (3H, m), 8.35 (1H, d, *J* = 7.7 Hz), 8.79 (1H, d, *J* = 4.8 Hz), 12.08 (1H, s). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 110.1, 112.1, 120.2, 125.1, 127.2, 127.3, 132.4, 133.6, 134.5, 135.2, 141.2, 142.7, 145.4, 154.9, 155.6, 182.4, 185.4.

HRMS (ESI) *m/z* calculated for $[C_{19}H_{11}N_3O_4+H]^+$: 346.0822; found 346.0820.

Due to poor solubility in $CDCl_3$ of **28e**, a major peak of water is visible in the spectrum of this compound. Other deuterated solvents (toluene-d8, THF-d8, DMSO-d6, $C_2Cl_4D_2$ and pyridine-d5) also failed to properly dissolve **28e**. An attempt to create the HCl-salt of **28e** was also made by adding 5 mL of a 1.25 M HCl in ethanol solution to the purified product and the resulting suspension was stirred at room temperature for two hours. The volatiles were removed to produce a brown oil, after which 20 mL of diethyl ether was added to induce formation of a dark brown solid. The volatiles were then removed. This solid, however, did not dissolve either in DMSO-d6 or D_2O.

3,3'-(*p*-Tolylazanediyl)bis(benzo[*g*]isoquinoline-5,10-dione) (30a)

Purified by flash chromatography over silica gel applying a heptane-ethyl acetate gradient, subsequent recrystallization in heptane and subsequent purification by preparative TLC using DCM/MTBE 98/2 as eluent. R_f = 0.33 (Hept/EtOAc 75/25). Yield 22 mg (42 %, purity 99.7%), yellow solid, mp 306 °C (decomposition). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 2.46 (3H, s, C*H*$_3$), 7.19 (2H, d, *J* = 8.1 Hz, CH_3CCHCH), 7.34 (2H, d, *J* = 8.0 Hz, CH_3CCH), 7.80 (2H, s, CHNCC*H*), 7.81 (2H, dd, *J* = 7.5, 8.0 Hz, NCCHCCOCCHC*H*), 7.87 (2H, dd, *J* = 7.4, 8.0 Hz, NCHCCOCCHC*H*), 8.28 (2H, d, *J* = 7.7 Hz, NCCHCCOCC*H*), 8.35 (2H, d, *J* = 7.6 Hz, NCHCCOCC*H*), 9.29 (2H, s, NC*H*). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 21.3 (*C*H$_3$), 111.8 (CHNC*C*H), 122.2 (NCCH*C*), 127.3 (NCHCCOC*C*H), 127.4 (NCCHCCOC*C*H), 128.2 (CH_3CCHCH), 131.4 (CH_3CCH), 133.3 (NCHCCO*C*), 133.6 (NCCHCCOC*C*H), 134.2 (NCCHCCOCCH*C*H), 135.0 (NCHCCOCCH*C*H), 138.5 (CH_3C), 139.7 (CH_3CCHCH*C*), 140.4 (NCH*C*), 150.1 (N*C*H), 161.3 (CHN*C*), 181.2 (NCHC*C*O), 182.5 (CHNCCHC*C*O).

HRMS (ESI) *m/z* calculated for $[C_{33}H_{19}N_3O_4+H]^+$: 522.1448; found 522.1443.

3,3'-(*o*-Tolylazanediyl)bis(benzo[*g*]isoquinoline-5,10-dione) (30b)

Purified by flash chromatography over silica gel applying a heptane-ethyl acetate gradient. R_f = 0.74 (Hept/EtOAc 4/6). Yield 41 mg (77 %, purity 98.9%), orange solid, mp 276 °C. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 2.09 (3H, s), 7.29 (1H, s), 7.37-7.48 (3H, m), 7.78 (2H, s), 7.81 (2H, dd, *J* = 7.4, 8.1 Hz), 7.86 (2H, dd, *J* = 7.4, 8.1 Hz), 8.26 (2H, d, *J* = 7.5 Hz), 8.32 (2H, d, *J* = 7.6 Hz), 9.29 (2H, s). ^{13}C NMR δ_C (100 MHz, $CDCl_3$) 18.1, 110.9, 122.0, 127.3, 127.4, 128.3, 129.3, 129.5, 132.3, 133.3, 133.6, 134.1, 135.0, 136.8, 140.4, 140.7, 150.1, 160.4, 181.2, 182.4.

