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Synthesis of New Quinolinequinone Derivatives and Preliminary Exploration of their Cytotoxic Properties

Charles M. Keyari, Alison K. Kearns, Nathan S. Duncan, Emily A. Eickholt, Geoffrey Abbott, Howard D. Beall* and Philippe Diaz*

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ABSTRACT

A series of 7-amino- and 7-acetamidoquinoline-5,8-diones with aryl substituents at the 2-position were synthesized, characterized and evaluated as potential NAD(P)H:quinone oxidoreductase (NQO1)-directed antitumor agents. The synthesis of lavendamycin analogs is illustrated. Metabolism studies demonstrated that 7-amino- analogues were generally better substrates for NQO1 than 7-amido- analogues as were compounds with smaller heteroaromatic substituents at the C-2 position. Surprisingly, only two compounds, 7-acetamido-2-(8'-quinolinyl)quinoline-5,8-dione (**11**) and 7-amino-2-(2-pyridinyl)quinoline-5,8-dione (**23**) showed selective cytotoxicity toward the NQO1-expressing MDA468-NQ16 breast cancer cells versus the NQO1-null MDA468-WT cells. For all other compounds, NQO1 protected against quinoline-5,8-dione cytotoxicity. Compound **22** showed a potent activity against human breast cancer cells expressing or not expressing NQO1 with IC50 values of respectively 190 nM and 140 nM and a low NQO1 mediated reduction rate, which suggests that the mode of action of **22** differs from lavendamycin and involves an unidentified target(s).

KEYWORDS: Lavendamycin, Suzuki coupling, microwave irradiation, palladium (0) catalysis, quinolinequinones, NQO1, antitumor, cytotoxicity

INTRODUCTION

Lavendamycin (Figure 1) is a quinolinequinone antibiotic with antitumor activity first isolated from *Streptomyces lavendulae* by Balitz et al in 1982.[1-2] It is structurally related to Streptonigrin which was first isolated from *Streptomyces flocculus*.[3-4] Streptonigrin is known for its potent cytotoxic properties, antitumor activity, and *in vitro* and *in vivo* antiviral properties and potent, broad spectrum antimicrobial properties. Although lavendamycin is not suitable for clinical use due to its toxicity, its analogs are less

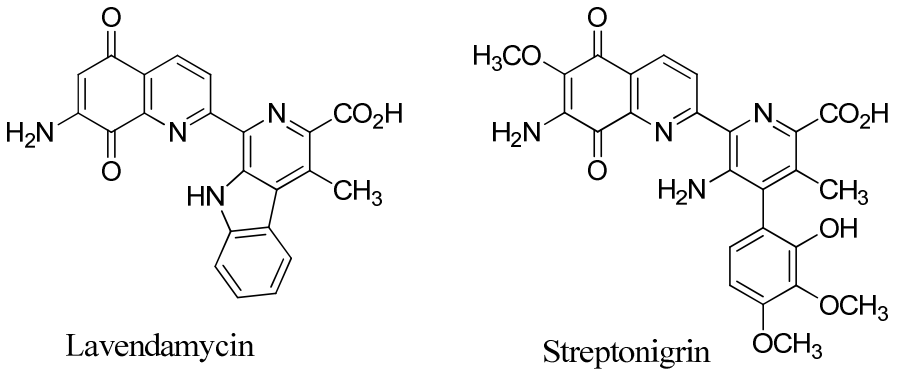


Figure 1. Natural quinolinequinone antibiotics

toxic and hence have potential as antitumor agents.[5] Recent findings[6-11] suggest that some indolequinones and quinolinequinones are excellent substrates for the quinone reductase enzyme, NAD(P)H:quinone oxidoreductase 1 (NQO1), and are selectively cytotoxic to cancer cell lines that overexpress NQO1. NQO1 is a ubiquitous flavoenzyme that catalyzes the 2-electron reduction of quinones to hydroquinones, and it is highly expressed in many solid tumors.[12] This forms the basis for the synthesis of novel quinolinequinones structurally related to lavendamycin as potential NQO1-directed antitumor agents.

Behforouz *et al.* (1996)[13] first demonstrated that 7-aminoquinoline-5,8-diones can be efficiently prepared from commercially available 8-hydroxy-2-methylquinoline. Fryatt and co-workers[7] also showed that by starting with 6-methoxyquinoline, 6-methoxy-2-chloroquinoline-5,8-dione was prepared and subsequent palladium(0) catalyzed reaction with boronic acids gave novel quinoline quinones under reflux for 24 hours. Further, in 2004, [14] arylboronic acids were shown to be more reactive than their counterparts,

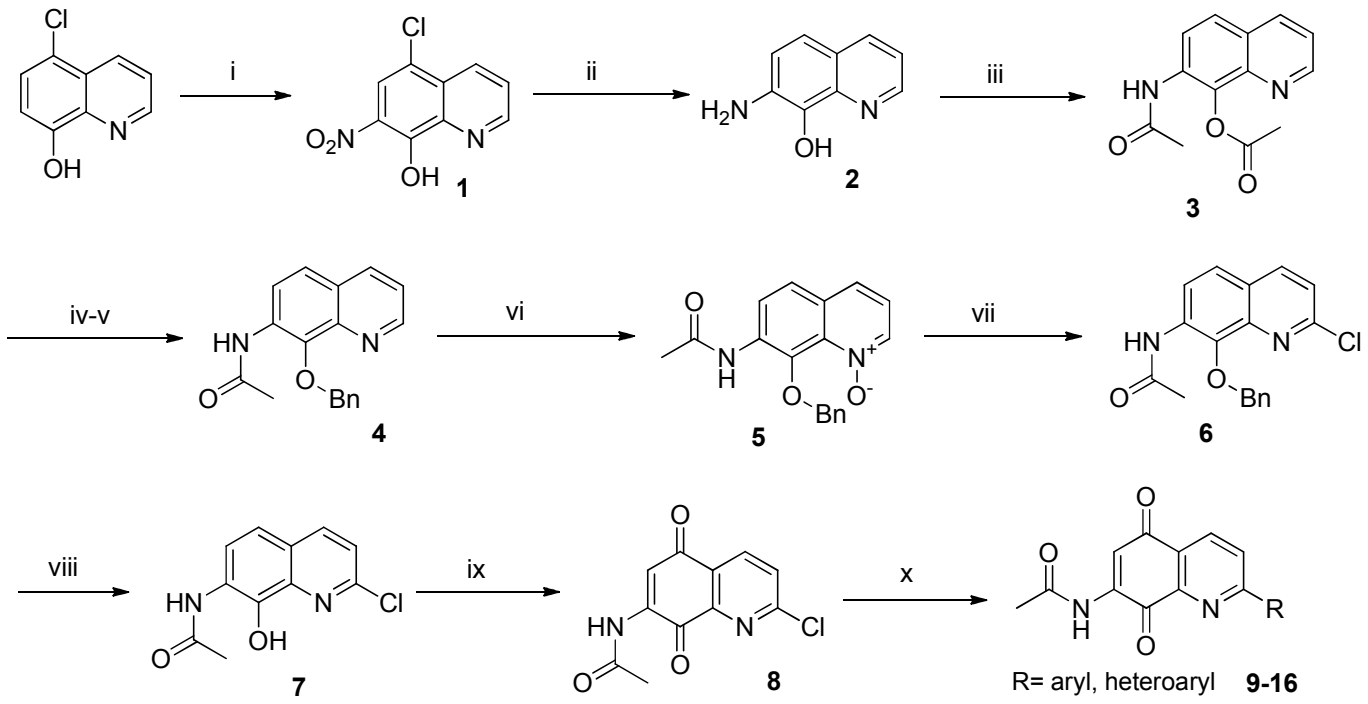
the arylpinacolboronate esters when reacted with indole bromides in Suzuki couplings under reflux. The lower reactivity was attributed to steric factors in the arylpinacolboronate esters. Also, 3-aryl-indazoles have been synthesized by the reaction of haloindazoles (3-bromoindazole and 3-iodoindazole) with aryl boronic acids under Pd(0) catalysis in Suzuki type cross couplings. [15] The reaction times ranged from 1 -18 hours under reflux conditions. In this present study we report a direct more efficient approach to 7-aminoquinoline quinones starting from commercially available 7-amino-8-hydroxyquinoline under microwave conditions where the reaction times are shorter. Computational, metabolism and cytotoxicity studies on the quinoline-5,8-diones are also described.

CHEMISTRY

The synthesis commenced with the nitration of 5-chloro-8-hydroxyquinoline under HNO_3/H_2SO_4 according to a procedure reported by Musser *et al.*[16] to give the 5-chloro-7-nitro-8-hydroxyquinoline (**1**) in good yield (79%). Hydrogenation under Pd/C-catalysis at 40-50 psi not only reduced the nitro group to the free amine but also removed the chloride to provide the desired 7-amino-8-hydroxyquinoline (**2**) in excellent yield (99%). A direct approach to the amino alcohol **2** involves the heating of a mixture of 8-hydroxyquinoline and N-methyl-N-phenylhydrazine at 90°C, albeit very low yields were obtained. [17]Our attempt to synthesize the amino alcohol by heating in a microwave between 130-160°C did not improve the yield. Acetylation proceeded smoothly where both the amino and hydroxyl groups were protected. The resulting diacetylated product (**3**) was hydrolyzed in $MeOH/H_2O$ under reflux to form 7-acetamido-8-hydroxyquinoline. Subsequent benzylation of the free hydroxyl was effected by reacting with $BnBr/K_2CO_3$ in DMF at 50°C for 24 hrs to give the 7-acetamido-8-benzyloxyquinoline (**4**) in 90% yield. Oxidation using *m*CPBA in 1,2-dichloroethane at rt for 48 hrs gave the N-oxide (**5**) in 82% yield.[18] The key intermediate in the synthesis, the 2-chloro-7-acetamido-8-benzyloxyquinoline (**6**), was obtained in 62% yield by refuxing the N-oxide with $POCl_3$ in $CHCl_3$.[19] The high regioselectivity of the reaction can be rationalized in terms of sterics as well as formation of an oxyphosphorane adduct anion in a rapid concerted mechanism. [20] We also

attempted refluxing the N-oxide **5** with SO_2Cl_2 as reported in literature,[9] but only resulted in massive decomposition of the starting material. Deprotection of the benzyl group was effected with $BCl_3 \bullet SMe_2$ in CH_2Cl_2 and subsequent oxidation using Fremy's salt (potassium nitrosodisulfonate-$(KO_3S)_2NO$) gave the 7-acetamido-2-chloro-quinoline-5,8-dione (**8**) in 71% yield. [7] The results are summarized in Scheme 1 below.

