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Synthesis, metabolism and in vitro cytotoxicity studies on novel lavendamycin antitumor agents

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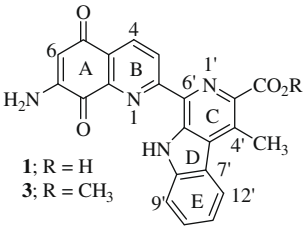
ABSTRACT

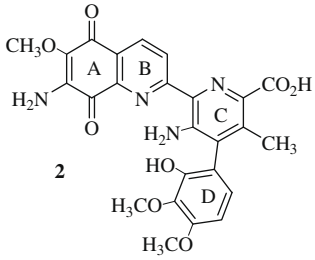
A series of lavendamycin analogues with two, three or four substituents at the C-6, C-7 *N*, C-2′, C-3′ and C-11′ positions were synthesized via short and efficient methods and evaluated as potential NAD(P)H:quinone oxidoreductase (NQO1)-directed antitumor agents. The compounds were prepared through Pictet–Spengler condensation of the desired 2-formylquinoline-5,8-diones with the required tryptophans followed by further needed transformations. Metabolism and toxicity studies demonstrated that the best substrates for NQO1 were also the most selectively toxic to NQO1-rich tumor cells compared to NQO1-deficient tumor cells.

1. Introduction

Lavendamycin (**1**), a naturally occurring 7-aminoquinoline-5,8-dione antitumor antibiotic, was first isolated from the fermentation broth of *Streptomyces lavendulae* by Balitz et al.[1] and its structure was determined by Doyle et al.[2] The latter study determined that lavendamycin is a pentacyclic structure with two moieties including quinoline-5,8-dione and indolopyridine (β-carboline) (Chart).[2] Lavendamycin is similar to another potent antibiotic antitumor agent streptonigrin (**2**).[1–5] The clinical use of both of these antibiotics has been precluded because of their toxicity toward human cells.[2,6–8] Initial structure–activity relationship (SAR) studies have demonstrated that the essential moiety for the cytotoxic activity of lavendamycin and streptonigrin is the 7-aminoquinoline-5,8-dione moiety.[7]

As it was thought that some analogues of the naturally occurring antibiotic **1** might show activity toward human tumors, efficient methods were needed to prepare these derivatives. There were two previous reports on the synthesis of lavendamycin methyl ester (**3**) (above and Table 1) by Kende[9] and Boger[10] but both were impractical, since they produced compound **3** in an overall yield of less than 2% and in 9- and 20-steps, respectively. Our group was able to develop two synthetic methods[11,12] that produced **3** in an overall yield of about 40% in only 5-steps. These efficient methods, particularly those reported in 1996,[12] made it possible to prepare a large number of substituted lavendamycins needed for various in vitro and in vivo screening tests.





We previously reported that a significant number of these lavendamycin analogues have potent biological activity including anti-HIV reverse transcriptase[13] and antitumor activity.[14–16] More importantly, several screening tests on a number of lavendamycin analogues have shown that a significant number of these compounds possess low animal toxicity.[13,17] The NCI in vivo studies

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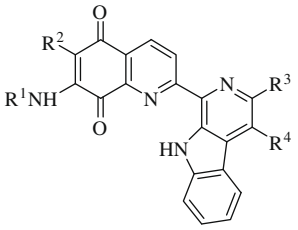
Table 1
Structures of lavendamycins discussed in Sections 1 **and** 2



No.	R^1	R^2	R^3	R^4
3	H	H	CO_2CH_3	CH_3
4	CH_3CO	H	$CONH_2$	H
5	H	H	$CONH_2$	H
6	CH_3CO	H	$CO_2C_8H_{17}$-*n*	H
7	CH_3CO	H	$CO_2(CH_2)_2CH(CH_3)_2$	H
8	CH_3CO	H	CO_2CH_3	CH_3
9	2-Furyl-CO	H	CO_2CH_3	H
10	CH_3CO	H	$CONHC_4H_9$-*n*	H
11	H	H	CH_2OH	H
12	H	H	$CO_2C_8H_{17}$-*n*	H
13	CH_3CO	H	CO_2CH_3	H
14	H	Cl	CO_2CH_3	CH_3
15	H	OCH_3	CO_2CH_3	CH_3
16	H	H	$CO_2(CH_2)_2OH$	H

have shown that the maximum tolerated dose of three derivatives **3**, **4** and **5** (Table 1) in mice is 400 mg/kg which is 31 and 1000 times higher than that for lavendamycin and streptonigrin, respectively.[13,17] The lavendamycin analogue **6** reduced tumor volume (up to 80%) in mice bearing tumor xenografts at a daily dose of 300 mg/kg for 10 days without exhibiting drug-related weight loss or lethality.[17] A recent study also demonstrated that the normal rat kidney epithelial cell line (NRK-52E) exhibited much less sensitivity to several lavendamycin analogues compared to the tumor cells with the same origin.[17] When lavendamycin analogues **7** and **6** at a daily dose of 300 mg/kg and **8** at a daily dose of 100 mg/kg were administered to nude mice for eight and seven days, respectively, no drug-related deaths or toxicity were observed.[17] Analogues **8**, **7** and **6** (Table 1) inhibited tumor growth in nude mice by 69%, 88% and 78%, respectively, when administered at 100, 150 and 300 mg/kg for 7, 8 and 8 days, respectively.[17]

NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-diaphorase) is a two-electron reductase, characterized by its capacity for utilizing either NADH or NADPH as reducing cofactors and by its inhibition by dicoumarol.[18] It is primarily located in the cytosol (>80%), but a recent study has reported a substantial pool of NQO1 in the nuclei of cancer cells.[19] NQO1 is generally categorized as a detoxification enzyme, and it can protect the cell from a broad range of chemically reactive metabolites including electrophiles and reactive oxygen species.[20] NQO1 can also function as an activating enzyme, specifically for the reductive activation of antitumor quinones and other bioreductive anticancer agents.[21]

NQO1 can be induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and polycyclic aromatic hydrocarbons.[21] Induction by procarcinogens and the capacity of NQO1 for detoxifying reactive metabolites suggest that changes in expression of NQO1 may occur during carcinogenesis.[22] Marked elevations in NQO1 activity and mRNA content have been documented in both preneoplastic tissues and established tumors. Tumors or cancer cell lines where increased NQO1 expression has been observed include those from the lung, liver, colon, and breast.[23] In addition, advanced, metastatic tumors appear to express higher levels of NQO1 than non-metastatic tumors.[24]

Correlations between NQO1 activity in cancer cells and cytotoxicity of antitumor quinones to those cells have been reported.[25–27] In our previous studies with several series of indole- and quinolinequinone antitumor agents, including analogues of lavendamycin, those compounds that were the best substrates for NQO1 were also found to be selectively toxic to cell lines with high levels of NQO1 compared to cell lines that were deficient in NQO1.[15,16,28–30] In this report, we describe the synthesis, metabolism by recombinant human NQO1 and anticancer activity of novel analogues of lavendamycin with functional group substitutions on both the quinoline-5,8-dione and indolopyridine moieties.

2. Results and discussion

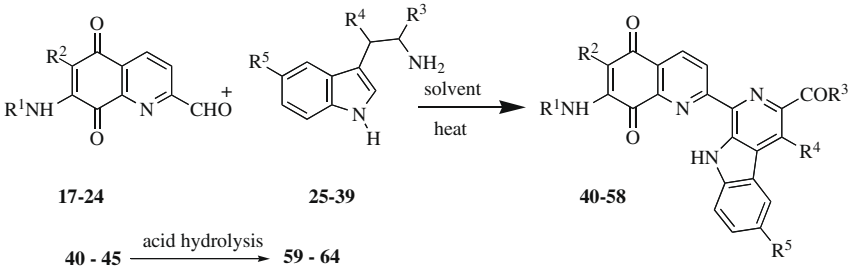
2.1. Synthetic chemistry

Table 4 presents the structures of a total of 25 lavendamycins that were the subject of our biological studies in this report. As shown in Scheme 1, Pictet–Spengler (P–S) condensation of the quinolinedione aldehydes (Table 2) with tryptophans (Table 3) yielded the target lavendamycins **40**–**58** listed in Table 4. The amino lavendamycins **59**–**64** were prepared respectively by the acid hydrolysis of the corresponding 7-acylamino lavendamycins **40–45**.

High resolution mass spectroscopy of **67** (Scheme 2) did not produce a signal for the molecular ion M^+, but rather gave one at 354.109. This corresponds to a molecular formula of $C_{18}H_{16}N_3O_5$ $(M-2H_2O+H)^+$, that may be due to the fragment shown in Scheme 2, produced by the dehydration of **67** under HRMS conditions.

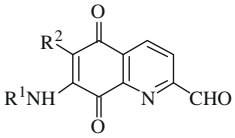
Similar to our reported synthesis of β-methyltryptophan methyl ester,[31] the nitrobutanoate **71** (Scheme 3) and the precursor to the ester **28**, existed in two stereoisomers **A** and **B** in a ratio of 1/1 as shown by NMR. However, upon recrystallization of the mixture, stereoisomer **B** was completely converted and only the less soluble isomer **A** was obtained in pure form. When **71A** was reduced with H_2 in the presence of Ra–Ni the product was 5-benzyloxy β-methyltryptophan methyl ester (73%).

Amides **30**, **31** and **32** were prepared as shown in Scheme 4. Alkyl esters **27**, **33**, **34** and **36** (Scheme 5) were prepared by the Fischer esterification of the corresponding tryptophan or hydroxytryptophan with the required alcohol. The preparation of 7-foramido-2-formylquinoline-5,8-dione (**20**), necessary for the synthesis of lavendamycin **53** is described in Section 4.



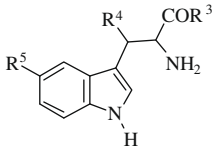
Scheme 1.

