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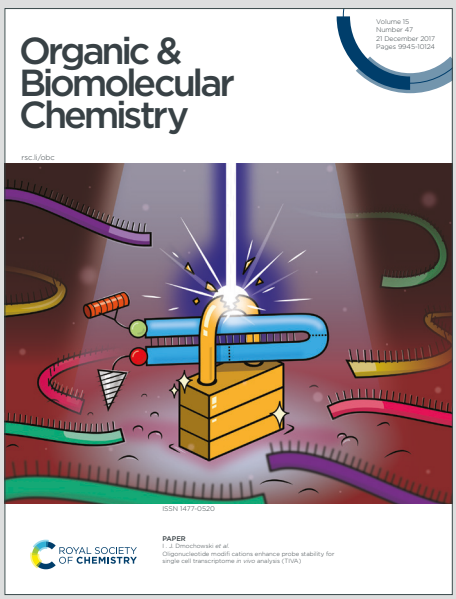

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Unified synthesis and cytotoxic activity of 8-*O*-methylfusarubin and its analogues

Pongsit Vijitphan [a], Vatcharin Rukachaisirikul [a], Chatchai Muanprasat [b,c], Panata Iawsipo [d,e], Jiraporn Panprasert [d,e] and Kwanruthai Tadpetch [*a]

[a]*Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*

[b]*Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

[c]*Excellent Center for Drug Discovery, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

[d]*Department of Biochemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Burapha University, Chonburi 20131, Thailand*

[e]*Unit of Bioactive Natural Compounds for Healthcare Products Development, Faculty of Science, Burapha University, Chonburi 20131, Thailand*

ABSTRACT

A simple and unified synthesis of four related pyranonaphthoquinone natural products e.g. 8-*O*-methylfusarubin, 8-*O*-methylanhydrofusarubin, fusarubin and anhydrofusarubin is reported. The key synthetic features include the precedented Diels-Alder cycloaddition to assemble the naphthalene skeleton, selective formylation and acetonylation and intramolecular acetalization to construct the pyran ring. Manipulation of the oxidation state of the naphthoquinone core was utilized to construct the two analogues, fusarubin and anhydrofusarubin. This work also highlights an unprecedented directing effect of the hydroxymethylene group in the selective hypervalent iodine-mediated quinone oxidation. The four synthetic compounds were evaluated for *in vitro* cytotoxic activity against six human cancer cells. 8-*O*-Methylfusarubin is the most potent analogue and displays excellent cytotoxic activity against MCF-7 breast cancer cells with an IC_{50} value of 1.01 μM with no cytotoxic effect to noncancerous Vero cells, which could potentially be a promising lead compound for anti-breast cancer drug discovery.

* Corresponding author. Tel.: +66 74 288437; fax: +66 74 558841.

Email address: kwanruthai.t@psu.ac.th (K. Tadpetch).

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Introduction

Naphthoquinones are secondary metabolites isolated from various natural sources such as plants, fungi, algae and bacteria.[1] The core structure of naphthoquinones consists of a naphthalene ring containing two carbonyl groups at 1,4 or 1,2 positions which can be named as 1,4-napthoquinones and 1,2-naphthoquinones, respectively (Figure 1). Pyrano-naphthoquinones are subgroups of naphthoquinones containing a pyran ring attached to naphthoquinones. The basic skeleton of pyranonaphthoquinones is naphtho[2,3-*c*]pyran-5,10-dione (Figure 1).[2] This group of metabolites has been reported to exhibit diverse biological activities particularly antimicrobial activity against a broad range of microbial and fungal species e.g. *Staphylococcus aureus*, *Escherichia coli* and *Candida rugosa*.[3] Furthermore, some pyranonaphthoquinones were shown to exhibit antiplasmodial, antimalarial and anticancer activities.[4]

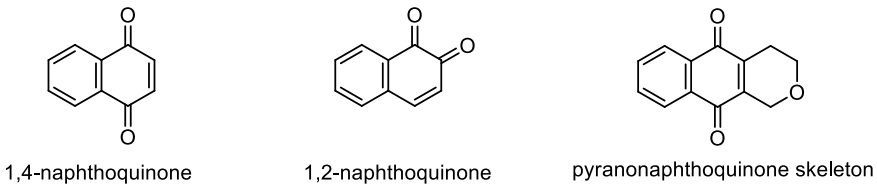

Figure 1. The core structures of 1,4-, 1,2-naphthoquinones and pyranonaphthoquinones.