HRMS (ESI) *m/z* calculated for $[C_{33}H_{19}N_3O_4+H]^+$: 522.1448; found 522.1469.

General Biology

Evaluation of antitubercular activity

In vitro anti-mycobacterial activity of the synthesized compounds was evaluated by a luminometric assay, which is based on a *Mtb* H37Ra laboratory strain (ATCC® 25177™) transformed with a pSMT1 luciferase reporter plasmid (H37Ra^{lux}). The tested compounds were solubilized in DMSO (Sigma-Aldrich) at stock concentration of 10 mM. A two-fold serial dilution of each compound was made in liquid Middlebrook 7H9 broth (Sigma-Aldrich) with 10% oleic acid, albumin, dextrose, catalase (OADC) enrichment (BD Biosciences) with final concentrations ranging from 64 to 0.25 µM. Volumes of 20 µL of the serial dilutions were added in triplicate to flat-bottomed 96-well plates. Then, a bacterial suspension was made by thawing and dissolving a frozen glycerol-stock of H37Ra^{lux} in 7H9-10% OADC. The dissolved pellet was passed through a 5.0 µM filter (Millipore) to eliminate clumps and left to recover at 37 °C. Next, the bacterial suspension was diluted in 7H9-10% OADC to obtain a suspension of 50.000 relative light units (RLU)/mL. Subsequently, a volume of 180 µL of the bacterial suspension was added to each well. Isoniazid was used as a positive control. Bacterial replication was analyzed by luminometry after 7 days of incubation at 37°C. 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. Light emission in each well was measured using a luminometer (Promega Discover).

Cytotoxicity evaluation

Acute cytotoxic effects on the J774 A.1 cell line (ATCC® TIB-67™) was determined for the derivatives by a neutral red uptake assay. The neutral red uptake assay relies on the ability of viable cells to bind and incorporate the neutral red dye, i.e. toluene red. The acute cytotoxic concentration (CC_{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%. The J774 A.1 cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco) with 10% inactivated fetal calf serum (iFCS; Gibco) until a semi-confluent layer of cells was obtained. The cells were trypsinized, washed with sterile phosphate-buffered saline (PBS; Gibco), seeded at 40.000 cells per well in a transparent, flat-bottomed 96-well plate and left for recovery at 37°C, 5% CO_2. The following day, a two-fold serial dilution of each compound was made in DMEM with 10% iFCS with final concentrations ranging from 128 to 0.50 µM. Subsequently, the J774 A.1 cells were washed and exposed to the derivatives in triplicate by adding 100 µL of the serial dilutions to the wells. The plates were left for incubation at 37°C, 5% CO_2 for 24 hours. Tamoxifen was used as a positive control. After exposure, the cells were washed with 200 µL of PBS, and 200 µL of neutral red working solution (Sigma) was added to each well. Subsequently, the plates were incubated for 3 hours at 37°C, 5% CO2. Then, the cells were washed twice with 200 µL of PBS and 150 µL of an ethanol/acetic acid (50%) mixture was added to each well. The plates were left on

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the shaker until the color became homogeneous purple and the optical density was measured at 530 nm (NR max) and 620 nm (reference wavelength) using a plate reader (Promega Discover)).

Assessment of genotoxicity by VITOTOX™

Observations on early signs of genotoxicity were assessed with the VITOTOX™ test and the included protocol was followed. Briefly, both the Genox (RecN2-4) and the Cytox pr1 strain were diluted 250 times and cultivated at 36°C for 16 hours in poor 869 medium. After incubation, the bacterial cultures were diluted 10 times more and incubated for 1 hour at 36°C. To test the genotoxic properties of the metabolites of the analogs, S9-mix was added to the designated +S9 cultures. The bacterial suspensions were exposed to the analogs at a final concentration of 100 µM and then incubated with shaking at 36°C. As a positive control, 4-nitroquinolone-1-oxide was used in samples without S9 liver fraction, whereas benzo[*a*]pyrene was used in samples with S9 fraction. The luminescent signal was measured in real time for 4 hours with a 5 minute interval.

Conflicts of interest

There are no conflicts to declare.

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