Table 2
Structures of aldehydes for Scheme 1



No.	R^1	R^2	
17	CH_3CO	H	See Refs. 11,12
18	$CH_3(CH_2)_2CO$	H	See Ref. 17
19	$(CH_3)_2CHCO$	H	See Ref. 17
20	HCO	H	
21	$ClCH_2CO$	H	See Ref. 15
22	2-Furyl-CO	H	See Ref. 16
23	$HO_2C(CH_2)_2CO$	H	
24	H	Cl	See Ref. 15

Table 3
Structures of tryptophans for Scheme 1



No.	R^3	R^4	R^5	
25	OCH_3	H	H	See Ref. 15
26	$N[(CH_2CH_2)_2]O$	H	H	See Ref. 15
27	$O(CH_2)_2CH(CH_3)_2$	H	OH	
28	OCH_3	CH_3	OH	
29	$N[(CH_2)_4]$	H	H	See Ref. 15
30	$NHCH_2CH_2OH$	H	H	
31	$NHCH_2CH(OH)CH_2OH$	H	H	
32	$N[(CH_2CH_2)_2]NCH_2Ph$	H	H	
33	$O(CH_2)_2CH_3$	H	H	
34	$OCH(CH_3)CH_2CH_3$	H	H	
35	OCH_2CH_3	H	H	See Ref. 17
36	OCH_2CH_3	H	OH	
37	OCH_3	CH_3	H	See Ref. 31
38	OC_4H_9-*n*	H	H	See Ref. 13
39	NH_2	H	H	See Ref. 13

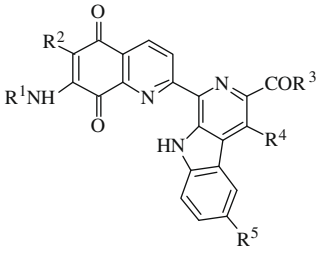
2.2. Biological studies

2.2.1. Metabolism

Metabolism of the lavendamycin analogues by recombinant human NQO1 was examined. Reduction rates by NQO1 were measured using a spectrophotometric assay that employs cytochrome *c* as the terminal electron acceptor[15] and gives initial rates of lavendamycin analogue reduction (Table 5). The initial reduction rates (μmol cytochrome *c* reduced/min/mg NQO1) were calculated from the linear portion (0–30 s) of the reaction graphs.

Compound **59** with NH_2 and $CON[(CH_2CH_2)_2]O$ groups at R^1 and R^3 positions, respectively, displayed the highest metabolism rate by NQO1 among the lavendamycin analogues (Table 5). This could be due to the fact that the NH_2 group, a small substituent, did not produce steric hindrance with the internal wall of the NQO1 active site resulting in favorable positioning of **59** for hydride ion reception from FAD and quinone reduction. Also, the morpholino group at R^3 could potentially form hydrogen bonding or van der Waals interactions with the residues of the active site to increase **59** binding affinity in the active site. Our recent molecular modeling studies have demonstrated that lavendamycin analogues with a small to medium size substituent at the R^1 position and substituents at R^3 that are capable of hydrogen bonding or van der Waals interactions with the key residues of the active site or FAD are good

Table 4
Structures of lavendamycin analogues



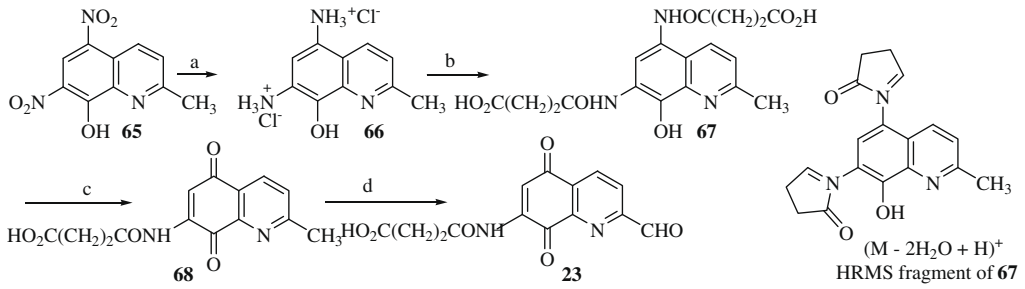
No.	R^1	R^2	R^3	R^4	R^5	
40	CH_3CO	H	$N[(CH_2CH_2)_2]O$	H	H	See Ref. 15
41	CH_3CO	H	$O(CH_2)_2CH(CH_3)_2$	H	OH	
42	CH_3CO	H	OCH_3	CH_3	OH	
43	CH_3CO	H	$N[(CH_2)_4]$	H	H	See Ref. 15
44	CH_3CO	H	$NH(CH_2)_2OH$	H	H	
45	$CH_3(CH_2)_2CO$	H	OCH_3	H	H	See Ref. 17
46	CH_3CO	H	$OCH(CH_3)CH_2CH_3$	H	H	
47	CH_3CO	H	$NHCH_2CH(OH)CH_2OH$	H	H	
48	CH_3CO	H	$O(CH_2)_2CH_3$	H	H	
49	CH_3CO	H	$N[(CH_2CH_2)_2]NCH_2Ph$	H	H	
50	CH_3CO	H	OCH_2CH_3	H	H	See Ref. 17
51	CH_3CO	H	OCH_2CH_3	H	OH	
52	$(CH_3)_2CHCO$	H	OCH_3	H	H	See Ref. 17
53	HCO	H	OCH_3	H	H	
54	$ClCH_2CO$	H	NH_2	H	H	
55	$ClCH_2CO$	H	$N[(CH_2)_4]$	H	H	
56	2-Furyl-CO	H	OCH_3	CH_3	H	
57	$HO_2C(CH_2)_2CO$	H	OC_4H_9-*n*	H	H	
58	H	Cl	OCH_2CH_3	H	H	
59	H	H	$N[(CH_2CH_2)_2]O$	H	H	
60	H	H	$O(CH_2)_2CH(CH_3)_2$	H	OH	
61	H	H	OCH_3	CH_3	OH	
62	H	H	$N[(CH_2)_4]$	H	H	
63	H	H	$NH(CH_2)_2OH$	H	H	
64	H	H	OCH_3	H	H	

NQO1 substrates.[15,16] Compounds **43**, **47**, **55**, **61** and **64** displayed good metabolism rates in the range of 28–43 μmol/min/mg NQO1 (Table 5).

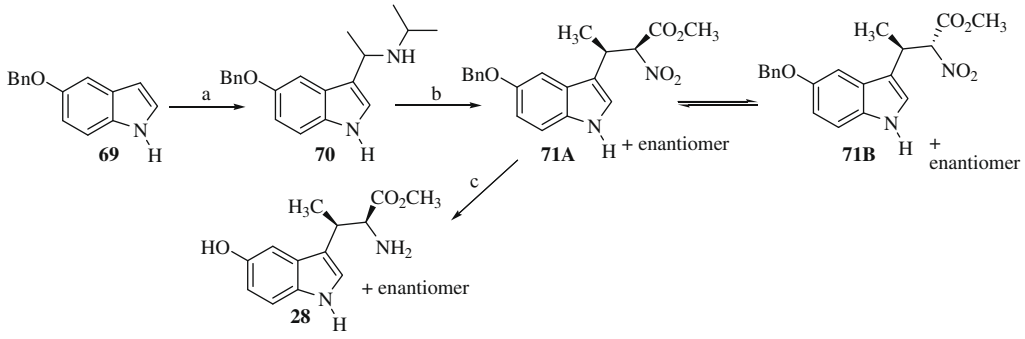
Compound **52**, however, with $(CH_3)_2CHCONH$ and CO_2CH_3 groups at the R^1 and R^3 positions, respectively, exhibited the lowest metabolism rate by NQO1 and ranked as the poorest substrate (Table 5). The decreased reduction rate of **52** can be explained by steric hindrance between the quinolinedione moiety of **52** and the NQO1 active site due in part to the large and branched isobutyramide group at R^1. This steric interaction could result in unfavorable positioning of **52** in the active site with subsequent poor hydride ion reception and quinone reduction capability. Lavendamycin analogue **56** also displayed a low metabolism rate by NQO1 (Table 5) possibly due to the failure of the 2-furyl-CONH group at the R^1 position to intercalate between and form van der Waals interactions with internal wall residues such as Trp-105 and Phe-106.[16] A similar 2-furyl-CONH compound **9** (Table 1) with H instead of CH_3 at R^4 was also ranked as a poor NQO1 substrate by our recent molecular docking study.[16] We previously demonstrated that the presence of substituents at R^2 also increases steric interactions of lavendamycin ligands with the internal wall of the active site.[15,16] Accordingly, compound **58** with a Cl group at this position was ranked as a poor substrate for NQO1 (Table 5).

2.2.2. In vitro cytotoxicity

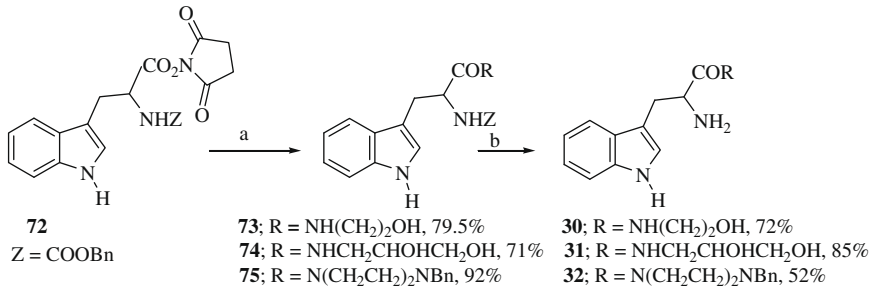
Cytotoxicity studies were also performed on the lavendamycin analogues with cell survival being determined by the MTT colorimetric assay. We demonstrated an excellent positive linear correlation between the IC_{50} values for clonogenic and MTT assays in a previous study with lavendamycin analogues.[15] We utilized the



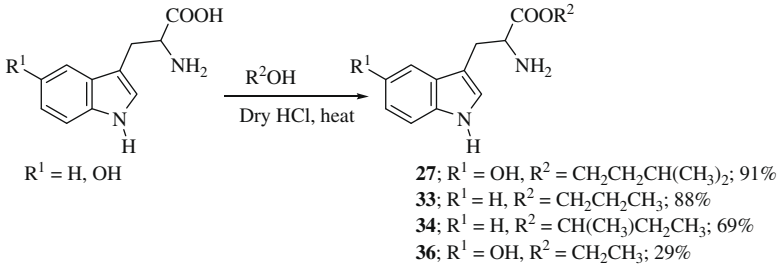
Scheme 2. Reagents and conditions: (a) see Ref. 16; (b) succinic anhydride, NaOAc, Na_2SO_3, DMF, Ar, 18 h, rt, 82%; (c) $K_2Cr_2O_7$, CH_3COOH, 20 h, rt, 44%; (d) SeO_2, 1,4-dioxane, H_2O, 11 h, reflux, 57%.