Scheme 1[a]

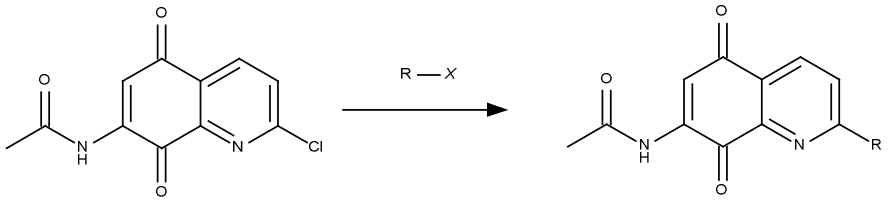


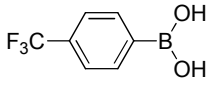
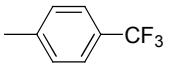
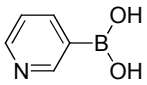
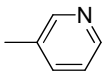
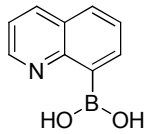
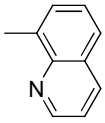
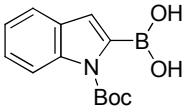
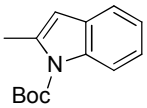
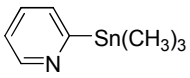
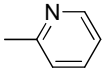
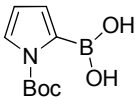
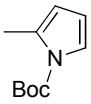
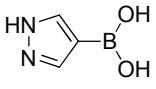
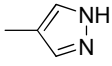
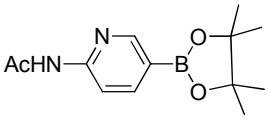
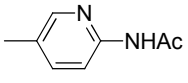
[a]Reagents and conditions: (i) HNO_3/H_2SO_4; (ii) H_2/Pd-C, MeOH, 40-50psi, overnight; (iii) CH_3COCl, DIEA, THF, 2hrs.; (iv) H_2O-MeOH, reflux, 1hr; (v) BnBr, K_2CO_3, DMF, 50°C, 24 hrs; (vi) *m*CPBA, $ClCH_2CH_2Cl$, 48hrs; (vii) $POCl_3$, $CHCl_3$, reflux, 2 hrs; (viii) $BCl_3 \cdot SMe_2$, CH_2Cl_2, overnight; (ix) Fremy's salt, rt, 1 hr; (x) $RB(OH)_2$, $Pd(PPh_3)_4$, 110-140°C, μW 20-25 min.

After the successful formation of the quinolinequinone **8**, the stage was now set for Suzuki coupling chemistry. This was accomplished by reaction with different boronic acids under Pd(0) catalysis in a microwave as illustrated in Table 1 below. Generally, the reactions were complete within 20-30 minutes in good yields except for the arylboronate ester where only 27% of the product (**16**) was obtained. The mechanistic details of the reaction have been well studied in which case oxidative addition, transmetallation

and reductive elimination being the most critical steps.[21] Interestingly, the 7-amino-2-(2-pyridyl)quinoline-5,8-dione was prepared in 9 steps starting from 3-hydroxybenzoic acid where the key step was a

Table 1. Suzuki coupling products



R-X	Reaction conditions	R	Yield (%)
	$Pd(PPh_3)_4$, DME/Na_2CO_3 140°C, 20 min	 (**9**)	70
	$Pd(PPh_3)_4$, DME/Na_2CO_3 120°C, 20 min	 (**10**)	41
	$Pd(PPh_3)_4$, DME/Na_2CO_3 120°C, 20 min	 (**11**)	51
	$Pd(PPh_3)_4$, DME/Na_2CO_3 110°C, 25 min	 (**12**)	67
	*$Pd(PPh_3)_4$, p-dioxane 120°C, 20 min	 (**13**)	71
	$Pd(PPh_3)_4$, DME/Na_2CO_3 110°C, 25 min	 (**14**)	53
	$Pd(PPh_3)_4$, DME/Na_2CO_3 120°C, 20 min	 (**15**)	42
	$PdCl_2$(dppf), p-dioxane,K_3PO_4 120°C,30 min	 (**16**)	27

* Stille coupling reaction

Friedlander condensation of 2-acetylpyridine and 2-amino-3-benzyloxy-4-bromobenzaldehyde to give the 8-benzyloxy-7-bromo-2-(2'-pyridyl)quinoline.[22] Although this seems an attractive strategy, the method lacks the flexibility needed to create a library of lavendamycin analogs.

The final step in the synthesis involved the removal of the acetate protecting group which was effected by reaction with H_2SO_4-MeOH at rt. The Boc-protected derivatives were also subjected to TFA/CH_2Cl_2 at rt for 2 hrs to provide the 7-acetamido derivatives.

ELECTROCHEMISTRY

Eletrochemistry was performed to compare the electrochemical behavior of the quinolinequinones with their reduction rates by NQO1, and the data are shown in Table 2. Tetrahydrofuran was used as the solvent for all compounds except **15**, which was run in DMSO. The compounds were run against a Ag/AgCl electrode cathodic and anodic peak potentials, E_{pc} and E_{pa}, respectively, were measured at a potential sweep rate of 50 mV/sec, and the midpoint of the peak potentials was used to determine $E_{1/2}$ values, (E_{pc} + E_{pa})/2. Unfortunately, many of the analogues did not show reversible electrochemistry, and in some cases, there were multiple reduction peaks. This makes interpretation of the numbers somewhat difficult, but some conclusions can be drawn. For instance, most of the acetylated quinolinequinones had a reduction peak between -1.08V and -1.18V, an indication that they are easier to reduce than the non-acetylated compounds due to the presence of this electron-withdrawing group. This is consistent with what we reported previously for lavendamycins. However, there was no correlation between reduction potentials and reduction rates by NQO1, in line with previous publications on this topic.[6-8, 23, 24] This suggests that steric interactions are more likely to be predictive of substrate efficiency than reduction potentials.

NMR SPECTROSCOPY AND SPECTROPHOTOMETRY

Complexation of zinc(II) triflate by compounds **19**, **22** and **23** was studied using ^{1}H NMR spectroscopy. No new peaks were observed in NMR spectra, indicating that free and complexed forms of zinc(II) triflate

were in a rapid exchange relative to NMR timescale. The aromatic region of the NMR spectrum of compound **19** in THF-D_8 at room temperature is shown below in Figure 2.

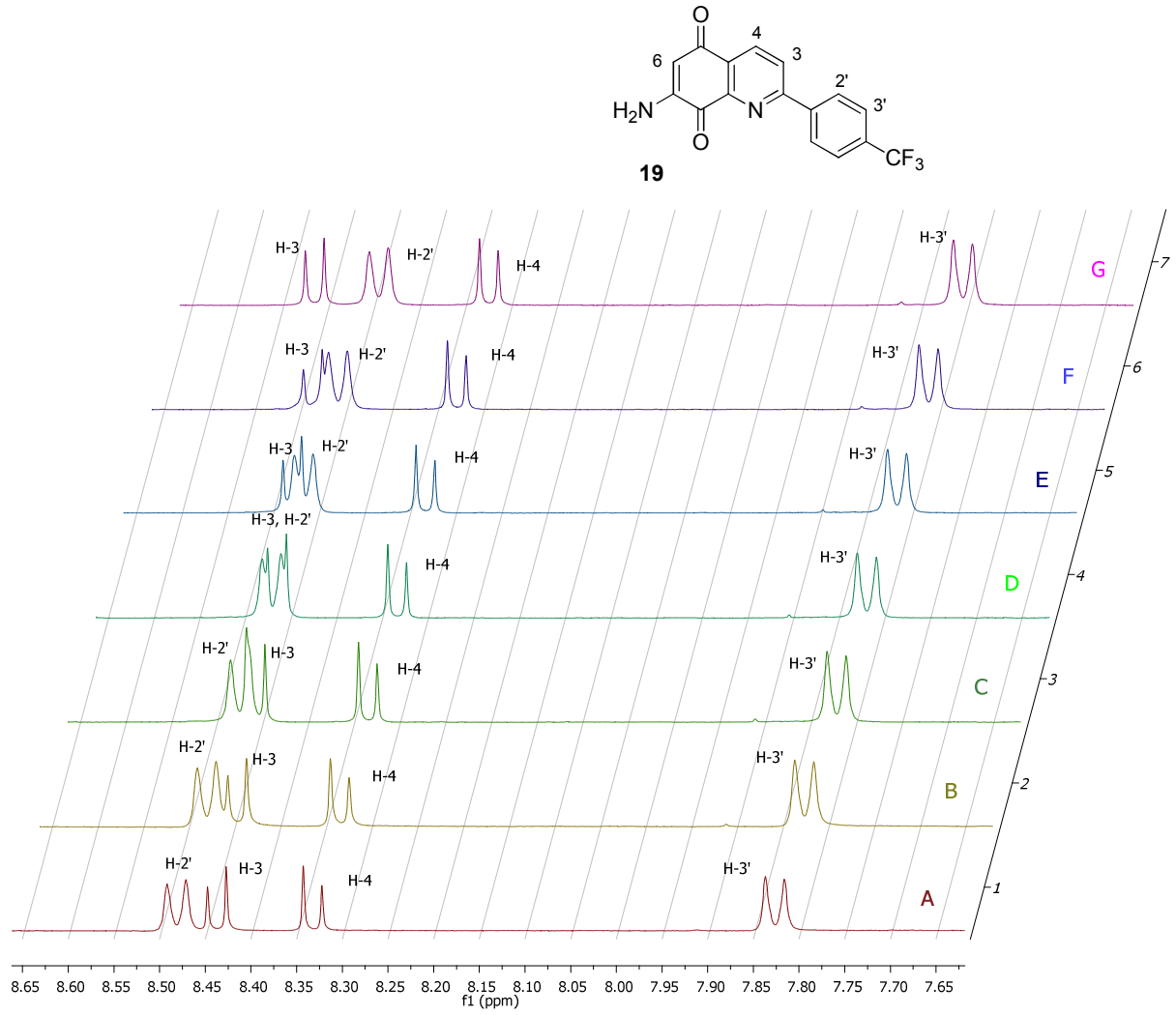


Figure 2. Aromatic proton region NMR spectra of **19** upon addition of increasing equivalent of $Zn(SO_3CF_3)_2$ in [$^2H_8$]THF. Note the change in δ of H-2' and H-3 on addition of Zn^{2+}. Equivalents of Zn^{2+}: A=0, B=1, C=2, D=3, E=4, F=5 and G=10.

There was a small difference in chemical shifts of H-2' (moving upfield) and H-3 (moving downfield) after addition of one equivalent zinc(II) triflate to the NMR solution (Table S1 and Figure 2) whereas the changes in δ of the other protons were barely noticeable. The biggest change in δ of H-2' (-0.04 ppm) and H-3' (+0.07 ppm) occurs after addition of ten equivalents of $Zn(SO_3CF_3)_2$. This suggests that weak binding occurs at low Zn^{2+} concentration.