In 2010, our research group reported the isolation of new anthraquinone and naphthoquinone derivatives along with known fusarubin[5] (**1**) and anhydrofusarubin[6] (**2**) from sea fan-derived fungi, *Fusarium* spp. PSU-F14 and PSU-F135.[7] Concurrently, our group also isolated a known related pyranonaphthoquinone, 8-*O*-methylfusarubin[8] (**3**) from a seagrass-derived fungus *Pestalotiopsis* sp. PSU-ES180 (Figure 2).[9] The isolated compounds **1**, **2** and **3** were examined for selected biological activities and were found to exhibit excellent *in vitro* cytotoxic activity against MCF-7 human breast cancer cells with IC_{50} values of 9.8, 1.06, 0.9 µM, respectively. Notably, the cytotoxic activities of compounds **2** and **3** were superior to a standard drug doxorubicin (IC_{50} = 2.18 µM). In addition, compounds **1**, **2** and **3** displayed very low cytotoxic effect against noncancerous African green monkey kidney (Vero) cells with IC_{50} values of 79, 49 and 58 µM, respectively. This finding has renewed the interest in the anticancer activity of this class of naphthoquinones. Owing to promising cytotoxic activity of these pyranonaphthoquinones and as part of our ongoing program for anticancer drug discovery, we have been focusing on a synthetic course of compounds in this class.

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Herein, we report a unified synthetic approach to pyranonaphthoquinone natural products **1**–**3** as well as a related analogue, 8-*O*-methylanhydrofusarubin[10] (**4**) and the cytotoxic activities against six human cancer cell lines of the synthetic compounds.

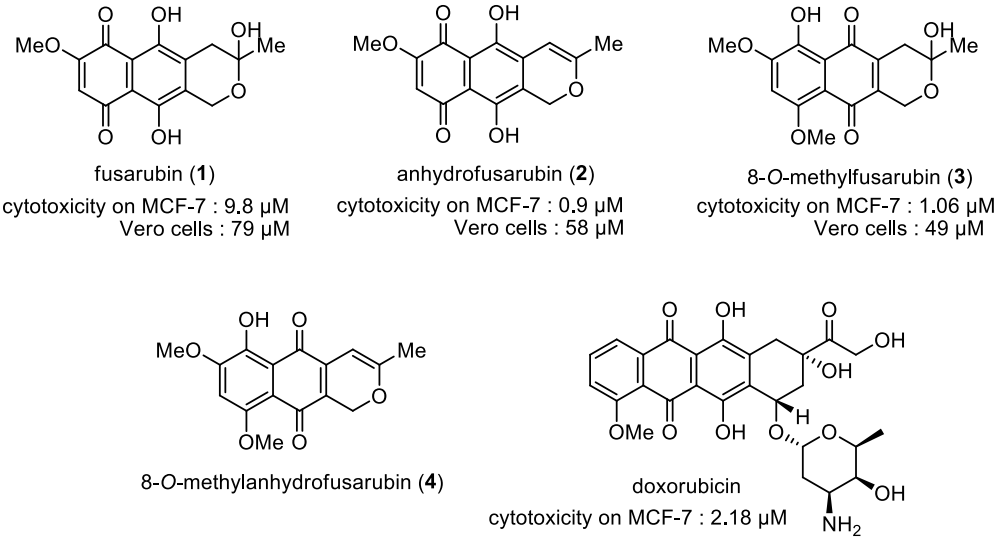

Figure 2. Structures of fusarubin (**1**), anhydrofusarubin (**2**), 8-*O*-methylfusarubin (**3**) and 8-*O*-methylanhydrofusarubin (**4**) and cytotoxic activities against MCF-7 cells of natural compounds **1**-**3** compared with doxorubicin.