Scheme 3. Reagents and conditions: (a) $CH_3CH{=}N(CH_3)_2$, CH_3COOH, toluene, 4 days, 5 °C, 38%; (b) $NaCH_2CH_3CO_2CH_3$, Et_3N, toluene, Ar, 1 h, rt, then 9 h at 103–105 °C, 97%; (c) H_2, Pd–C 10%, CF_3COOH, 21 h, rt, 93%.



Scheme 4. Reagents and conditions: (a) $H_2N(CH_2)_2OH$ for **73**, $H_2NCH_2CHOHCH_2OH$ for **74**, $N(CH_2CH_2)_2NBn$ for **75**, $CHCl_3$, Et_3N, alcohol, Ar, 29 h for **73**, 24 h for **74**, and 48 h for **75**, rt; (b) $HCOONH_4$, Pd–C 10%, isopropanol for **30**, **32** and methanol for **31**, Ar, 4.5 h for **30**, 30 min for **31** and **32**, rt.

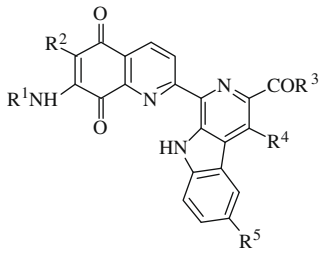


Scheme 5.

BE human colon adenocarcinoma cells stably transfected with human NQO1 cDNA.[32] The BE cells had no measurable NQO1 activity whereas activity in the transfected cells (BE-NQ) was 337 nmol/min/mg total cell protein using dichlorophenolindophenol (DCPIP) as the standard electron acceptor. In the present study, the cytotoxicity of the lavendamycin analogues (Table 6) was compared using these cell lines.

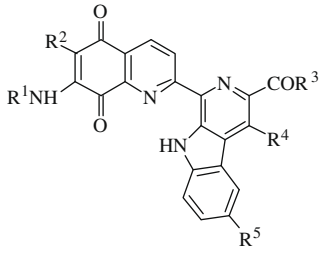
Lavendamycin analogues such as **47**, **55**, **59** and **61** that were good substrates for NQO1 (Table 5) were also more toxic to the NQO1-rich BE-NQ cell line than the NQO1-deficient BE cell line (Table 6). Compound **59**, the best substrate for NQO1 (Table 1), had the greatest differential toxicity with a selectivity ratio [IC_{50} (BE)/IC_{50} (BE-NQ)] of 9 (Table 6). Our previous studies also determined that good lavendamycin substrates for NQO1 were

Table 5
Metabolism of lavendamycin analogues by recombinant human NQO1 monitored by spectrophotometric cytochrome *c* assay



No.	R^1	R^2	R^3	R^4	R^5	Metabolism by NQO1 (μmol/min/mg)
43	CH_3CO	H	$N[(CH_2)_4]$	H	H	43 ± 7
44	CH_3CO	H	$NH(CH_2)_2OH$	H	H	2.2 ± 0.7
46	CH_3CO	H	$OCH(CH_3)CH_2CH_3$	H	H	3.9 ± 2.0
47	CH_3CO	H	$NHCH_2CH(OH)CH_2OH$	H	H	36 ± 6
48	CH_3CO	H	$O(CH_2)_2CH_3$	H	H	6.8 ± 3.5
49	CH_3CO	H	$N[(CH_2CH_2)_2]NCH_2Ph$	H	H	8.7 ± 1.5
50	CH_3CO	H	OCH_2CH_3	H	H	3.6 ± 0.3
51	CH_3CO	H	OCH_2CH_3	H	OH	21 ± 6
52	$(CH_3)_2CHCO$	H	OCH_3	H	H	0.04 ± 0.08
53	HCO	H	OCH_3	H	H	20 ± 8
54	$ClCH_2CO$	H	NH_2	H	H	13 ± 2
55	$ClCH_2CO$	H	$N[(CH_2)_4]$	H	H	34 ± 5
56	2-Furyl-CO	H	OCH_3	CH_3	H	5.8 ± 1.7
57	$HO_2C(CH_2)_2CO$	H	OC_4H_9-*n*	H	H	5.2 ± 2.6
58	H	Cl	OCH_2CH_3	H	H	6.9 ± 1.1
59	H	H	$N[(CH_2CH_2)_2]O$	H	H	103 ± 8
60	H	H	$O(CH_2)_2CH(CH_3)_2$	H	OH	2.9 ± 1.4
61	H	H	OCH_3	CH_3	OH	31 ± 7
62	H	H	$N[(CH_2)_4]$	H	H	35 ± 7
63	H	H	$NH(CH_2)_2OH$	H	H	26 ± 4
64	H	H	OCH_3	H	H	28 ± 2

Table 6
Cytotoxicity of lavendamycin analogues toward BE (NQO1-deficient) and BE-NQ (NQO1-rich) human colon adenocarcinoma cell lines



No.	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)		Selectivity ratio
						BE-NQ	BE	BE/BE-NQ
43	CH_3CO	H	$N[(CH_2)_4]$	H	H	6.2 ± 0.2	13 ± 1	2.1
44	CH_3CO	H	$NH(CH_2)_2OH$	H	H	>50	>50	—
46	CH_3CO	H	$OCH(CH_3)CH_2CH_3$	H	H	40 ± 10	33 ± 8	0.8
47	CH_3CO	H	$NHCH_2CH(OH)CH_2OH$	H	H	18 ± 2	>50	>2.8
48	CH_3CO	H	$O(CH_2)_2CH_3$	H	H	2.4 ± 0.1	3.2 ± 0.2	1.3
49	CH_3CO	H	$N[(CH_2CH_2)_2]NCH_2Ph$	H	H	9.8 ± 0.8	6.5 ± 0.5	0.7
50	CH_3CO	H	OCH_2CH_3	H	H	10 ± 1	13 ± 1	1.3
51	CH_3CO	H	OCH_2CH_3	H	OH	4.8 ± 0.2	4.0 ± 0.4	0.8
52	$(CH_3)_2CHCO$	H	OCH_3	H	H	>50	>50	-
53	HCO	H	OCH_3	H	H	10 ± 1	14 ± 1	1.4
54	$ClCH_2CO$	H	NH_2	H	H	5.8 ± 0.6	12 ± 1	2.1
55	$ClCH_2CO$	H	$N[(CH_2)_4]$	H	H	3.0 ± 0.5	8.0 ± 1.6	2.7
56	2-Furyl-CO	H	OCH_3	CH_3	H	>50	>50	—
57	$HO_2C(CH_2)_2CO$	H	OC_4H_9-*n*	H	H	7.6 ± 0.2	12 ± 1	1.6
58	H	Cl	OCH_2CH_3	H	H	>50	>50	—
59	H	H	$N[(CH_2CH_2)_2]O$	H	H	2.0 ± 0.1	18 ± 1	9.0
60	H	H	$O(CH_2)_2CH(CH_3)_2$	H	OH	>50	>50	—
61	H	H	OCH_3	CH_3	OH	5.7 ± 0.6	14 ± 1	2.5
62	H	H	$N[(CH_2)_4]$	H	H	45 ± 3	>50	>1.1
63	H	H	$NH(CH_2)_2OH$	H	H	1.1 ± 0.1	1.1 ± 0.1	1.0
64	H	H	OCH_3	H	H	22 ± 3	45 ± 3	2.0

selectively toxic towards BE-NQ versus BE cells.[15,16] Recently studied lavendamycin analogues **10**, **11**, **12**, **5**, **3** and **16** (Table 1) exhibited high selective toxicity toward BE-NQ cells (selectivity ratios = 30, 11, 10, 9, 9 and 7, respectively).[15,16] Four analogues, **8**, **13**, **14** and **15** (Table 1), suppressed the clonogenic survival of A549 human lung carcinoma cells at concentrations less than 100 nM with **14** being the most potent analogue that inhibited A549 colony growth by 70% at a concentration of 100 nM.[14] Also, the colony outgrowth of the cells was reduced by 70% at 10 nM and by 100% at 100 nM concentration of the lavendamycin analogue **5**.[14] Although the PC-3 human prostate cancer cell line is rather resistant to antitumor effects of many anticancer agents, these cells displayed sensitivity toward the cytotoxic properties of **5** at a concentration as low as 10 nM.[14] Compound **5** also exhibited promising antiproliferative activities against cancer cell lines of the National Cancer Institute (NCI) 60-cell line panel including colon, ovarian and renal cells, and cancer cells in a hollow fiber tumorigenesis assay.[14]

Lavendamycin analogues **44**, **46**, **48**, **49**, **50**, **52**, **56**, **57** and **58** that were poor substrates for NQO1 (Table 5) demonstrated no selective toxicity toward BE-NQ cells or had no measurable cytotoxicity (IC_{50} >50 μM) (Table 6). Overall, our results suggest that the best lavendamycin substrates for NQO1 were also the most selectively toxic to the high-NQO1 BE-NQ cell line compared to NQO1-deficient BE cells, consistent with our previous studies.[15,16]

3. Conclusions

Twenty-five novel lavendamycin analogues were synthesized, characterized and evaluated as NQO1-directed antitumor agents. In line with our earlier computational and structure–activity studies,[15,16] analogues with small substituents at the R^1 position and groups capable of hydrogen bonding/van der Waals interactions at the R^3 position generally made the best substrates for NQO1. Consequently, many of these compounds were also selectively toxic to the cancer cells with elevated NQO1 activity. The best substrate for NQO1 was **59**, the 2′-CON[$(CH_2CH_2)_2$]O-7-NH_2 derivative that also was nine times more toxic to the high-NQO1 BE-NQ cells than the NQO1-deficient BE cells. These data advance our understanding of the structure–activity relationships for NQO1-directed antitumor quinones.