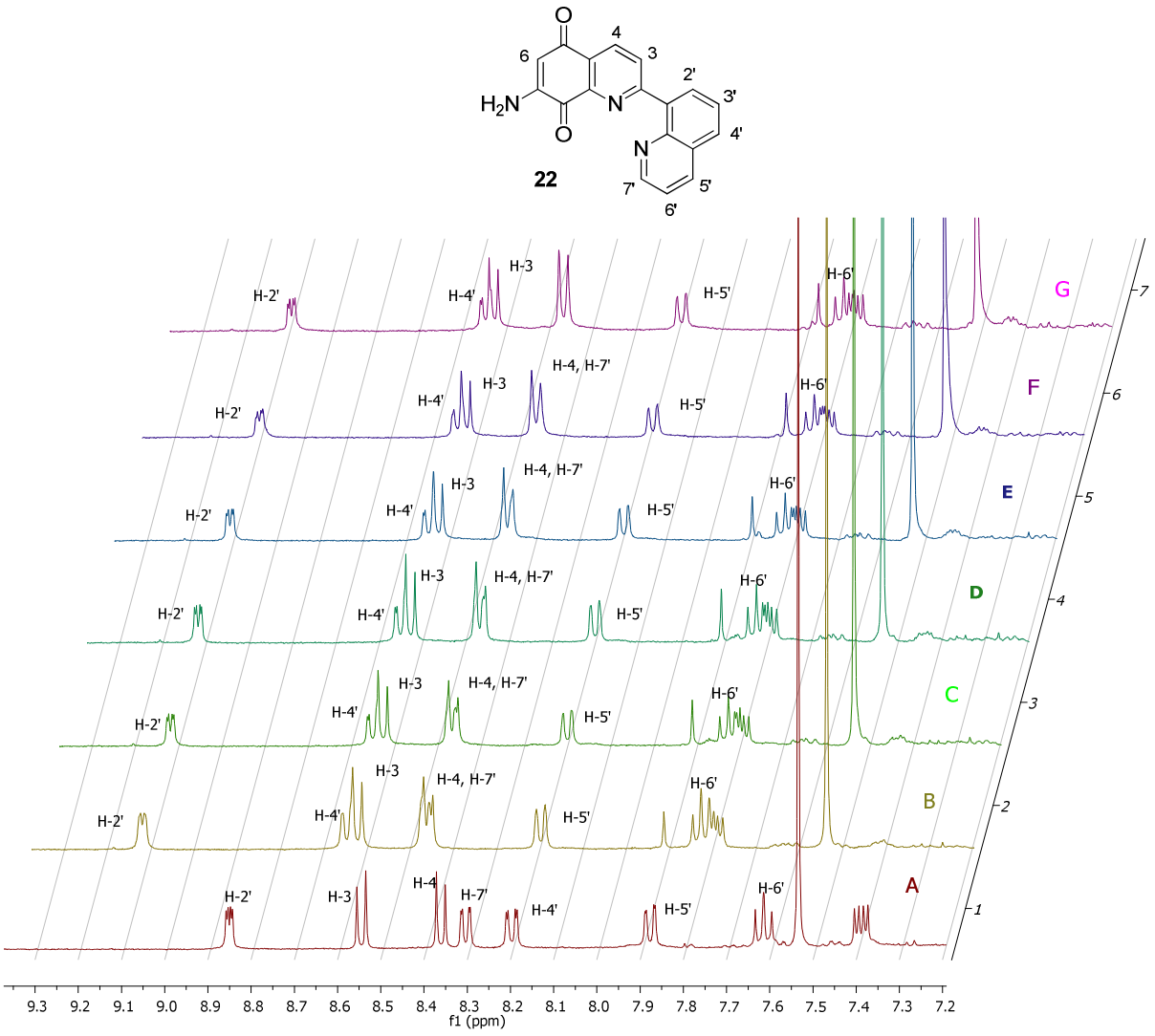


Figure 3. Aromatic protons region NMR spectra of **22** upon addition of increasing equivalent of $Zn(SO_3CF_3)_2$ in [$^2H_8$]THF. Equivalents of Zn^{2+}: A=0, B=1, C=2, D=3, E=4, F=5 and G=10.

In contrast, addition of only one equivalent of $Zn(SO_3CF_3)_2$ to compound **22** caused larger chemical shift variations of all the protons (Table S2 and Figure 3) Increasing the amount of Zn^{2+} (2-10 equiv.) added to compound **22** made little or no difference in δ afterwards (>0.01 ppm). This means that the quinoline derivative binds the Zn^{2+} more efficiently than compound **19** and only one equivalent of Zn^{2+} is enough to cause chemical shift variations.

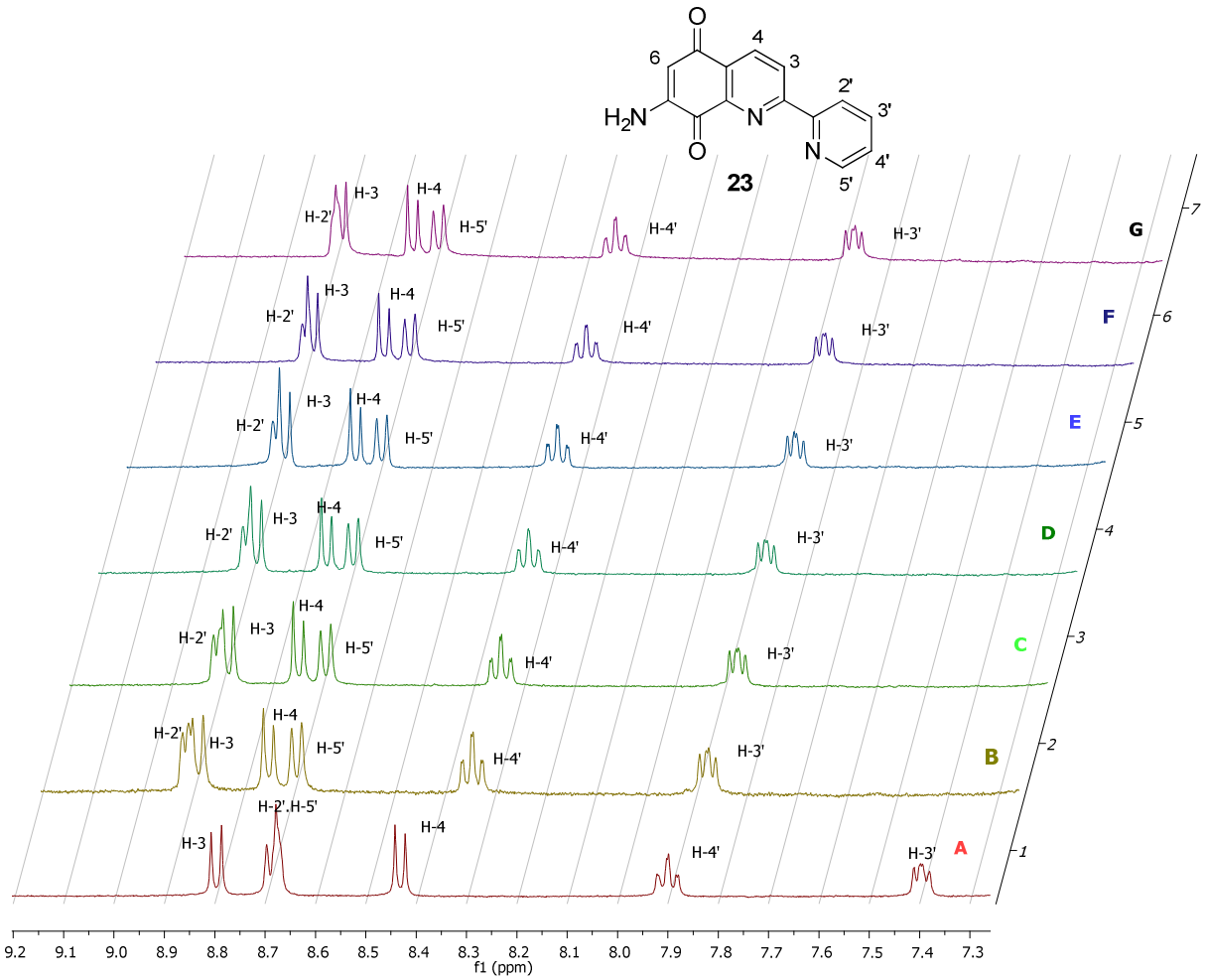


Figure 4. Aromatic proton region NMR spectra of **23** upon addition of increasing equivalent of $Zn(SO_3CF_3)_2$ in [$^2H_8$]THF. Equivalents of Zn^{2+}: A=0, B=1, C=2, D=3, E=4, F=5 and G=10.

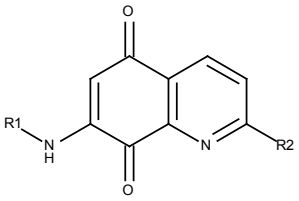
Similar observations were made with compounds **23** (Table S3 and Figure 4) and **13** (Table S4). This is consistent with the results reported by Long and Harding[25] where they demonstrated that the 1:1 bipyridyl complex of streptonigrin was the major complex formed at room temperature by perfoming an NMR study in [$^2H_8$]THF with addition of Zn^{2+}. Titration of compound **23** with Zn^{2+} in a mixture of dimethylsulfoxide-methanol (1:3) was monitored by a spectrophotometer as reported in literature.[26] A plot of $\Delta\lambda_{355}$ against Zn^{2+} concentration gave a moderate affinity constant of $1.41\times10^4\ M^{-1}$ for compound **23** binding with Zn^{2+}.

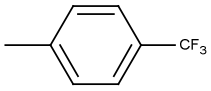
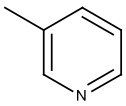
RESULTS AND DISCUSSION

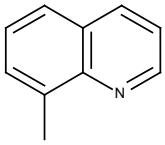
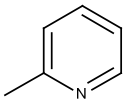
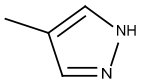
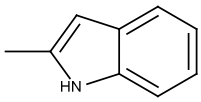
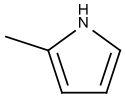
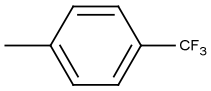
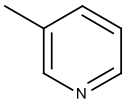
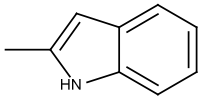
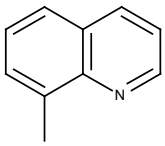
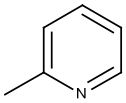
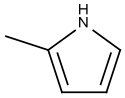
Quinolinequinone metabolism by recombinant human NQO1 was examined using a spectrophotometric assay that employs cytochrome c as the terminal electron acceptor. Initial rates of reduction (μmol

cytochrome c reduced/min/mg NQO1) were calculated from the linear portion (0-30 s) of the reaction graphs. The 7-acetamido-2-(2-pyridinyl) compound **13** displayed the highest reduction rate by NQO1 (Table 2), although it was the only acetylated analogue with a high reduction rate. In all other cases, 7-amino compounds had much higher reduction rates than corresponding 7-acetamido compounds with identical substituents at the quinoline-2-position. Although unusual, higher rates for acetylated analogues have been observed in other series.[6, 11] With regard to the aromatic substituents at the quinoline-2-position, no clear trend in reduction rates was observed except that bulkier groups generally decreased reduction rates. Oxygen consumption is a measure of the ability of the reduced quinone (hydroquinone) to redox cycle following reduction by NQO1. This could lead to production of toxic reactive oxygen species and ultimately to cell death. Oxygen consumption was measured for select quinolinequinones, and the trend, if not the magnitude, mirrored the reduction rates. (Table 2).