4. Experimental

4.1. Chemistry

4.1.1. General methods

For general methods, see Ref. 16. In nearly all of the experimental work-ups, solvents were evaporated under reduced pressure and heat using a rotaevaporator. In each experiment, the progress of the reaction was monitored by TLC and the reaction stopped when TLC showed its completion. In some instances, no NMR signals were observed for active H's, especially when DMSO-d_6 was the solvent of use.

4.1.2. 7-*N*-Formamido-2-formylquinoline-5,8-dione (20)

In a 25 mL two-necked round-bottomed flask, equipped with a stirring bar, a condenser and an argon filled balloon, 7-formamido-2-methylquinoline-5,8-dione[33] (**7**, 0.246 g, 1.14 mmol), selenium dioxide (0.202 g, 1.82 mmol) were added to 18 mL of dried distilled 1,4-dioxane and 0.2 mL water and refluxed for 24 h. Solid black selenium metal was allowed to settle and the supernatant was filtered. The filter paper containing selenium was placed in a beaker, 10 mL of dioxane was added, and heated for a few minutes on a steam bath to boiling. The mixture was filtered and the solid was again washed with 15 mL of dichloromethane. The combined filtrates were evaporated and to the resulting solid, 50 mL of dichloromethane was added and placed in a separatory funnel. The solution was washed with sodium bicarbonate (2 × 50 mL), the organic layer was dried ($MgSO_4$) and evaporated. The brown yellow solid was dissolved in dichloromethane, and then concentrated to a small volume. Addition of enough *n*-hexane gave the yellow solid of pure **5** (78.6 mg, 30%): mp 192–195 °C; ^{1}H NMR ($CDCl_3$) δ 8.07 (s, 1H), 8.34 (d, 1H, J = 7.0), 8.60 (s, 1H), 8.64 (d, 1H, J = 6.6), 8.80 (s, 1H), 10.31 (s, 1H); HRMS *m*/*e* calcd for $C_{11}H_6N_2O_4$, 230.032; found, 230.032.

4.1.3. 7-*N*-(3-Carboxypropionyl)amino-2-formylquinoline-5,8-dione (23)

Dione **68** (225.1 mg, 0.781 mmol) was added to 4 mL of dioxane and 0.1 mL of water. To the mixture 167.9 mg (1.5 mmol) of selenium dioxide was added and refluxed with stirring under argon for 11 h. The reaction mixture was filtered hot and the filter cake was returned to the flask and refluxed for 5 min with 5 mL of dioxane and then filtered. This process was repeated once more and the combined filtrates were evaporated to produce 130 mg (55%) of the yellow orange solid **23**: mp 225.5 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.50 (m, 2H), 2.88 (m, 2H), 7.8 (s, 1H), 8.28 (d, 1H, J = 8.0), 8.54 (d, 1H, J = 8.0), 10.16 (s, 1H), 10.23 (s, 1H), 12.17 (br s, 1H); HRMS *m*/*e* $C_{14}H_{10}N_2O_6$, 303.061; found, 303.062.

4.1.4. 7-*N*-(3-Carboxypropionyl)amino-2-methylquinoline-5,8-dione (68)

To a solution of potassium dichromate (1.10 g) in 29 mL of water/glacial acetic acid mixture (13.4:15.6), 0.51 g (1.3 mmol) of amide **67** was added and the reaction mixture was stirred at rt for 20 h and then extracted with dichloromethane (5 × 50 mL). The combined extracts were dried ($MgSO_4$) and evaporated to give 0.26 g (69%) of the yellow solid **53**: mp 144 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.73 (m, 7H), 7.71 (s, 1H), 7.73 (d, 1H, J = 8.3), 8.24 (d, 1H, J = 7.9), 9.98 (s, 1H); HRMS *m*/*e* calcd for $C_{14}H_{12}N_2O_5$, 288.075; found, 288.077.

4.1.5. 5,7-Bis(3-carboxypropionylamino)-8-hydroxy-2-methylquinoline (67)

5,7-Diamino-8-hydroxy-2-methylquinoline dihydrogen chloride salt[16] (**66**, 1 g, 3.81 mmol) was added to stirred solution of sodium acetate (2 g), sodium sulfite (2 g) in 80 mL DMF under argon. A solution of succinic anhydride (3.05 g) in 30 mL DMF was dropwise added and stirred for 18 h at rt. The reaction mixture was filtered, water (3 mL) added to the filtrate and after1 h, 500 mL of dichloromethane was added and kept in the refrigerator for 36 h. The orange red solid was filtered and dried (1.2034 g, 81%): mp 180–180.6 °C; ^{1}H NMR (DMSO-d_6) δ 2.54 (m, 4H), 2.64 (m, 4H), 2.70 (s, 3H), 7.35 (d, 1H, J = 8.8), 8.06 (s, 1H), 8.18 (d, 1H, J = 8.8), 9.65 (br s, 1H), 9.98 (br s, 1H); HRMS *m*/*e* calcd for $C_{18}H_{16}N_3O_5$ (M−2 H_2O+H), 354.109; found, 354.108.

4.1.6. 5-Hydroxytryptophan isoamyl ester (27)

5-Hydroxytryptophan (1.101 g, 5 mmol) in 60 mL anhydrous isoamyl alcohol with 10 mL of hydrogen chloride ether solution (1.0 M) was refluxed for 22 h. The reaction mixture was evaporated, the solid salt was mixed with 50 mL of dichloromethane and neutralized with a solution of 14% ammonium hydroxide. The aqueous layer was extracted with EtOAc (3 × 75 mL) and the combined organic extracts were dried ($MgSO_4$) and then evaporated to give 1.28 g (91%) of product **27** as a gel: ^{1}H NMR (DMSO-d_6) δ 0.89 (d, 6H, J = 6.2), 1.30 (m, 2H), 1.60 (m, 1H), 2.8 (m, 1H), 2.9 (m, 1H), 4.14 (t, 2H, J = 7.0), 6.55 (s, 1H), 6.75 (m, 1H), 6.95 (m, 1H), 7.10 (d, 1H, J = 8.8), 8.56 (m, 1H), 10.51 (s, 1H); HRMS *m*/*e* calcd for $C_{16}H_{22}N_2O_3$, 290.163; found, 290.162.

4.1.7. Methyl (2*RS*,3*SR*)-2-amino-3-[3-(5-hydroxyindolyl)]butanoate (28)

In a 500 mL heavy-walled hydrogenation flask equipped with a magnetic stirring bar, a cool solution of trifluoroacetic acid (0.96 g, 8.44 mmol) in 145 mL absolute ethanol was placed. To this, powdered benzyloxy nitro ester **71A** (1.03 g, 2.81 mmol) was added and the mixture was stirred for 1 h for most of the solid to be dissolved. The magnetic bar was removed and 1.65 g of 10% Pd–C was added. Using a Parr hydrogenator, the mixture was hydrogenated at 40 psi at rt for 21 h, then filtered through a layer of Celite. The filter cake was washed with absolute ethanol (3 × 15 mL) and the filtrate was evaporated to dryness. To the residue, 60 mL ether, 2 mL water, 3 mL 14% ammonium hydroxide were added and the yellow ether layer was separated. The aqueous layer was extracted with ether (5 × 50 mL), then the combined ether extracts were washed with 10% sodium chloride solution (2 × 10 mL), dried (MgSO4) and evaporated to give a solid. The product was redissolved in some ether and evaporated. This process was repeated nine more times and the off-white crystals were dried on a vacuum pump, producing 0.65 g (93%) of **28**: mp 88–95 °C (unable to recrystallize **28** further): ^{1}H NMR ($CDCl_3$) δ 1.28 (d, 3H, J = 7.1), 2.15 (br s, 2H), 3.57 (m, 1H), 3.65 (s, 3H), 3.89 (d, 1H, J = 3.8), 6.74 (m, 1H), 6.98 (d, 1H, J = 2.2), 7.03 (d, 1H, J = 2.5), 7.04 (s, 1H), 7.17 (d, 1H, J = 3.7), 7.95 (br s, 1H); HRMS m/e calcd for $C_{13}H_{16}N_2O_3$; 248.115; found, 248.115.

4.1.8. Methyl 3-[3-(5-benzyloxyindolyl)]nitrobutanoate (71)

In a 500 mL three-necked round-bottomed flask equipped with a magnetic stirring bar, thermometer, condenser under argon flow was placed indole **70** (1.72 g, 5.54 mmol) in 19.5 mL anhydrous toluene. To this, 0.74 g (7.29 mmol) of freshly distilled triethylamine and 1.0 g (8.42 mmol) of methyl nitroacetate were added. The solution was stirred for 1 h at rt, then heated at 103–105 °C for 9 h and allowed to cool to rt. The solution was extracted with a10% hydrochloric acid solution (3 × 24 mL) followed by water (2 × 13 mL). The organic layer was dried ($MgSO_4$), evaporated and the solid was dried under a vacuum pump at 60 °C overnight (1.98 g, 97%). NMR showed the product to be a mixture of the two stereoisomers **71A** and **71B**. Careful recrystallization of this product with a mixture of chloroform: hexane (60:40) produced only **71A**. An analytical sample was obtained by the recrystallization of the product with 95% ethanol giving **71A** as off-white crystals: mp 110–111 °C; ^{1}H NMR ($CDCl_3$) δ 1.53 (d, 3H, J = 3.5), 3.53 (s, 3H), 4.10 (m, 1H), 5.11 (s, 2H), 5.60 (d, 1H, J = 9.0), 6.94 (dd, 1H, J = 9.0, 2.3), 7.05 (dd, 1H, J = 9.0, 2.5), 7.10 (m, 1H), 7.23 (s, 1H), 7.38 (m, 5H), 9.01 (br s, 1H). Anal. Calcd for $C_{20}H_{20}N_2O_5$: C, 65.21; H, 5.47; N, 7.60. Found: C, 65.04; H, 5.54; N, 7.57; HRMS m/e calcd, 368.141; found, 368.137.

4.1.9. 3-(Isopropylaminoethylidene)-5-benzyloxyindole (70)[31,34]

A 250 mL three-necked round-bottomed flask equipped with a magnetic stirring bar, thermometer, dropping funnel, Claisen head, a condenser with flowing ice-water (using an ice-water circulator) and a calcium chloride drying tube, was immersed in an ice-salt bath. A solution of 5-benzyloxyindole **69** (3.06 g, 0.014 mol) in 50 mL of glacial acetic acid was placed in the flask and then freshly distilled ethylideneisopropylamine[34] (1.31 g, 0.015 mol) in 23 mL anhydrous toluene was dropwise added over an h, maintaining the mixture temperature below 15 °C. The reaction mixture was stirred at this temperature for 30 more minutes and then kept in the refrigerator for four days. With vigorous stirring, the mixture was added to 115 mL of ice-water, 38 mL of ether and then the dark upper ether layer was separated from the aqueous layer. The ether solution was extracted with 1 M potassium hydrogen sulfate (3 × 23 mL) and all the aqueous solutions were combined and basified with 10 M NaOH to pH 11 or higher, while the solution temperature was kept below 25 °C. Enough base was added to produce an oily layer at the top of the solution. Solidification of the oil was facilitated by the addition of 5 mL methylcyclohexane and scratching the side of the beaker inside the oil. The mixture was allowed to stand in the refrigerator overnight, the resulting solid filtered off, and washed thoroughly with cold water to remove any remaining NaOH. The crystals of **70** were vacuum dried (1.61 g, 38%): mp 132–132.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.01 (d, 3H, J = 4.0), 1.04 (d, 3H, J = 4.0), 1.40 (br s, 1H), 1.46 (d, 3H, J = 6.6), 2.82 (m, 1H), 4.17 (q, 1H, J = 6.6), 5.10 (s, 2H), 6.92 (m, 1H), 7.06 (d, 1H, J = 2.3), 7.22 (s, 2H), 7.37 (m, 5H), 7.89 (br s, 1H). Anal. Calcd for $C_{20}H_{24}N_2O$: C, 77.89; H, 7.84; N, 9.08. Found: C, 77.55; H, 7.74; N, 8.86; HRMS m/e calcd, 308.193; found, 308.189.

4.1.10. Tryptophan 2-hydroxyethanamide (30)

In a 100 mL round-bottomed flask equipped with a magnetic stirring bar and an argon filled balloon, a mixture of amide **73** (1.45 g, 3.82 mmol), isopropanol (66 mL), 10% Pd–C (1.785 g) and dry ammonium formate (1.204 g, 19 mmol) was stirred at rt for 5 h. The reaction mixture was filtered and the filtrate was evaporated to give **30** as a gel (774 mg, 82%); ^{1}H NMR ($CDCl_3$) δ 3.02 (m, 2H), 3.39 (m, 2H), 3.65 (m, 2H), 3.67 (m, 1H) 7.09 (s, 1H), 7.15 (m, 1H), 7.21 (m, 1H), 7.37 (d, 1H, J = 8.0), 7.62 (br s, 1H), 7.83 (d, 1H, J = 7.3), 8.07 (br s, 1H); HRMS m/e calcd for $C_{13}H_{17}N_3O_2$, 248.139; found, 248.139.

4.1.11. *N*-Carbobenzyloxytryptophan 2-hydroxyethanamide (73)

In a 25 mL round-bottomed flask equipped with a magnetic stirring bar and an argon filled balloon, 544 mg (1.25 mmol) of succinimide ester **72**, and 76.4 mg (1.25 mmol) of 2-hydroxyethanol were added to a mixture of isopropyl alcohol (5.2 mL), chloroform (5.2 mL) and triethylamine (0.8 mL). The reaction mixture was stirred at rt for 23 h and then mixed with 60 mL EtOAc. The organic layer was washed with 10 mL portions of brine, 10% citric acid and water, dried ($MgSO_4$) and then evaporated to give tryptophan hydroxyamide **73** as a gel (464.6 mg, 98%): ^{1}H NMR ($CDCl_3$) δ 2.65 (br s, 2H), 3.40 (m, 1H), 3.48 (m, 1H), 3.67 (s, 2H), 5.10 (m, 5H), 5.43 (m, 1H), 7.08 (m, 1H), 7.15 (m, 1H), 7.29 (m, 7H), 7.55 (m, 1H), 8.41 (br s, 1H); HRMS m/e calcd for $C_{21}H_{24}N_3O_4$ (MH^+), 382.176; found, 382.176.

4.1.12. Tryptophan 2,3-dihydroxypropanamide (31)

In a set up similar to that for **30** and in a 50 mL flask, amide **74** (411 mg, 1 mmol), anhydrous methanol (18 mL), 10% Pd–C (315.3 mg) and anhydrous ammonium formate were mixed and stirred at rt for 30 min. The mixture was filtered and the filtrate evaporated to give **31** as a colorless gel (236 mg, 85%); ^{1}H NMR (DMSO-d_6) δ 2.80 (m, 1H), 2.97 (m, 2H), 3.11 (m, 1H), 3.25 (m, 3H), 3.46 (m, 2H), 3.56 (m, 1H), 6.98 (t, 1H, J = 8.1), 7.07 (t, 1H, J = 8.1), 7.17 (s, 1H), 7.34 (d. 1H, J = 8.4), 7.58 (d, 1H, J = 8.4), 8.06 (br s, 1H), 8.29 (s, 1H), 10.89 (br s, 1H); HRMS m/e calcd for $C_{14}H_{20}N_3O_3$ (MH^+), 278.150; found, 278.150.

4.1.13. *N*-Carbobenzyloxytryptophan 2,3-dihydroxypropanamide (74)

In a similar procedure to that of **73**, succinimide ester **72** (1.74 g, 4 mmol), 2,3-dihydroxypropanamine (364 mg, 4 mmol) were added to a mixture of 8 mL of isopropyl alcohol, 16 mL of chloroform and 1.12 mL of triethylamine and stirred at rt for 24 h. To the mixture, 120 mL EtOAc was added and the solution was washed consecutively with 10 mL portions of brine, 10% citric acid and then water. The organic layer was dried (Mg SO_4) and evaporated to give 1.66 g (71%) of **74** as a gel: ^{1}H NMR (DMSO-d_6) δ 3.10 (m, 5H), 4.25 (m, 2H), 4.50 (m, 1H), 4.72 (m, 1H), 5.17 (m, 4H), 7.00 (m, 2H), 7.13 (s, 1H), 7.35 (m, 5H), 7.60 (m, 1H),

7.98 (br s, 1H), 10.80 (br s, 1H); HRMS *m*/*e* calcd for $C_{22}H_{26}N_3O_5$ MH^+), 412.187; found, 412.187.

4.1.14. Tryptophan *N*-benzylpiperazine amide (32)

In a setup similar to that for **30**, and in a 50 mL flask, a mixture of amide **75** (250 mg, 0.5 mmol), ammonium formate (158 mg, 2.5 mmol) and 10% Pd–C (790 mg) were added to 20 mL anhydrous isopropyl alcohol and stirred at rt for 35 min. The reaction mixture was filtered and the filter cake was washed with 20 mL isopropanol. The solid obtained upon the evaporation of the filtrate was suspended in 3 mL of dichloromethane and introduced on top of a 4 in diameter flash chromatography column. The column was first eluted with a mixture of dichloromethane/methanol (10:1) to remove the unreacted starting materials and then the product was eluted with methanol/dichloromethane (5:1). Evaporation of the eluant gave a yellowish white solid that was dried under a vacuum pump at 70–80 °C over 48 h, yielding 95 mg (52%) of the white solid **32**: mp 86–87 °C; ^{1}H NMR (DMSO-d_6) δ 1.70 (m, 2H), 2.16 (m, 2H), 2.80 (m, 1H), 2.90 (m, 1H), 3.29 (m, 8H), 3.91 (m, 1H), 6.95 (m, 1H), 7.01 (m, 1H), 7.12 (s, 1H), 7.27 (m, 5H), 7.35 (d, 1H, *J* = 8.0), 7.47 (d, 1H, *J* = 7.8), 10.85 (s, 1H); HRMS *m*/*e* calcd for $C_{22}H_{27}N_4O$ (MH^+), 363.217; found, 363.218.

4.1.15. *N*-Carbobenzyloxytryptophan *N*-benzylpiperazine amide (75)

In a similar set up to that for **73**, succinimide ester **72** (435 mg, 1 mmol), *N*-benzylpiperazine (176 mg, ~ 0.18 mL, 1 mmol) were added to 5 mL chloroform, absolute ethanol (3 mL), and 0.14 ml (1 mmol) of triethylamine and was stirred at rt for 48 h. The mixture was evaporated and the solid was dissolved in 90 mL dichloromethane and the solution was washed with 30 mL of water followed by 2 × 30 mL of 10% citric acid. The aqueous layer was extracted with 2 × 10 mL dichloromethane and the combined organic extracts were washed with 30 mL of 1 M sodium bicarbonate solution, followed by 5 mL of water, dried ($MgSO_4$) and evaporated to dryness to give a gel. This material was further dried under a vacuum pump at 60 °C overnight to produce the white solid of **75** (455 mg, 92%): mp = 134–136 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (m, 4H), 2.01 (m, 4H), 3.16 (m, 2H), 3.30 (s, 2H), 4.96 (m, 1H), 5.11 (s, 2H), 5.76 (m, 1H), 7.25 (m, 14H) 7.64 (d, 1H, *J* = 7.7), 8.08 (s, 1H); HRMS *m*/*e* calcd for $C_{30}H_{32}N_4O_3$, 496.247; found, 496.247.

4.1.16. DL-Tryptophan *n*-propyl ester (33)

Using a similar procedure to that for ester **27**, ester **33** was obtained in 88% yield by the reaction of *n*-propyl alcohol with DL-tryptophan in the presence of anhydrous HCl in ether as a thick honey colored paste. TLC showed the product to be pure: R_f = 0.58 (3:2 acetone/EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.90 (m, 3H), 1.6 (s, 2H), 1.75 (m, 2H), 3.04 (m, 1H), 3.27 (m, 1H), 3.83 (m, 1H), 4.05 (m, 2H), 7.06 (s, 1H), 7.12 (m, 1H), 7.20 (m, 1H), 7.35 (d, 1H, J = 8.1), 7.63 (d, 1H, J = 8.1), 8.30 (br s, 1H).