Table 2. Reduction rates[a] and oxygen consumption[b] as a result of quinoline-5,8-dione metabolism by recombinant human NQO1 and electrochemical reduction potentials vs ferrocene[c]. Reduction rates were determined by spectrophotometric cytochrome *c* assay and oxygen uptake by oxygen electrode.



No.	R1	R2	Reduction rate by hNQO1 (µmol cyt *c* reduced /min/mg NQO1	Oxygen Consumption (µmol/min/mg NQO1)	Reduction Potential ($E_{1/2}$(V) vs Fc)
9	CH_3CO		4.5 +/- 0.5	5.2 +/- 1.0	-1.93 nr[d]
10	CH_3CO		25 +/- 4	-	-1.17, -1.92 nr

11	CH_3CO		7.0 +/- 0.3	-	-1.18, -1.68, -1.77
13	CH_3CO		480 +/- 200	34 +/- 3	-1.90 nr
15	CH_3CO		16 +/- 1	-	-1.08, -1.36, -1.58
17	CH_3CO		2.8 +/- 0.2	-	-1.10, -1.60, -1.94
18	CH_3CO		31 +/- 9	-	-
19	H		78 +/- 7	-	-
20	H		170 +/- 30	-	-1.99 nr
21	H		80 +/- 8	-	-1.84 nr
22	H		18 +/- 6	-	-1.53, -1.65
23	H		71 +/- 13	-	-1.85 nr
24	H		120 +/- 10	8.5 +/- 1.6	-

[a]Spectrophotometric assay using cytochrome c as terminal electron acceptor (550 nm).

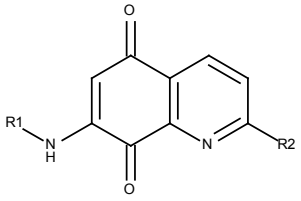
[b]Oxygen concentration monitored using an oxygen electrode.

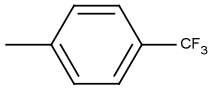
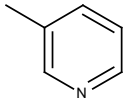
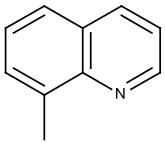
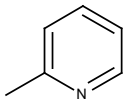
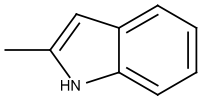
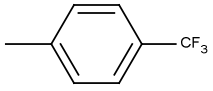
[c]$E_{1/2}$ values calculated as $(E_{pc}+E_{pa})/2$ are average values from voltammograms recorded potential sweep rate of 50 mV/sec. E_{pc} = cathodic peak potential; E_{pa} = anodic peak potential.

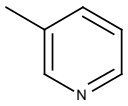
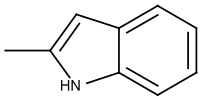
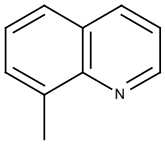
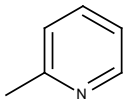
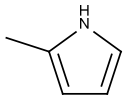
[d]nr = non-reversible, anodic peak only.

Cell survival was measured using the MTT colorimetric assay. In previous work, we demonstrated that IC_{50} values generated from standard clonogenic assays and MTT assays were positively correlated suggesting that the MTT assay is a reliable indicator of cytotoxicity. [6] We utilized MDA-MB-468 human breast cancer cells stably transfected with human NQO1 cDNA (MDA468-NQ16) along with the non-transfected wild type cells (MDA468) to compare the cytotoxicity of the quinolinequinones (Table 3).[27]

Table 3. Cytotoxicity of quinoline-5,8-diones toward MDA468-WT (NQO1-deficient) and MDA468-NQ16 (NQO1-rich) human breast cancer cell lines.



No.	R1	R2	IC_{50} (μM) MDA468-WT	IC_{50} (μM) MDA468-NQ16	Selectivity Ratio IC_{50} (WT)/ IC_{50} NQ16
9	CH_3CO		1.7 +/- 0.8	2.4 +/- 1.9	0.73
10	CH_3CO		3.3 +/- 0.1	6.3 +/- 0.2	0.52
11	CH_3CO		0.80 +/- 0.33	0.64 +/- 0.41	1.2
13	CH_3CO		0.53 +/- 0.27	2.2 +/- 0.5	0.24
17	CH_3CO		7.4 +/- 5.0	19.1 +/- 5.9	0.39
19	H		5.3 +/- 0.8	17 +/- 5	0.31

20	H		5.6 +/- 1.3	15 +/- 2	0.37
21	H		4.8 +/- 0.9	10 +/- 1	0.47
22	H		0.14 +/- 0.02	0.19 +/- 0.04	0.75
23	H		19 +/- 12	5.3 +/- 2.1	3.5
24	H		4.5 +/- 1.9	17 +/- 2	0.26

Quinolinequinone cytotoxicity (IC_{50}) to MDA468 cells was generally in the single digit micromolar range following 2-h exposures with some in the high nanomolar range (**11**, **13**, **22)**. Surprisingly, selectivity ratios [IC_{50} (MDA468) / IC_{50} (MDA468-NQ16)] were generally <1 meaning that the quinolinequinones were less cytotoxic to the NQO1-rich MDA468-NQ16 cells rather than more cytotoxic. This suggests that NQO1 was protective to the cells rather than functioning as an activating enzyme.[27] Only two compounds (**11**, **23**) were selectively cytotoxic to the MDA468-NQ16 cells. The reason for the general absence of selective cytotoxicity with this particular series of compounds is unclear, but it is consistent with NQO1's primary role as a detoxification enzyme.[27]

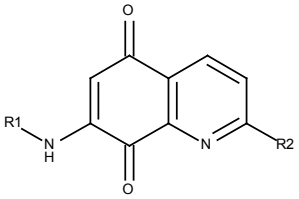
Molecular docking of the quinolinequinones in the NQO1 active site was performed using Sybyl 8.1.1 and GOLD 5.1 for scoring. Three good NQO1 substrates (**13**, **20**, **24**) and three poor NQO1 substrates (**9**, **11**, **17**) were docked and scored using ChemPLP and ChemScore (Table 4). The highest scores representing a good fit for the model were found for **20** and **24** consistent with the metabolism data. The exception again was **13**, which scored the lowest, but was the best substrate. Interatomic distances between quinolinequinone carbonyl groups and FAD N5 and His161 were shortest for **20**, but all were within a

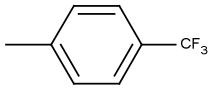
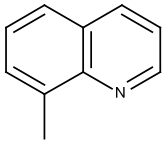
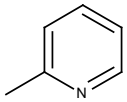
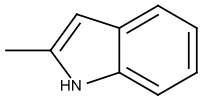
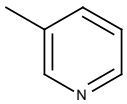
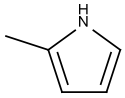
reasonable distance for hydride transfer from FAD when the dynamic effects of the quinone-enzyme interaction are considered. Figure **5** shows possible docking conformations for **20** and **11** with NQO1. All quinolinequinones orient with the quinone ring above the FAD isoalloxazine ring as needed for hydride transfer.

The mechanism of action of lavendamycin and streptonigrin is not clearly understood. However previous studies demonstrated that quinone moieties are reduced by NQO1 to the corresponding hydroquinones which undergo auto-oxidation producing activated oxygen species including not only the semiquinone derivatives but also superoxide and hydroxyl radicals.[28] In addition, both streptonigrin and lavendamycin chelate divalent cationic metal ions. This property might confer to streptonigrin and lavendamycin the ability to shuttle iron cations into the cells which in turn can catalyze production of reactive oxygen species through a Fenton reaction. On the other hand, this chelation can result in depletion of intracellular cationic metals which might result in cell death. [29]Generation of the semiquinone radical, after reduction of the quinone to the hydroquinone followed by auto-oxidation, results in a decrease of activity in 9 compounds. The best NQO1 substrates are less active compounds (**13**, **20**, **24**) in NQO1 expressing cells. In contrast, poor NQO1 substrates such as compound **22** or **11**, exhibit the best activity in both cancer cells expressing NQO1 and not expressing NQO1. According to the NMR experiments, the quinoline derivative **22** and compound **13** binds the Zn^{2+} more efficiently than compound **19;** and only one equivalent of Zn^{2+} is enough to cause important chemical shift variations. Similar observations were made with compound **23,** which was less cytotoxic than compound **22**. Even though metal chelation by these compounds is still a plausible mechanism to explain their activity against breast cancer cells, another mode of action cannot be discarded. Most active compounds (**11**, **13** and **22**) are potential tridentate ligands for metals. Compound **23** exhibits lower activity than the corresponding acetylated amino analogue **13**. It was proposed that metals can assist tautomeric shift from the active quinone analogues to the quinoid analogue which has an isoelectronic structure with the biologically inactive azastreptonigrin.[24] This tautomeric shift can explain the decrease of activity of the amino derivative compared to the amido derivative. In our series of aryl

substituted quinonequinolines the active molecule is the quinone derivatives and not the semiquinone derivatives. A similar mode of action than the bidentate metal ligands derivatives 8-hydroxyquinone is currently under investigation. [30, 31]

Table 4. Computational parameters for selected quinoline-5,8-diones.