4.1.17. L-Tryptophan *sec*-butyl ester (34)

L-Tryptophan (1 g, 4.9 mmol) and *sec*-butyl alcohol (60 mL) were placed in a 100 mL round-bottomed flask equipped with a magnetic stirring bar, a condenser and an oil bubbler. The flask was cooled in an ice-bath for 10 min and then 12 mL of an anhydrous HCl–ether solution was added via a syringe through a rubber septum at the top of the condenser. The flask was removed from the ice-bath, placed in an oil bath and then refluxed for 22 h. Three drops of concd sulfuric acid was added and the solution was refluxed for an additional 2 h. When thin layer chromatography indicated the completion of the reaction, the mixture was allowed to cool to rt to provide a solid residue. Dichloromethane (50 mL) was added to the residue, then placed in a separatory funnel and treated with a 5% solution of sodium bicarbonate until pH ~8. The mixture was extracted with dichloromethane (4 × 40 mL), washed with 40 mL of brine, dried ($MgSO_4$) and then evaporated to yield 0.88 g (69%) of **34** as a light brown gel. ^{1}H NMR ($CDCl_3$) δ 0.90 (m, 3H), 1.20 (m, 3H), 1.60 (m, 4H), 3.00 (m, 1H), 3.30 (m, 1H), 3.80 (m, 1H), 4.87 (m, 1H), 7.07 (s, 1H), 7.20 (m, 2H), 7.35 (d, 1H, *J* = 7.7), 7.65 (d, 1H, *J* = 7.7), 8.22 (br s, 1H); HRMS *m*/*e* calcd for $C_{15}H_{20}N_2O_5$, 260.152; found, 260.151.

4.1.18. 5-Hydroxytryptophan ethyl ester (36)

In a 100 mL round-bottomed flask, 5-hydroxtrypyophan (1.101 g, 5 mmol), ethanol (50 mL) and 10 mL of hydrogen chloride ether solution (1.0 M) was heated in an oil bath at 88 °C for 24 h. The reaction mixture was evaporated, the solid salt was mixed with 50 mL of dichloromethane and neutralized with a solution of 14% ammonium hydroxide. The aqueous layer was extracted with EtOAc (3 × 75 mL) and the combined organic extracts were dried ($MgSO_4$) and then evaporated to give 367 mg (29%) of ester **36** as a gel: ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3H, *J* = 7.2), 1.80 (br s, 2H), 2.02 (s, 1H), 3.00 (m, 1H), 3.22 (m, 1H), 3.77 (m, 1H), 4.15 (q, 2H, *J* = 7.2), 6.72 (m, 1H), 7.01 (m, 2H), 7.22 (m, 1H), 7.95 (br s, 1H); HRMS *m*/*e* calcd for $C_{13}H_{16}N_2O_3$, 248.115; found, 248.115.

4.1.19. 7-*N*-Acetyl-11′-hydroxydemethyllavendamycin isoamyl ester (41)

In a 250 mL three-necked round-bottomed flask, equipped with a magnetic stirring bar and a condenser under an argon flow, isoamyl ester **27** (84 mg, 0.3 mmol) in 10 mL anhydrous DMF and 100 mL anhydrous anisole were placed. To this solution, aldehyde 17 (73.2 mg, 0.3 mmol) was added and heated to 150 °C over 3 h and then the heat was maintained for an additional 5 h. The golden yellow solution was allowed to cool to rt and then evaporated to give an orange solid that was washed with 3 mL of acetone followed by 3 mL of ether. The filtrate also gave some product as a brown solid. Total weight of the product **41** was 80.2 mg (53%): mp >290 °C; R_f = 0.23 (0.2:5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 1.01 (d, 6H, *J* = 6), 1.70 (m, 1H), 2.30 (m, 2H), 2.42 (s, 3H), 4.38 (t, 2H, *J* = 6.4), 7.20 (m, 1H), 7.35 (d, 1H, *J* = 8.8), 7.65 (d, 1H, *J* = 1.4), 7.71 (s, 1H), 8.40 (d, 1H, *J* = 8.1), 8.72 (d, 1H, *J* = 8.1), 8.79 (s, 1H), 9.45 (s, 1H), 10.12 (br s, 1H), 11.48 (br s, 1H); HRMS *m*/*e* calcd for $C_{28}H_{24}N_4O_6$ 512.170; found, 512.169.

4.1.20. 7-*N*-Acetyl-11′-hydroxylavendamycin methyl ester (42)

In a set up similar to that for **41**, in a 500 mL flask, aldehyde **17** (180.7 mg, 0.58 mmol), 5-hydroxy-β-methyltryptophan methyl ester (**28**, 198.8 mg, 0.8 mmol) in 390 mL of anhydrous anisole were slowly heated to 162 °C over a period of 3 h and then allowed to continue at this temperature for 26 h. In the last 2 h of the reaction, argon flow was replaced by an oxygen flow. The reaction mixture was evaporated to give a dark red solid (201 mg, 59%): mp 180–181 °C (dec); R_f = 0.49 (0.4:5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H), 2.96 (s, 3H), 3.94 (s, 3H), 7.16 (d, 1H, *J* = 8.8), 7.38 (d, 1H, *J* = 8.8), 7.61 (br s, 1H), 7.71 (s, 1H), 8.36 (d, 1H, *J* = 8.2), 8.69 (d, 1H, *J* = 8.3), 9.42 (s, 1H), 9.42 (s, 1H), 10.12 (s, 1H), 11.56 (br s, 1H); HRMS *m*/*e* calcd for $C_{25}H_{18}N_4O_6$, 470.122; found, 470.121.

4.1.21. 7-*N*-Demethyllavendamycin 2-hydroxyethanamide (44)

Using a similar set up as that for **41**, in a 500 mL flask, tryptophan (2-hydroxyethan)amide (**30**, 125.8 mg, 0.5 mmol) was dissolved in a mixture of 10 mL anhydrous DMF and 220 mL anisole, heated to 84 °C and to this, 122 mg (0.5 mmol) of 7-acetamido-2-formylquinoline-5,8-dione (**17**) was added. The reaction mixture was gradually heated to 150–155 °C over 3 h and kept at this temperature for another 3 h. The mixture was evaporated, the solid was washed consecutively with 5 mL portions of acetone, ether and finally petroleum ether to give 131 mg of a product. NMR

showed that this product contained some of the 5,8-dihydroxy derivative that was converted to **44** by treating with 63.65 mg of DDQ in 23 mL THF and allowed to stir at rt for 20.5 h. The solid was consecutively washed with 3 mL portions of THF, acetone and petroleum ether yielding 124 mg (53%) of **44** as an orange solid: mp >280 °C; R_f = 0.47 (0.2:5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H), 3.53 (m, 2H), 3.62 (m, 2H), 4.86 (m, 1H), 7.42 (dd, 1H, J = 7.0, 7.0), 7.67 (m, 2H), 7.84 (s, 1H), 8.54 (m, 2H), 9.07 (s, 1H), 9.09 (m, 1H), 9.42 (d, 1H, J = 8.4), 10.32 (br s, 1H), 11.98 (br s, 1H); HRMS m/e calcd for $C_{25}H_{19}N_5O_5$, 469.138; found, 469.137.

4.1.22. *N*-Acetyldemethyllavendamycin *sec*-butyl ester (46)

In a set up similar to that for **41**, anhydrous and freshly distilled xylene (145 mL) was placed in the flask and to this, 7-acetamido-2-formylquinoline-5,8-dione (**17**, 0.2401 g, 1 mmol) was added and heated to 100 °C. To the mixture, a solution of L-tryptophan *sec*-butyl ester (**34**, 0.2568 g, 1 mmol) in 12 mL of anhydrous and freshly distilled xylene under argon was added via a syringe and then heated for 16 h at 130 °C until the reaction was completed (TLC). The mixture was filtered off to produce a greenish solid and the filtrate was kept in the refrigerator overnight to give more product (total, 0.2785 g, 57%). An analytical sample of **46** was obtained by recrystallization with DMSO: mp = 268 °C; R_f = 0.46 (1.7:10 MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3H, J = 7.7), 1.42 (d, 3H, J = 6.2), 1.8 (m, 1H), 2.30 (s, 3H), 5.2 (m, 1H), 7.34 (t, 1H, J = 8.1), 7.62 (t, 1H, J = 8.1), 7.69 (d, 1H, J = 8.4), 7.93 (m, 2H), 8.21 (d, 1H, J = 7.7), 8.35 (br s, 1H), 8.52 (d, 1H, J = 8.4), 8.88 (s, 1H), 9.17 (d, 1H, J = 8.4), 11.23 (br s, 1H); HRMS m/e cacld for $C_{27}H_{25}N_4O_5$ $(M + 3H)^+$, 485.182; found, 485.183.

4.1.23. 7-*N*-Acetyldemethyllavendamycin 2,3-dihydroxypropanamide (47)

In a set up similar to that for **41**, tryptophan 2,3-dihydroxypropanamide (**31**, 114.8 mg, 0.44 mmol) was dissolved in 40 mL anhydrous DMF and 240 mL anhydrous anisole and heated to 80 °C in a 500 mL flask. To this, 7-acetamido-2-formylquinoline-5,8-dione (**17**, 97.6 mg, 0.44 mmol) was added and the mixture was gradually heated to 150–154 °C over a period of 3 h, and then continued at this temperature for five additional hours. The reaction mixture was concentrated to a small volume producing **47** as orange crystals. The crystals were washed with ethyl ether and dried (104.2 mg, 52%): mp 214–215 °C (dec); R_f = 0.4 (0.3:5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H), 3.37 (m, 1H), 3.45 (m, 2H), 3.60 (m, 1H), 4.75 (m, 1H), 5.00 (m, 1H), 7.45 (m, 1H), 7.74 (m, 2H), 7.84 (s, 1H), 8.55 (m, 2H), 9.07 (s, 1H), 9.12 (br s, 1H), 9.34 (d, 1H, J = 8.4), 11.97 (br s, 1H); HRMS m/e calcd for $C_{26}H_{21}N_5O_6$, 499.149; found, 499.147.

4.1.24. 7-*N*-Acetyldemethyllavendamycin *n*-propyl ester (48)

In a set up similar to that of **41**, in a 500 mL flask, 7-acetamido-2-formylquinoline-5,8-dione (**17**, 149 mg, 0.61 mmol), tryptophan *n*-propyl ester (**33**, 162 mg, 0.66 mmol) in 90 mL anhydrous and distilled xylene were heated to 100 °C over 1 h and then allowed to reflux for 3 h. While hot, some solid impurities were filtered and the filtrate was evaporated to a small volume and allowed to cool giving an orange red solid. The product was dried on a vacuum pump, producing 80 mg (30%) of **48**: mp 265 °C (dec): R_f = 0.48 (0.1:5 MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J = 7.3), 1.94 (m, 2H), 2.36 (s, 3H), 4.46 (t, 2H, J = 7.4), 7.40 (t, 1H, J = 8.1), 7.67 (t, 1H, J = 6.9), 7.75 (d, 1H, J = 8.0), 8.00 (s, 1H), 8.26 (d, 1H, J = 7.6), 8.44 (s, 1H), 8.58 (d, 1H, J = 8.4), 8.97 (s, 1H), 9.22 (d, 1H, J = 7.1), 11.83 (br s, 1H); HRMS m/e calcd for $C_{26}H_{20}N_4O_5$; 468.143; found, 468.144.

4.1.25. 7-*N*-Acetyldemethyllavendmycin *N*-benzylpiperazine amide (49)

In a set up similar to that for **41**, in a 100 mL flask, aldehyde **17** (122 mg, 0.5 mmol) in 40 mL anhydrous anisole was stirred and heated to 70 °C until it was dissolved. A solution of *N*-benzylpiperazine amide of tryptophan (**32**, 180 mg, 0.5 mmol) in 5 mL dried and freshly distilled pyridine was introduced to the reaction flask using a syringe. The reaction mixture was heated to reflux (30 min) and then maintained at this temperature for 6 h. The mixture was allowed to cool to rt, the yellow lemon precipitate was filtered off and the filter cake was washed with THF (3 mL), followed by petroleum ether (3 mL). The solid was vacuum dried, suspended in 10 mL THF and then treated with DDQ (94 mg, 0.41 mmol), stirred at rt for 24 h in order to oxidize any 5,8-dihydroxy lavendamycin present in the mixture to **49**. The brown reaction mixture was filtered off, the filter cake washed with THF (3 mL) followed by petroleum ether (3 mL) and dried on a vacuum pump giving 115 mg (40%): mp >260 °C; R_f = 0.88 (1:7 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H), 3.30 (m, 4H), 3.57 (s, 2H), 3.76 (m, 4H), 7.32 (m, 6H), 7.70 (m, 2H), 7.81 (s, 1H), 8.47 (d, 1H, J = 7.7), 8.55 (d, 1H, J = 8.2), 8.70 (s, 1H), 8.86 (d, 1H, J = 8.2), 10.30 (br s, 1H), 11.85 (br s, 1H); HRMS m/e calcd for $C_{34}H_{28}N_6O_4$; 584.217; found, 584.219.

4.1.26. 7-*N*-Acetyl-11′-hydroxydemethyllavendamycin ethyl ester (51)

Using a similar set up to that for **41**, in a 100 mL flask, 5-hydroxytryptophan ethyl ester (36, 29.76 mg, 0.12 mmol) in 4 mL of DMF was added to 40 mL anhydrous anisole and then with 29.8 mg (0.12 mmol) of 7-acetamido-2-formylquinoline-5,8-dione[9,10] (**17**) was gradually heated to 160 °C over 3 h and kept at this temperature for 23.5 h. The orange solution was evaporated to give a dark solid. The crude product was washed with 3 mL acetone and dried under vacuum producing the dark violet **51** (27 mg, 48%): mp >280 °C; R_f = 0.69 (0.2:5 MeOH/ CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 1.44 (m, 3H), 2.30 (s, 3H), 4.40 (m, 2H), 7.17 (m, 1H), 7.40 (m, 1H), 7.67 (br s, 1H), 7.74 (s, 1H), 8.44 (d, 1H, J = 8.4), 8.78 (d, 1H, J = 8.4), 8.84 (s, 1H), 9.45 (s, 1H), 10.15 (br s, 1H), 11.53 (br s, 1H); HRMS m/e calcd for $C_{25}H_{18}N_4O_6$, 470.122; found, 470.121.

4.1.27. 7-*N*-Formyldemethyllavendamycin methyl ester (53)

In a similar set up as that for **41**, 7-formamido-2-formylquinoline-5,8-dione (**20**, 50 mg, 0.22 mmol), and tryptophan methyl ester (46 mg, 0.21 mmol) were dissolved in 125 mL of anhydrous anisole in a 250 mL flask, heated to 130 °C over an hour and continued for another 1.5 h. Anisole was evaporated and the solid residue was washed with 5 mL of acetone. The brownish solid was filtered off and washed with a few mL acetone and dried. The product **53** weighed 18.45 mg (19.6%): mp >300 °C; R_f = 0.79 (0.2:2.3 MeOH/EtOAC/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 4.1 (s, 3H), 7.42 (m, 1H), 7.69 (m, 1H), 7.78 (d, 1H, J = 8.1), 8.02 (s, 1H), 8.26 (d, 1H, J = 8.1), 8.53 (br s, 1H), 8.61 (d, 1H, J = 8.4), 8.76 (br s, 1H), 9.02 (s, 1H), 9.26 (d, 1H, J = 8.4), 11.85 (br s, 1H); HRMS m/e calcd for $C_{23}H_{14}N_4O_5$, 426.099; found, 426.096.

4.1.28. 7-*N*-Chloroacetyldemethyllavendamycin amide (54)

In a set up similar to that of **53**, 7-*N*-chloroacetyl-2-formylquinoline-5,8-dione[15] (**21**, 82.7 mg, 0.3 mmol), tryptophan amide[13] (**39**, 61 mg, 0.3 mmol), and anhydrous anisole (120 mL) were mixed, stirred and heated to 155 °C over a period of 3 h. Upon the completion of the reaction after 20 h, the mixture was allowed to cool to room temperature and the yellow solid was filtered off. The product was washed with petroleum ether and dried on a vacuum pump overnight to give 68 mg (49%) of pure **54**: mp >280 °C; R_f = 0.35 (0.25:5 MeOH/CH_2Cl_2). ^{1}H NMR (DMSO-d_6) δ 4.55 (s,

2H), 7.39 (t, 1H, J = 8.4), 7.70 (t, 1H, J = 8.4), 7.82 (d, 1H, J = 8.0), 8.49 (s, 1H), 8.49 (d, 1H, J = 8.1), 8.61 (d, 1H, J = 8.8), 9.02 (s, 1H), 9.12 (d, 1H, J = 9.2), 9.75 (s, 1H), 9.93 (s, 1H), 10.19 (s, 1H), 12.26 (br s, 1H); HRMS m/e calcd for $C_{23}H_{14}ClN_5O_4$, 462.097; found, 462.097.

4.1.29. 7-*N*-Chloroacetyldemethyllavendamycin pyrrolidine amide (55)

In a similar set up to that of **53**, quinolinedione aldehyde **21** (42.5 mg, 0.15 mmol), tryptophan pyrrolidine amide[15] (**29**, 39 mg, 0.15 mmol) and 55 mL anhydrous anisole were mixed in a 100 mL flask. The mixture was gradually heated to 155 °C over a period of 3 h and then heated at this temperature for an additional 18 h. The reaction mixture was filtered off hot and the filter cake was washed with 4 mL of dichloromethane followed by 4 mL of chloroform. The filtrate was evaporated, the crude product was introduced on a small column of silica gel and eluted with ethyl acetate to produce 11 mg (21%) of pure **55** as a yellow solid: mp 264 °C (dec); R_f = 0.77 (0.25:5 MeOH/CH_2Cl_2). ^{1}H NMR (DMSO-d_6) δ 1.96 (m, 4H), 3.64 (m, 2H), 3.98 (m, 2H), 4.66 (s, 2H), 7.41 (t, 1H, J = 8.1), 7.71 (m, 2H), 7.81 (s, 1H), 8.48 (d, 1H, J =8.1), 8.57 (d, 1H, J = 8.1), 8.88 (s, 1H), 8.94 (d, 1H, J = 8.4), 10.65 (s, 1H), 11.87 (br s, 1H); HRMS m/e calcd for $C_{27}H_{20}ClN_5O_4$, 513.124; found, 513.120.

4.1.30. 7-*N*-(2-Furyl)carbonyllavendamycin methyl ester (56)

Using a similar set up to that of **53**, in a 50 mL flask, a mixture of 7-furoylamino-2-formylquinoline-5,8-dione[16] (**22**, 29.7 mg, 0.10 mmol), β-methyltryptophan methyl ester[31] (**37**, 23.1 mg, 0.10 mmol) and 32 mL freshly distilled anhydrous xylene was gradually heated to 145 °C over a 3 h period. The heat was continued for an additional 19 h and then the mixture was evaporated to dryness. The dark orange solid was washed with a small amount of chloroform producing the light orange solid **56** (30.11 mg, 60%): mp 327–328 °C; R_f = 4.1 (0.1:5 MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 3.21 (s, 3H), 4.09 (s, 3H), 6.68 (m, 1H), 7.41 (m, 2H),7.68 (m, 2H), 7.82 (m, 1H), 8.06 (m, 1H), 8.36 (m, 1H), 8.53 (d, 1H, J = 8.4), 9.12 (d, 1H, J = 8.4), 9.36 (s, 1H), 11.92 (br s, 1H). HRMS m/e calcd for $C_{28}H_{18}N_4O_6$, 506.122; found, 506.122.