No.	R^1	R^2	ChemPLP	ChemScore	C=O8… NH5 ((Å)	C=O5… His161NE2 (Å)
9	CH_3CO		63.2	22.6	3.9	3.6
11	CH_3CO		63.8	22.6	4.7	3.3
13	CH_3CO		57.6	21.4	4.3	3.5
17	CH_3CO		63.8	22.1	4.2	3.3
20	H		72.8	26.0	3.6	3.2
24	H		67.3	22.7	4.1	3.3

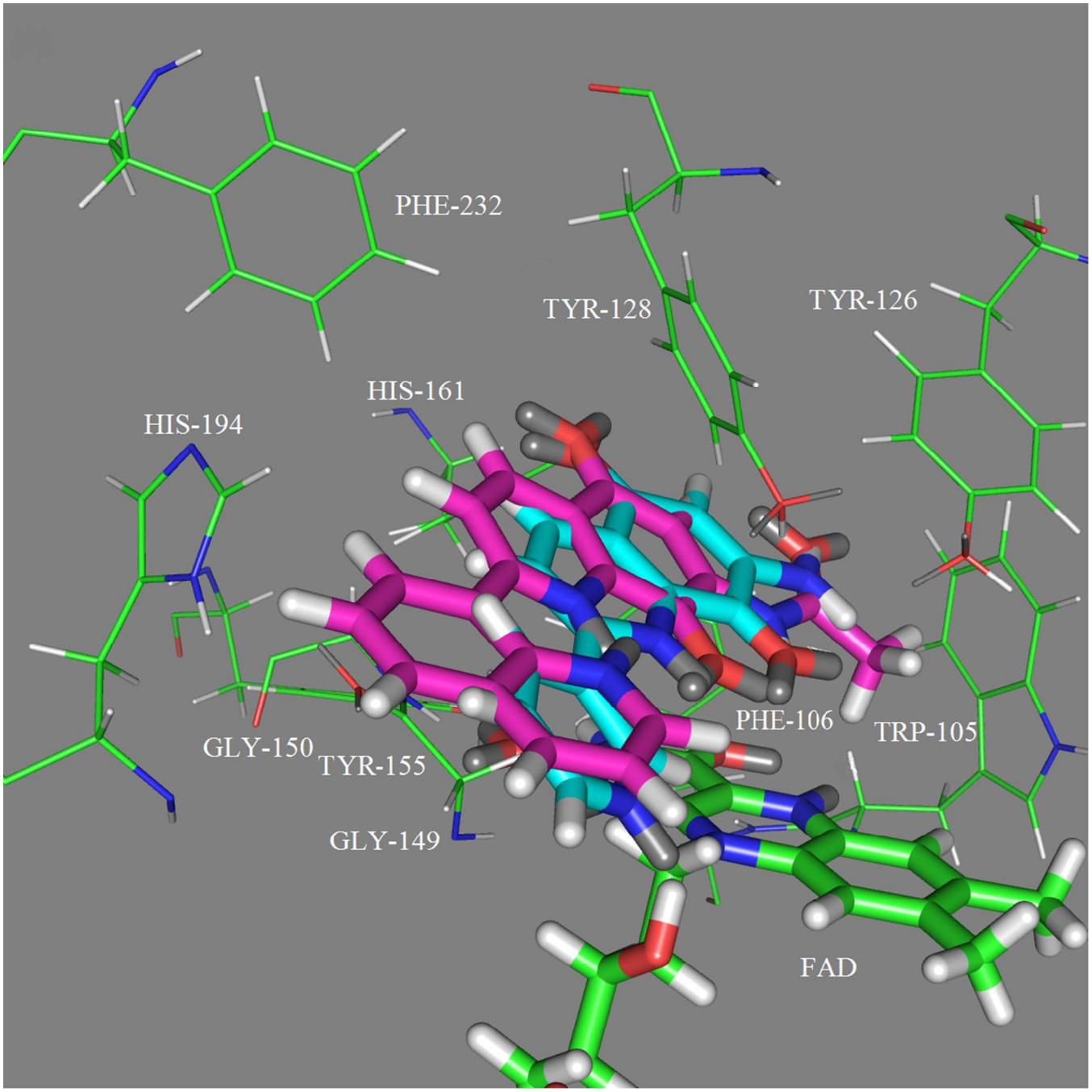


Figure 5. Quinolinequinones docked in NQO1 active site: **20**, cyan; **11**, magenta; FAD, green.

CONCLUSIONS

A six step synthetic scheme led to good yields for quinolinequinone analogs of lavendamycin projected as NQO1-directed antitumor agents. Unexpectedly, ten of eleven analogs demonstrated excellent

cytotoxicity (IC_{50} values of single digit micromolar or better) towards MDA468 breast cancer cells, but only two were selectively cytotoxic to NQO1-expressing MDA468-NQ16 cells. Compounds **22** and **11** are poor NQO1 substrates and exhibit the best activity against breast cancer cells. In our novel series of aryl substituted quinonequinolines the active molecule appears to be the quinone derivatives and not the semiquinone derivatives resulting from NQO1 reduction, suggesting that the mode of action of this novel series differs from lavendamycin and involves an unidentified target. Quinolinequinone derivatives **11**, **13** and **22** cytotoxicities (IC50) to MDA468 cells were in the high nanomolar range. Our results seem to indicate that compounds **11**, **13** and **22** effects could be also, at least partially, mediated by metal chelation. These aryl quinonequinoline derivatives represent a novel promising class of cytotoxic agents with a potential novel therapeutic value.

EXPERIMENTAL SECTION

Cell Culture. MDA-MB-468 (MDA468) human breast cancer cells and stably NQO1-transfected MDA468-NQ16[32] were a gift from Dr. David Ross (University of Colorado-Denver, Denver, CO). MDA468 cells had no measurable NQO1 activiy whereas activity in MDA468-NQ16 cells was 1070 nmol/min/mg total cell protein using dichlorophenolindophenol (DCPIP) as the standard electron acceptor. Cells were grown in RPMI 1640 medium with L-glutamine and penicillin/streptomycin, and supplemented with 10% fetal bovine serum (FBS). Cell culture medium and supplements were obtained from Invitrogen (Carlsbad, CA). The cells were incubated at 37 °C under a humidified atmosphere containing 5% CO_2.

Spectrophotometric cytochrome c assay. Quinolinequinone reduction was monitored using a spectrophotometric assay in which the rate of reduction of cytochrome c was quantified at 550 nm. Briefly, the assay mixture contained cytochrome c (70 μM), NADH (1 mM), recombinant human NQO1 (0.1-10 μg) (gift from Dr. David Ross, University of Colorado-Denver, Denver, CO) and quinonlinequinones (25 μM) in a final volume of 1 mL Tris-HCl (25 mM, pH 7.4) containing 0.7 mg/mL BSA and 0.1% Tween-20. Reactions were carried out at room temperature and started by the addition of NADH. Rates of reduction

were calculated from the initial linear part of the reaction curve (0-30 s) and results were expressed in terms of μmol of cytochrome c reduced/min/mg of NQO1 using a molar extinction coefficient of 21.1 mM^{-1} cm^{-1} for cytochrome c. All reactions were carried out at least in triplicate.

Oxygen Consumption. Oxygen concentration was monitored using a MI-730 Micro-Oxygen Electrode (Microelectrodes, Bedford, NH) with concentrations adjusted for temperature (25 °C). Assay mixtures contained 25 μM quinonlinequinones, 200 μM NADH and 1 μg/mL NQO1 in a 2 mL Tris-Hcl-BSA/Tween (0.1%) buffer system. Reactions were started with NADH and measured over 3 minute intervals at room temperature. All reactions were carried out in triplicate.

Electrochemistry. Cyclic Voltammagrams were collected for 10 analogues using a BAS CV-50W electrochemical analyzer using a standard 3 electrode cell. Experiments were performed using an Ag/AgCl reference electrode, a glossy carbon working electrode and a platinum wire auxiliary electrode. The reported potentials are referenced by the Ferrocene (0/+) couple in the solvent used, primarily THF, which occurs at +.60V vs. Ag/AgCl. The compounds were run at concentrations of 1mM in THF, except compound 15 which was run in DMSO, with a.1M concentration of tetrabutylammonium hexafluorophosphate as a supporting electrolyte. All samples were purged and run under an Ar atmosphere during the course of the experiment, and the electrodes were washed and wiped down between each sample. Each CV was collected at a sweep rate of 50mV/s in the potential range of 0V to -2V at room temperature of 21°C.

NMR spectroscopy. One-dimensional ^{1}H NMR spectra were recorded at room temperature on Bruker Avance IIITM spectrometer (The Woodlands, Texas) at 400 MHz using a 5-mm probe and a simple pulse-acquire sequence. Acquisition parameters consisted of spectral width of 4000 Hz with an acquisition time 3.98 s, number of scans of 128, and relaxation delay of 1 s. Complexes were prepared in a mixture of $CDCl_3$ and THF-D_8.

Cell Viability Assay. Growth inhibition was determined using the MTT colorimetric assay. Cells were plated in 96-well plates at a density of 10,000 cells/mL and allowed to attach overnight (16 h). Quinolinequinone solutions were applied in medium for 2 hours, removed and replaced with fresh medium, and the plates were incubated at 37 °C under a humidified atmosphere containing 5% CO_2 for 3-5 days. MTT (50 μg) was added and the cells were incubated for another 4 hours. Medium/MTT solutions were removed carefully by aspiration, the MTT formazan crystals were dissolved in 100 μL DMSO, and absorbance was determined on a plate reader at 560 nm. IC_{50} values (concentration at which cell survival equals 50% of control) were determined from semi-log plots of percent of control vs. concentration. Selectivity ratios were defined as the IC_{50} value for the MDA468 cell line divided by the IC_{50} value for the MDA468-NQ16 cell line.

Molecular Modeling. For docking purposes, the crystallographic coordinates of the human NQO1 complex with 3-(hydroxymethyl)-5-(2-methylaziridin-1-yl)-1-methyl-2-phenylindole-4,7-dione (**25**) were obtained from the Brookhaven Database (PDB code 1H69[33] and resolution 1.86Å) and was edited accordingly to provide a monomer of the protein. The protein complex was then minimized within Sybyl 7.3 (Tripos Ltd., St Louis) while holding all heavy atoms stationary. The ligand was then removed to leave the receptor complex which was used for the subsequent docking studies. For preparation of ligand structures, fragments from Sybyl 8.1.1 were used to construct the compounds and all symmetric compounds were prepared as monoanionic ligands. Ligands were subject to 1000 iterations of energy minimization using the Powell method with MMFF94s force field standard method. For computational docking, the GOLD 5.1 software was used in combination with the ChemPLP[34] scoring function (rescoring with ChemScore.[35] The active site was defined as being any volume within 8Å of the quinone scaffold of **25** in its crystal pose in 1H69. Each GA run comprised using the default parameters of: 100000 genetic operations on an initial population of 100 members divided into five subpopulations with weights for crossover, mutation, and migration being set to 95, 95, and 10, respectively. GOLD allows a user-definable number of GA runs per ligand, each of which starts from a different orientation. For these experiments, the

number of GA runs was set to 10, and scoring of the docked poses was performed with the ChemPLP scoring function using ChemScore rescore. Each GOLD run was saved and the strongest scoring binding pose of each ligand (subject to a rmsd default distance threshold of 1.5Å) was compared to that of the reference ligand position observed in the crystal structure. The best output pose (orientations) of the ligands generated were analyzed based on its ChemPLP/ChemScore score, feasibility of hydride transfer process and H-bonding to the enzyme. The best pose(s) were visualized using PyMOL Molecular Graphics System version 1.3.