4.1.31. 7-*N*-(3-Carboxypropionyl)demethyllavendamycin *n*-butyl ester (57)

Using the same set up as that of **53**, 60 mL of anhydrous anisole was placed in a 100 mL flask and then tryptophan n-butyl ester[13] (**38**, 39.5 mg, 0.15 mmol) was added followed by 40 mg (0.13 mmol) of 7-*N*-(3-carboxypropionyl)-2-formylquinoline-5,8-dione (**23**). With stirring, this mixture was heated at 120 °C for 19.5 h. The reaction mixture was filtered to remove the solid impurity and the filtrate was evaporated. The amorphous solid was recrystallized from 10 mL ethyl acetate to give 22 mg (29%) of **57** as a red crystalline solid: mp 232.4 °C (dec); R_f = 0.83 (2.5:3.5 MeOH/$CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 1.01 (t, 3H, J = 7.3), 1.54 (m, 2H), 1.83 (m, 2H), 2.50 (m, 2H), 2.90 (m, 2H), 4.50 (m, 2H), 7.50 (m, 1H), 7.70 (m, 2H), 7.80 (s, 1H), 8.54 (d, 1H, J = 8.0), 8.59 (d, 1H, J = 8.4), 8.93 (d, 1H, J = 8.4), 9.09 (s, 1H), 10.35 (br s, 1H), 11.97 (br s, 1H); HRMS m/e calcd for $C_{29}H_{25}N_4O_7$ (MH^+), 541.172; found, 541.172.

4.1.32. 6-Chlorodemethyllavenamycin ethyl ester (58)

In a 500 mL flask with similar set up to that of **53**, a mixture of 7-amino-6-chloro-2-formylquinoline-5,8-dione (**24**, 47.4 mg. 0.2 mmol) and tryptophan ethyl ester[17] (**35**, 46.4 mg, 0.2 mmol) and 120 mL anhydrous anisole was slowly heated to 130 °C over 3 h. The reaction mixture was refluxed for 1 h and then allowed to cool to rt. The solvent was evaporated to dryness, the solid washed with acetone (20 mL), filtered and dried under vacuum giving 47 mg (49%) of **58**: mp >270 °C; R_f = 0.26 (0.1:5 MeOH/CH_2Cl_2); ^{1}H NMR $CDCl_3$) δ 1.57 (m, 3H), 4.57 (q, 2H, J = 7.2), 5.86 (br s, 2H), 7.42 (m, 1H), 7.68 (m, 1H), 7.77 (d, 1H, J = 7.3), 8.27 (d, 1H, J = 7.8), 8.67 (d, 1H, J = 8.2), 9.00 (s, 1H), 9.22 (d, 1H, J = 8.3), 11.86 (br s, 1H); HRMS m/e calcd for $C_{23}H_{16}ClN_4O_4$ (MH^+), 447.086; found, 447.086.

4.1.33. Demethyllavendamycin morpholine amide (59)

In a 25 mL two-necked round-bottomed flask, equipped with a magnetic stirring bar, a condenser and an argon filled balloon, 7-*N*-acetyldemethyllavendamycin morpholine amide[15] (**40**, 163 mg, 0.33 mmol) was placed and kept for a few min in an ice-bath. To this 15.8 mL of 70% sulfuric acid was dropwise added until the solid was dissolved. The mixture was heated at 61 °C for 6 h. To the reaction mixture, 10 mL water was added and neutralized to a pH of 8.5 with a saturated solution of sodium carbonate. The mixture was evaporated to give a solid material, that was finely ground by mortar and pestle and extracted with 160 mL of chloroform in a Soxhlet extractor overnight. The resulting solution was evaporated and dried to give 91 mg (63%) of the red orange **59**: mp 275–276 °C; R_f = 0.39 (0.01:5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 3.74 (m, 8H), 5.95 (s, 1H), 7.39 (t, 1H, J = 8.1), 7.48 (br s, 2H), 7.70 (m, 2H), 8.47 (d, 1H, J = 8.1), 8.49 (t, 1H, J = 8.1), 8.71 (s, 1H), 8.84 (d, 1H, J = 8.4) 11.87 (br s, 1H). HRMS m/e calcd for $C_{25}H_{20}N_5O_4$ (MH^+), 454.154; found, 454.151.

4.1.34. 11′-Hydroxydemethyllavendamycin isoamyl ester (60)

In a procedure similar to that of **59**, 7-*N*-acetyldemethyllavendamycin isoamyl ester (**41**, 25.1 mg, 0.05 mmol) and 2.4 mL of sulfuric acid (70%) was heated at 60 °C for 3.5 h. The reaction mixture was carefully neutralized with a saturated solution of sodium bicarbonate to pH 8, and then extracted with chloroform (4 × 100 mL). The organic extracts were washed with water followed by brine and then dried ($MgSO_4$). Evaporation of the solvent produced 16 mg (70%) of the orange product **60**: mp > 280 °C; R_f = 0.23 (0.2:5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 1.01(d, 6H, J = 6.2), 1.74 (m, 3H), 4.43 (m, 2H), 5.95 (s, 1H), 7.25 (d, 1H, J = 8.8), 7.61 (d, 1H, J = 8.8), 7.78 (br s, 1H), 8.54 (d, 1H, J = 8.4), 8.90 (d, 1H, J = 8.4), 8.97 (s, 1H), 9.46 (s, 1H), 11.82 (br s, 1H); HRMS m/e calcd for $C_{26}H_{22}N_4O_5$, 471.167; found, 471.169.

4.1.35. 11′-Hydroxylavendamycin methyl ester (61)

In a similar set up and procedure to that of **59**, acetyllavendamycin ester **42** (98.4 mg, 0.21 mmol) was slowly added to 15 mL of a 70% solution of sulfuric acid at 0 °C and then heated at 60 °C for 3 h. The reaction mixture was treated with a saturated solution of sodium carbonate to pH 8 and extracted with 8 × 75 mL of ethyl acetate, dried and evaporated to give **61** as red crystals. The aqueous layer was evaporated to dryness, the solid was ground and extracted with 150 mL ethyl acetate for 24 h in a Soxhelet extractor. Evaporation of the solution afforded more of **61** (total weight 33.7 mg, 38%): mp >280 °C; R_f = 0.72 (0.4:5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 2.85 (s, 3H), 3.90 (s, 3H), 5.85 (s, 1H), 7.06 (d, 1H, J = 8.8), 7.30 (d, 1H, J = 8.8), 7.41 (br s, 2H), 7.49 (s, 1H), 8.23 (d, 1H, J = 8.1), 8.50 (d, 1H, J = 8.1), 9.30 (s, 1H), 11.36 (s, 1H); HRMS m/e calcd for $C_{23}H_{16}N_4O_5$, 428.112; found, 428.113.

4.1.36. Demethyllavendamycin pyrrolidine amide (62)

In a similar procedure to that for **59**, 51 mg (0.11 mmol) of 7-*N*-acetyldemethyllavendamycin pyrrolidine amide[15] (**43**), was hydrolyzed with 5.2 mL of 70% sulfuric acid to give 24 mg (50%) of the orange red product **62**: mp 300 °C; R_f = 0.22 (0.1:5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 1.92 (m, 4H), 3.63 (m, 2H), 3.98 (m, 2H), 5.95 (s, 1H), 7.39 (m, 2H), 7.77 (m, 3H), 8.45 (m, 2H), 8.84 (m, 2H), 11.89 (br s, 1H); HRMS m/e calcd for $C_{25}H_{20}N_5O_3$ (MH^+) 438.156; found 438.156.

4.1.37. Demethyllavendamycin 2-hydroxyethanamide (63)

Lavendamycin **44** (70.5 mg, 0.15 mmol) was added slowly to 7.2 mL of 70% sulfuric acid and heated at 60 °C for 3.5 h. The mixture was treated with a saturated solution of sodium carbonate to pH 8 and evaporated to dryness. The residue was extracted with 70 mL of methanol/dichloromethane (10:60) and then with 35 mL of the same mixture. The extracts were concentrated to a small volume and the red crystals of **63** were filtered off (18.9 mg, 30%): mp >280 °C; R_f = 0.31 (0.3:5 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 3.83 (m, 2H), 4.40 (m, 2H), 5.02 (br s, 2H), 5.96 (s, 1H), 7.40 (m, 1H), 7.73 (m, 2H), 7.78 (d, 1H, J = 8.0), 8.5 (d, 1H, J = 8.0), 8.85 (d, 1H, J = 8.1), 8.92 (d, 1H, J = 8.1), 9.12 (s, 1H), 12.01 (br s, 1H); HRMS m/e calcd for $C_{23}H_{17}N_5O_4$, 427.128; found, 427.130.

4.1.38. Demethyllavendamycin methyl ester (64)

Using a similar set up as that for **59**, 7-*N*-butyryldemethyllavendamycin methyl ester[17] (**45**, 22 mg, 0.05 mmol) was slowly added to 2 mL of 70% sulfuric acid in a 5 mL flask with stirring. The mixture was heated at 60 °C for 4 h, then carefully neutralized with a saturated solution of sodium carbonate to pH 8 and extracted with chloroform (2 × 100 mL and 50 mL). The extracts were evaporated to give **64** as a red solid (17 mg, 85%): mp 220 °C (dec); R_f = 0.35 (0.1:5 MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 4.00 (s, 3H), 5.35 (br s, 2H), 6.15 (s, 1H), 7.41 (m, 1H), 7.70 (m, 2H), 7.81 (d, 1H, J = 8.0), 8.27 (d, 1H, J = 8.0), 8.60 (d, 1H, J = 8.3), 9.20 (d, 1H, J = 8.3), 12.00 (br s, 1H); HRMS m/e calcd for $C_{22}H_{14}N_4O_4$, 398.101; found, 398.099.

4.2. Biology

4.2.1. Cell culture

BE human colon adenocarcinoma cells and stably NQO1-transfected BE-NQ cells[32] were a gift from Dr. David Ross (University of Colorado-Denver, Denver, CO). Cells were grown in a minimum essential medium (MEM) with Earle's salts, non-essential amino acids, L-glutamine and penicillin/streptomycin, and supplemented with 10% fetal bovine serum (FBS), sodium bicarbonate and HEPES. Cell culture medium and supplements were obtained from Gibco, Invitrogen Co., Grand Island, NY. The cells were incubated at 37 °C under a humidified atmosphere containing 5% CO_2.

4.2.2. Spectrophotometric cytochrome *c* assay

Lavendamycins analogue 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 (0.1–3 μg) (gift from Dr. David Ross, University of Colorado-Denver, Denver, CO) and lavendamycins (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.

4.2.3. 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). Lavendamycin analogue solutions were applied in medium for 2 h. Lavendamycin analogues solutions were 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 h. 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 versus concentration. Selectivity ratios were defined as the IC_{50} value for the BE cell line divided by the IC_{50} value for the BE-NQ cell line.

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