Chemistry. All moisture sensitive reactions were performed in an inert, dry atmosphere of argon in flame dried glassware. Air sensitive liquids were transferred via syringe or cannula through rubber septa. Reagent grade solvents were used for extraction and flash chromatography. THF was distilled from Na/benzophenone under argon; dichloromethane (CH_2Cl_2) and chloroform ($CHCl_3$) were distilled from CaH_2 under argon. All other reagents and solvents which were purchased from commercial sources, were used directly without further purification. The progress of reactions was checked by analytical thin-layer chromatography (Sorbent Technologies, Silica G TLC plates w/UV 254). The plates were visualized first with UV illumination followed by charring with ninhydrin (0.3% ninhydrin (w/v), 97:3 EtOH-AcOH). Flash column chromatography was performed using prepacked Biotage SNAP cartridges on a Biotage Isolera One instrument. Microwave reactions were performed using a Biotage Initiator instrument. The solvent compositions reported for all chromatographic separations are on a volume/volume (v/v) basis. ^{1}HNMR spectra were recorded at 400 or 500 MHz and are reported in parts per million (ppm) on the δ scale relative to tetramethylsilane as an internal standard. ^{13}CNMR spectra were recorded at 100 or 125 MHz and are reported in parts per million (ppm) on the δ scale relative to CDCl3 (δ 77.00). Melting points were determined on a Stuart melting point apparatus from Bibby Scientific Limited and are uncorrected. High Resolution mass spectrometry (HRMS) was performed on a Waters/Micromass LCT-TOF instrument. All compounds were more than 95% pure.

*5-chloro-8-hydroxy-7-nitroquinoline (**1**).* This compound was prepared according to the literature [12] procedure to yield a yellow solid, 4.40 g (79%). M.p. 198-200°C, [lit.[12], m.p. 192-194°C]; ^{1}H NMR (500 MHz, DMSO) δ 9.09 (dd, *J* = 4.2, 0.5 Hz, 1H), 8.58 (dd, *J* = 8.5, 0.8 Hz, 1H), 8.18 (s, 1H), 7.94 (dd, *J* = 8.5, 4.3 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 150.5, 150.1, 139.9, 133.6, 132.3, 128.5, 125.9, 122.0, 117.9. HRMS (TOF MS ES+) for $C_9H_6ClN_2O_3^+$ (MH+) calcd. 225.0067, found 225.0055.

*7-Amino-8-hydroxyquinoline (**2**).* Compound **1** (2.4 g, 10.69 mmol) was placed in a hydrogenation apparatus equipped with a magnetic stir bar and methanol added. Pd/C (150 mg) in a small amount of MeOH (60 mL) was added and stirring commenced. H_2 gas was introduced at a pressure of 40-50 psi and reacted at rt overnight. TLC showed full conversion. The black solution was filtered using a celite pad and concentrated under reduced pressure to yield **2** as a black oil, 99% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66 (dd, *J* = 4.4, 1.6 Hz, 1H), 8.03 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.24 (d, *J* = 8.7 Hz, 1H), 7.17 (dd, *J* = 8.2, 4.4 Hz, 1H), 7.10 (d, *J* = 8.7 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.0, 137.9, 136.6, 136.1, 132.1, 122.4, 119.3, 118.5, 117.7. HRMS (TOF MS ES+) for $C_9H_9N_2O^+$ (MH+) calcd. 161.0715, found 161.0707.

*7-acetamido-8-acetyloxyquinoline (**3**).* Compound **2** (330 mg, 2.06 mmol) was dissolved in dried THF (10 mL) and DIEA added with stirring. AcCl (176 μL) in 1mL THF was added drop wise while stirring and reacted at rt for 2 hrs. Then concentrated under reduced pressure followed by redissolving in CH_2Cl_2 (20 mL) and water (10 mL). The two layers were allowed to partition and extracted 2x 20 mL CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Then purified on a Biotage SNAP cartridge (25 g) at a flow rate of 25 mL/min to yield an orange solid, 382 mg (76%); m.p. 151-153°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.85 (dd, *J* = 4.1, 1.3 Hz, 1H), 8.49 (d, *J* = 9.1 Hz, 1H), 8.13 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.70 (d, *J* = 9.1 Hz, 1H), 7.67 (s, 1H), 7.36 (dd, *J* = 8.2, 4.2 Hz, 1H), 2.56 (s, 1H), 2.04 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.7, 168.5, 150.6, 140.7, 135.8, 134.9, 130.8, 125.8, 125.6, 121.3, 120.6, 24.5, 21.0; HRMS (TOF MS ES+) for $C_{13}H_{13}N_2O_3^+$ (MH+) calcd. 245.0926, found 245.0923.

*7-acetamido-8-benzyloxyquinoline (**4**).* To a solution of **3** (1.2 g, 4.91 mmol) in MeOH (100 mL) was added water (10 mL) and the reaction stirred under reflux for 1 hr. The black solution was concentrated and in vacuo and flash chromatographed on a KP-Sil 100 g Biotage SNAP cartridge using MeOH: DCM as the solvent (0-5% MeOH). A white solid (0.9 g) obtained and used for the next step directly. R_f= 0.11 (5% MeOH:CH_2Cl_2).

To a solution of 7-acetamido-8-hydroxyquinoline (2.27 g, 11.23 mmol) in 40 mL DMF was added K_2CO_3 (2.33 g, 16.80 mmol) and BnBr (2 mL, 16.80 mmol) respectively. The reaction was stirred at 50°C for 24 hrs after which TLC showed almost all the starting material was consumed. The reaction mixture was diluted with 30 mL CH_2Cl_2, filtered with a pad of celite and concentrated under reduced pressure. The residue was loaded onto a 100 g Biotage SNAP cartridge by dissolving in a small amount of CH_2Cl_2 and eluted with EtOAc:heptane gradient (0-50%). Yield 2.95 g (90%) of a yellow oil was obtained. R_f= 0.50 (60% EtOAc:heptane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.58 (d, *J* = 9.0 Hz, 1H), 8.14 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.77 (s, 1H), 7.57 (d, *J* = 9.0 Hz, 1H), 7.40 – 7.35 (m, 6H), 5.49 (s, 2H), 1.93 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.3, 150.0, 142.0, 141.0, 137.4, 136.2, 132.0, 128.9, 128.8, 128.8, 126.0, 124.0, 120.0, 120.0, 77.3, 24.6. HRMS (TOF MS ES+) for $C_{18}H_{17}N_2O_2^+$ (MH+) calcd. 293.1290, found 293.1264.

*7-acetamido-8-(benzyloxy)quinoline-1-oxide (**5**).* The starting material (**4**) (428 mg, 1.46 mmol) was dissolved in 4.3 mL 1,2-dichloroethane with stirring. The *m*CPBA (340 mg, 1.76 mmol) was added (0.5 M) and the reaction stirred at rt for 48 hrs. TLC showed almost all the starting material was consumed. The precipitated *m*CPBA was filtered and washed with 5 mL 1, 2-dichloroethane. The filtrate was concentrated under reduced pressure and flash chromatographed on a KP-sil 100 g Biotage SNAP cartridge using a 5% MeOH: DCM gradient at a flow rate of 25 mL/min to yield a yellow solid, 373 mg (82%). M.p. 145-147°C; R_f 0.24 (5%MeOH:DCM). ^{1}H NMR (500 MHz, DMSO) δ 9.45 (s, 1H), 8.46 (d, *J* = 6.1 Hz, 1H), 8.20 (d, *J* = 8.9 Hz, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 7.77 (d, *J* = 9.0 Hz, 1H), 7.58 – 7.50 (2H), 7.40 – 7.30 (aromatic, 4H). ^{13}C NMR (126 MHz, DMSO) δ 168.9, 139.8, 138.1, 137.1, 136.4, 133.3, 129.8, 129.1, 128.1, 128.0,

124.7, 124.4, 120.8, 77.7, 23.8. HRMS (TOF MS ES+) for $C_{18}H_{17}N_2O_3^+$ (MH+) calcd. 309.1239, found 309.1227.

*7-acetamido-8-benzyloxy-2-chloroquinoline (**6**).* Phosphoryl chloride (280 μL, 3.0 mmol) in $CHCl_3$ (1.0 mL) was added to a stirred solution of the oxide **5** (770 mg, 2.50 mmol) in 21 mL $CHCl_3$ and stirred for 15 min. The mixture was then refluxed for 2 hrs, cooled and poured into ice (50 g) and the pH adjusted to 12 with NaOH (aq.). The aq. layer was extracted with 2 x 50 mL CH_2Cl_2, washed with 2 x 20 mL H_2O, dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield a brown oil. Then purified on a HP-Sil 25 g Biotage SNAP cartridge using EtOAc:heptane gradient (0-50%) as the solvent. Yield 504 mg (62%) of an off-white solid was obtained. R_f= 0.58 (60% EtOAc:heptane); M.P. 92-94°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.60 (d, *J* = 9.0 Hz, 1H), 8.06 (d, *J* = 8.5 Hz, 1H), 7.81 (s, 1H), 7.54 (d, *J* = 9.0 Hz, 1H), 7.45 – 7.35 (m, 1H), 7.32 (d, *J* = 8.5 Hz, 1H), 5.48 (s, 1H), 1.96 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.4, 150.5, 141.4, 140.3, 139.0, 137.2, 133.0, 128.9, 128.8, 128.8, 124.3, 123.3, 121.1, 120.1, 77.4, 24.7. HRMS (TOF MS ES+) for $C_{18}H_{16}ClN_2O_2^+$ (MH+) calcd. 327.0900, found 327.0936.

*7-acetamido-2-chloro-8-hydroxyquinoline (**7**).* To a solution of **6** (330 mg, 1.01 mmol) in CH_2Cl_2 (10.1 mL) under an Ar atmosphere was added BCl_3•SMe_2 (10.1 mL) via a syringe and stirred at rt overnight. TLC showed the reaction was complete. The reaction was then quenched with saturated $NaHCO_3$(aq.) and extracted with 2x20 mL CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified on 50 g KP-Sil Biotage SNAP cartridge using a MeOH: CH_2Cl_2 gradient (0-5% MeOH) at a flow rate of 25 mL/minute to give a yellow solid, 198 mg (82%). M.P. 176-178°C; R_f= 0.50 (5% MeOH:CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, *J* = 9.0 Hz, 1H), 8.05 (d, *J* = 8.5 Hz, 1H), 7.82 (brs, 1H), 7.72 (s, 1H), 7.35 (d, *J* = 9.0 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 1H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.6, 149.7, 138.9, 138.2, 137.1, 124.4, 123.3, 121.5, 121.3, 118.0, 24.9. HRMS (TOF MS ES+) for $C_{11}H_{10}ClN_2O_2^+$ (MH+) calcd. 237.0431, found 237.0424.

*7-Acetamido-2-chloroquinoline-5,8-dione (**8**).* To a solution of **7** (300 mg, 1.27 mmol) in acetone (30 mL) was added a solution of Fremy's salt in NaH_2PO_4 buffer (0.3 M, 30 mL) and the mixture stirred at rt for 1

hr. A further solution of Fremy's salt in the buffer (0.3M, 30 mL) was added and stirring continued for 2 hrs. The acetone was removed under reduced pressure and the residue extracted with 2 x 50 mL CH_2Cl_2. The CH_2Cl_2 phases were combined, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified on a 25 g HP-Sil Biotage SNAp cartridge using EtOAc:heptanes gradient (0-60%) to obtain a yellow solid, 225 mg (71% over 2 steps); m.p. 224-226°C (decomposes into a black mass), R_f= 0.49 (60% EtOAc:heptane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.39 (d, *J* = 8.2 Hz, 1H), 7.97 (s, 1H), 7.74 (d, *J* = 8.2 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 183.4, 178.1, 169.5, 156.7, 145.9, 140.4, 137.2, 129.9, 128.0, 116.3, 25.1. HRMS (TOF MS ES+) for $C_{11}H_8ClN_2O_3^+$ (MH+) calcd. 251.0223, found 250.0203.

General procedure for Suzuki coupling under microwave conditions. The 7-acetamido-2-chloroquinoline-5,8-dione **8** (21 mg, 0.08 mmol) was dissolved in 4 mL dimethoxyethane (DME) and degassed under reduced pressure. The palladium (0) catalyst, $Pd(PPh_3)_4$ (10 mg, 0084 mmol) was added and the solution degassed further. The mixture was stirred under Ar atmosphere for 10 minutes. Na_2CO_3 solution (0.2 mL, 2.0 M) was added followed by the boronic acid (0.126 mmol). The mixture was then heated using a Biotage microwave initiator at 110-140°C for 20 minutes. After cooling, TLC showed all the starting material was consumed. The reaction mixture was poured into DCM and washed with 2 x 10 mL water. Then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified on HP-Sil 25 g Biotage SNAP cartridge using EtOAc:heptane gradient (0-50%) at a flow rate of 20 mL/min. For very polar products, MeOH:CH_2Cl_2 (0-10%MeOH) was used as solvent for purification.

*7-acetamido-2-(4-(trifluoromethyl)phenyl)quinoline-5,8-dione (**9**).* Yield 21 mg (70%) of a yellow solid was obtained. R_f= 0.47 (50% EtOAc:heptane); m.p. 250°C(decomposes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, *J* = 8.2 Hz, 1H), 8.45 (s, 1H), 8.27 (d, *J* = 8.1 Hz, 2H), 8.17 (d, *J* = 8.2 Hz, 1H), 7.99 (s, 1H), 7.80 (d, *J* = 8.2 Hz, 2H), 2.35 (s, 4H).^{13}C NMR (126 MHz, $CDCl_3$) δ 184.1, 179.1, 169.5, 160.1, 146.1, 140.6,

135.7, 128.3, 128.0, 126.0, 126.0, 126.0, 125.3, 116.5, 25.2; HRMS (TOF MS ES+) for $C_{18}H_{12}F_3N_2O_3^+$(MH+) calcd. 361.0800, found 361.0834.

*7-acetamido-2-(3-pyridinyl))quinoline-5,8-dione (**10**).* Yield 21 mg (41%) of a yellow solid obtained**,** R_f= 0.19 (5% MeOH:DCM); m.p. >300°C(decomposes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.72 (d, *J* = 3.9 Hz, 1H), 8.56 (d, *J* = 8.2 Hz, 1H),8.55 (m, 1H), 8.21 (d, *J* = 8.2 Hz, 1H), 8.00 (s, 1H), 7.56 (dd, *J* = 8.0, 4.9 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 184.2, 179.0, 170.4, 158.9, 150.7, 148.1, 146.1, 140.9, 135.7, 135.7, 128.1, 125.3, 116.6, 24.6 ; HRMS (TOF MS ES+) for $C_{16}H_{12}N_3O_3^+$MH+) calcd. 294.0879, found 294.0914. 7-amino-2-(3-pyridinyl)quinoline-5,8-dione: 6 mg (12%) of a red solid was obtained. R_f= 0.13 (5% MeOH:DCM); m.p. 195-197°C (decomposes, turns black); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.29 (d, *J* = 1.7 Hz, 1H), 8.67 (dd, *J* = 4.9, 1.4 Hz, 1H), 8.58 (ddd, *J* = 8.0, 2.2, 1.7 Hz, 1H), 8.52 (d, *J* = 8.2 Hz, 1H), 8.22 (d, *J* = 8.2 Hz, 1H), 7.59 (ddd, *J* = 8.0, 4.9, 0.7 Hz, 1H), 6.07 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 181.9, 179.8, 157.0, 150.4, 149.6, 147.6, 146.3, 135.4, 135.0, 133.5, 129.4, 124.7, 123.8, 102.1. HRMS (TOF MS ES+) for $C_{14}H_{10}N_3O_2^+$MH+) calcd. 252.0773, found 252.0795.

*7-acetamido-2-(8'-quinolinyl)quinoline-5,8-dione (**11**).* Yield 31 mg (51%) of a yellow solid was obtained. R_f= 0.25 (70% EtOAc:heptane), crystallized from MeOH/CH_2Cl_2; m.p. 295°C (decomposes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.93 (dd, *J* = 4.2, 1.8 Hz, 1H), 8.53 (d, *J* = 8.1 Hz, 1H), 8.48 (d, *J* = 8.1 Hz, 1H), 8.34 (dd, *J* = 8.3, 1.8 Hz, 1H), 8.22 (dd, *J* = 7.2, 1.4 Hz, 1H), 8.04 (dd, *J* = 8.2, 1.4 Hz, 1H), 8.00 (s, 1H), 7.76 (dd, *J* = 8.1, 7.3 Hz, 1H), 7.54 (dd, *J* = 8.3, 4.2 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 184.8, 179.1, 170.9, 161.9, 150.5, 145.7, 145.1, 140.9, 136.7, 136.5, 133.3, 131.9, 131.7, 130.2, 128.4, 127.6, 126.3, 121.4, 116.5, 24.2. HRMS (TOF MS ES+) $C_{20}H_{14}N_3O_3^+$ (MH+) calcd. 344.1035, found 344.1022.

*7-acetamido-2-(2-(1-tert-butoxycarbonylindolyl))quinoline-5,8-dione (**12**).* Yield 63mg (67%) of an orange was obtained. R_f= 0.40 (50% EtOAc:heptane); m.p. 191-193°C (decomposes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.45 (d, *J* = 8.1 Hz, 1H), 8.15 (d, *J* = 8.4 Hz, 1H), 7.97 (s, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.27 (dd, *J* = 9.1, 5.9 Hz, 1H), 6.98 (s, 1H), 2.33 (s, 3H), 1.41 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 184.2, 179.0, 169.6, 157.4, 149.7, 145.3, 140.4, 138.1, 137.5,

134.2, 128.6, 127.9, 127.6, 126.0, 123.3, 121.5, 116.5, 115.2, 114.0, 84.2, 27.8, 25.1. HRMS (TOF MS ES+) $C_{24}H_{22}N_3O_5^+$(MH+) calcd. 432.1559, found 432.1568.

*7-acetamido-2-(2-pyridinyl)quinoline-5,8-dione (**13**).* Yield 37 mg (71%) of a yellow solid was obtained. R_f= 0.19 (5% MeOH:CH2Cl2), crystallized from MeOH/CH_2Cl_2; m.p. 255-258°C (decomposes); ^{1}H NMR (500 MHz, DMSO) δ 10.08 (s, 1H), 8.78 (ddd, *J* = 4.8, 1.6, 0.8 Hz, 1H), 8.53 (d, *J* = 7.9 Hz, 1H), 8.46 (d, *J* = 8.2 Hz, 1H), 8.08 (td, *J* = 7.7, 1.8 Hz, 1H), 7.77 (s, 1H), 7.58 (ddd, *J* = 7.5, 4.7, 1.1 Hz, 1H), 2.28 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 184.6, 178.4, 171.5, 158.6, 153.6, 149.8, 146.4, 142.5, 137.8, 135.0, 128.5, 125.5, 124.6, 121.7, 115.3, 24.7. HRMS (TOF MS ES+) $C_{16}H_{12}N_3O_3^+$ (MH+) calcd. 294.0879, found 294.0914.

*7-acetamido-2-(2-(1-tert-butoxycarbonylpyrrolyl))quinoline-5,8-dione (**14**).* Yield 36 mg (53%) of a yellow solid was obtained. R_f= 0.30 (50% EtOAc:heptane); m.p. 191-193°C (decomposes), recrystallized from methanol; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.42 (s, 1H), 8.39 (d, *J* = 8.2 Hz, 1H), 7.95 (s, 1H), 7.79 (d, *J* = 8.2 Hz, 1H), 7.42 (dd, *J* = 3.2, 1.7 Hz, 1H), 6.64 (dd, *J* = 3.4, 1.7 Hz, 1H), 6.29 (t, *J* = 3.3 Hz, 1H), 2.32 (s, 3H), 1.43 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 184.3, 179.2, 169.5, 156.9, 148.8, 145.3, 140.3, 134.0, 132.5, 128.0, 127.3, 125.5, 118.6, 116.4, 111.2, 84.4, 27.7, 25.1. HRMS (TOF MS ES+) $C_{20}H_{20}N_3O_5^+$ (MH+) calcd. 382.1403, found 382.1381.

*7-acetamido-2-(4-pyrazolyl))quinoline-5,8-dione (**15**).* Yield 31 mg (42%) of a brown solid was obtained. R_f= 0.33 (5% MeOH:CH_2Cl_2); m.p. 270°C (decomposes), recrystallized from methanol; ^{1}H NMR (500 MHz, DMSO) δ 13.34 (s, 1H), 9.97 (s, 1H), 8.55 (s, 1H), 8.25 (d, *J* = 8.2 Hz, 1H), 8.21 (s, 1H), 8.11 (d, *J* = 8.2 Hz, 1H), 7.69 (s, 1H), 2.26 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 184.6, 178.6, 171.4, 156.1, 146.6, 142.1, 134.2, 126.1, 123.8, 121.2, 115.1, 24.6. HRMS (TOF MS ES+) $C_{14}H_{11}N_4O_3^+$ (MH+) calcd. 283.0831, found 283.0846.

*7-acetamido-2-(3-(2-acetamido-pyridinyl))quinoline-5,8-dione (**16**).* The quinone **8** (71 mg, 0.28 mmol) was dissolved in 2 mL 1,4-dioxane and degassed under reduced pressure. $PdCl_2$(dppf) (20 mg), K_3PO_4 (238 mg) and the boronate were added and the solution degassed further. The mixture was stirred under Ar

atmosphere for 10 minutes. The mixture was then heated heated using a Biotage microwave initiator at 120°C for 30 minutes. After cooling, the reaction mixture was poured into CH_2Cl_2 and washed with 2 x 10 mL water and extracted 2x 30 mL DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified on a HP-Sil 25 g Biotage SNAP cartridge using MeOH:CH_2Cl_2 gradient (0-5%) at a flow rate of 20 mL/min. Yield 23mg (23%) of a brown solid was obtained. R_f= 0.32 (5% MeOH:CH_2Cl_2); m.p. 249°C (decomposes); ^{1}H NMR (500 MHz, DMSO) δ 10.82 (s, 1H), 10.04 (s, 1H), 9.17 (d, *J* = 2.5 Hz, 1H), 8.60 (dd, *J* = 8.8, 2.5 Hz, 1H), 8.45 (d, *J* = 8.3 Hz, 1H), 8.38 (d, *J* = 8.2 Hz, 1H), 8.27 (d, *J* = 8.8 Hz, 1H), 7.75 (s, 1H), 2.28 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 184.6, 178.5, 171.5, 169.7, 157.4, 153.6, 147.3, 146.6, 142.4, 137.0, 134.8, 128.0, 127.2, 124.1, 115.3, 113.0, 24.7, 24.0. HRMS (TOF MS ES+) $C_{18}H_{15}N_4O_4^+$ (MH+) calcd. 351.1093, found 351.1064.

*7-acetamido-2-(2-indolyl)quinoline-5,8-dione (**17**).* The starting material **12** (39 mg, 0.09 mmol) was dissolved in 2.5 mL CH_2Cl_2 and cooled to 0°C using an ice bath. Trifluoroacetic acid (140 μL) was the added dropwise and reacted at rt for 2 hrs. TLC showed full conversion. Then quenched with sat. $NaHCO_3$ (10 mL) and extracted 2x20 mL CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified on a HP-Sil 25 g Biotage SNAP cartridge using EtOAc:heptane gradient (0-70%) at a flow rate of 20 mL/min. Yield 17 mg (59%) of a red solid was obtained after recrystallization from MeOH. M.p. 185°C, decomposes; R_f= 0.38 (70% EtOAc:heptane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (d, *J* = 8.3 Hz, 1H), 8.14 (d, *J* = 8.3 Hz, 1H), 7.92 (s, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 7.49 (d, *J* = 8.3 Hz, 1H), 7.29 (t, *J* = 7.6 Hz, 1H), 7.23 (s, 1H), 7.13 (t, *J* = 7.4 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 184.2, 180.4, 170.5, 154.7, 145.4, 140.3, 137.8, 134.7, 134.4, 128.3, 126.6, 124.4, 124.4, 121.5, 120.2, 117.0, 111.7, 104.4, 24.4. HRMS (TOF MS ES+) $C_{19}H_{14}N_3O_3^+$ (MH+) calcd. 332.1035, found 332.1030.

*7-acetamido-2-(2-(pyrrolyl))quinoline-5,8-dione (**18**).* The starting material **14** (30 mg, 0.08 mmol) was dissolved in 3 mL CH_2Cl_2 and cooled to 0°C using an ice bath. Trifluoroacetic acid (150 μL) was the added dropwise and reacted at rt for 2 hrs. TLC showed full conversion. Then quenched with sat. $NaHCO_3$ (10 mL) and extracted 2x20 mL CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified on a HP-Sil 25 g Biotage SNAP cartridge using EtOAc:heptane gradient (0-50%) at a flow rate of 20 mL/min. Yield 21 mg (93%) of a red solid was obtained after recrystallization from MeOH. M.P. 255°C, decomposes. R_f= 0.11 (50% EtOAc:heptane). ^{1}H NMR (500 MHz, DMSO) δ 11.65 (s, 1H), 9.95 (s, 1H), 8.20 (d, *J* = 8.4 Hz, 1H), 8.05 (d, *J* = 8.4 Hz, 1H), 7.69 (s, 1H), 7.07 – 7.04 (m, 2H), 6.28 – 6.22 (m, 1H), 2.27 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 184.6, 178.7, 171.4, 154.0, 146.6, 141.9, 133.9, 130.1, 125.3, 123.8, 121.8, 115.2, 111.9, 110.4, 24.7. HRMS (TOF MS ES+) $C_{15}H_{12}N_3O_3^+$ (MH+) calcd. 282.0879, found 282.0909.

General procedure for removal of the acetate group with MeOH-H_2SO_4. To the starting material (0.1 mmol) in a 20 mL vial was added 175μL of H_2SO_4 in 3.0 mL MeOH and stirred at rt for 3 hrs. The red solution was then neutralized with 5 mL 5% $NaHCO_3$ (aq.) and extracted with 5 X 10 mL CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Then purified on a HP-Sil 25 g Biotage SNAP cartridge using EtOAc:heptanes (0-70%) or MeOH:CH_2Cl_2 gradient (0-5%) at a flow rate of 20 mL/min.

*7-Amino-2-(4-(trifluoromethyl)phenyl)quinoline-5,8-dione (**19**).* The general procedure was used to obtain 6.0 mg (67%) of a red solid; R_f= 0.38 (60% EtOAc:heptane); m.p. 151-153°C (decomposes, turns black); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (d, *J* = 8.2 Hz, 1H), 8.05 (d, *J* = 8.2 Hz, 2H), 7.95 (d, *J* = 8.2 Hz, 1H), 7.58 (d, *J* = 8.3 Hz, 2H), 5.84 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.2, 180.1, 158.7, 150.3, 146.3, 140.6, 135.1, 129.4, 127.7, 125.5, 125.5, 125.1, 105.8, 102.4. HRMS (TOF MS ES+) $C_{16}H_{10}F_3N_2O_2^+$ (MH+) calcd. 319.0694, found 319.0666.

*7-amino-2-(3-pyridinyl)quinoline-5,8-dione (**20**).* The general procedure was used to obtain 10 mg (83%) of a red solid. R_f= 0.16 (5% MeOH:CH_2Cl_2); m.p. 195-197°C (decomposes, turns black). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.29 (d, *J* = 1.7 Hz, 1H), 8.67 (dd, *J* = 4.9, 1.4 Hz, 1H), 8.58 (ddd, *J* = 8.0, 2.2, 1.7 Hz, 1H), 8.52 (d, *J* = 8.2 Hz, 1H), 8.22 (d, *J* = 8.2 Hz, 1H), 7.59 (ddd, *J* = 8.0, 4.9, 0.7 Hz, 1H), 6.07 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 181.9, 179.8, 157.0, 150.4, 149.6, 147.6, 146.3, 135.4, 135.0, 133.5, 129.4, 124.7, 123.8, 102.1. HRMS (TOF MS ES+) for $C_{14}H_{10}N_3O_2^+$MH+) calcd. 252.0773, found 252.0795.

*7-amino-2-(2-indolyl)quinoline-5,8-dione (**21**).* The general procedure was used to obtain 19 mg (63%) of a dark-brown solid. R_f= 0.22 (70% EtOAc:heptane); m.p. 235°C decomposes. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (d, *J* = 8.3 Hz, 1H), 8.10 (d, *J* = 8.3 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.39 (s, 1H), 7.27 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1H), 7.19 (s, 1H), 7.12 (td, *J* = 7.5, 0.8 Hz, 1H), 6.01 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.6, 181.6, 153.6, 149.5, 145.9, 137.6, 135.0, 134.2, 128.3, 128.0, 124.1, 124.0, 121.3, 120.0, 111.7, 103.5, 102.9. HRMS (TOF MS ES+) for $C_{17}H_{12}N_3O_2^+$ (MH+) calcd. 290.0930, found 290.0900.

*7-amino-2-(8-quinolinyl)quinoline-5,8-dione (**22**).* The general procedure was used to obtain 55 mg (71%) of a brown solid. R_f= 0.29 (5% MeOH:CH_2Cl_2); m.p. 243-245°C, recrystallized from MeOH. ^{1}H NMR (500 MHz, CD_3OD) δ 8.92 (dd, *J* = 4.2, 1.8 Hz, 1H), 8.49 (d, *J* = 8.1 Hz, 1H), 8.40 (d, *J* = 8.1 Hz, 1H), 8.33 (dd, *J* = 8.3, 1.8 Hz, 1H), 8.21 (dd, *J* = 7.2, 1.5 Hz, 1H), 8.02 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.75 (dd, *J* = 8.1, 7.3 Hz, 1H), 7.53 (dd, *J* = 8.3, 4.2 Hz, 1H), 6.06 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.8, 180.1, 160.5, 150.3, 150.2, 146.2, 145.2, 136.7, 136.7, 133.2, 131.6, 131.5, 129.8, 129.1, 128.4, 126.3, 121.2, 102.4. HRMS (TOF MS ES+) for $C_{18}H_{12}N_3O_2^+$ (MH+) calcd. 302.0930, found 302.0939.

*7-amino-2-(2-pyridinyl)quinoline-5,8-dione (**23**).* The general procedure was used to obtain 16 mg (76%) of a red solid. R_f= 0.25 (20% MeOH:CH_2Cl_2), recrystallized from MeOH. ^{1}H NMR (500 MHz, DMSO) δ 8.75 (d, *J* = 4.1 Hz, 1H), 8.72 (d, *J* = 8.2 Hz, 1H), 8.50 (d, *J* = 7.9 Hz, 1H), 8.40 (d, *J* = 8.1 Hz, 1H), 8.05 (t, *J* = 7.7 Hz, 1H), 7.58 – 7.53 (m, 1H), 5.89 (s, 1H). HRMS (TOF MS ES+) for $C_{14}H_{10}N_3O_2^+$ (MH+) calcd. 252.0773 found 252.0749.

*7-Amino-2-(2-pyrrolyl)quinoline-5,8-dione (**24**).* The general procedure was used to obtain 11 mg (78%) of a red solid. R_f= 0.37 (5% MeOH:CH_2Cl_2); m.p. 230°C (decomposes), recrystallized from MeOH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.4 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 7.06 (dd, *J* = 2.5, 1.3 Hz, 1H), 6.91 (dd, *J* = 3.7, 1.3 Hz, 1H), 6.32 (dd, *J* = 3.7, 2.6 Hz, 1H), 5.97 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 183.0, 181.7, 153.5, 149.4, 145.7, 133.9, 129.9, 126.6, 122.7, 122.3, 110.8, 110.3, 102.5. HRMS (TOF MS ES+) for $C_{13}H_{10}N_3O_2^+$ (MH+) calc. 240.0773, found 240.0779.

ASSOCIATED CONTENT

Supporting Information. Additional characterization data, ^{1}H and ^{13}C-NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS

NQO1, NAD(P)H: quinone oxidoreductase 1; MeOH, Methanol; BnBr, Benzyl bromide; DME, 1, 2-Dimethoxyethane; dppf, 1,1'-Bis(diphenylphosphino)ferrocene; E_{pc} = cathodic peak potential; E_{pa} = anodic peak potential

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