To our surprise, there has been no report on the synthesis of fusarubin and 8-*O*-methylfusarubin whereas only one synthesis of anhydrofusarubin is precedented to date. In 2012, de Koning and co-workers disclosed the first total synthesis of **2** using Stobbe condensation to construct the naphthalene core and Wacker oxidation to assemble the isochromene ring as the key strategy.[11] Their synthetic approach led to **2** in 11 steps with an overall yield of 5%.

Results and Discussion

Structurally, fusarubin (**1**) and 8-*O*-methylfusarubin (**3**) only differ by the position of the 1,4-quinone moiety of the naphthoquinone core. From this inherent structural similarity, it was envisioned that compounds **1** and **3** could be synthesized by the same synthetic approach via manipulation of the oxidation state of the naphthoquinone core. We would begin with the syntheses of **3** and its dehydrated analogue **4**, of which their retrosynthetic analysis is depicted in Scheme 1. 8-*O*-methylanhydrofusarubin (**4**) would be derived from **3** via dehydration. The dihydropyran hemiacetal moiety of **3** was envisaged to be constructed from

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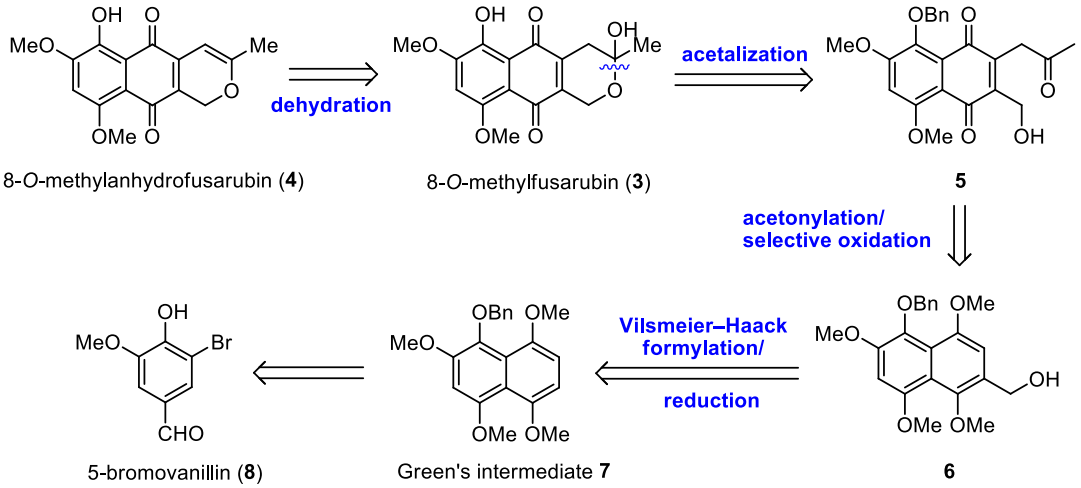
acetonylnaphthoquinone **5** via intramolecular acetalization. The acetalization precursor **5** would then be obtained from acetonylation of the corresponding naphthoquinone prepared from selective oxidation of naphthol derivative **6**. The hydroxymethylene moiety of **6** would be installed via Vilsmeier-Haack formylation/reduction of known pentaalkoxynaphthalene intermediate **7**, which in turn could be prepared from 5-bromovanillin following a protocol reported by Green and co-workers.[12]



Scheme 1. Retrosynthetic analysis of 8-*O*-methylfusarubin (**3**) and 8-*O*-methylanhydrofusarubin (**4**).

The Vilsmeier-Haack formylation precursor, pentaalkoxynaphthalene **7**, was prepared following a procedure reported by Green and co-workers with slight modification (Scheme 2). Starting with commercially available 5-bromovanillin (**8**), dibromobenzene **9** was obtained in 4 steps in 83% yield.[13] The key Diels-Alder cycloaddition between benzyne intermediate generated from **9** and 2-methoxyfuran (**10**) in THF at –78 °C constructed naphthols **11** and **12** in 62% combined yield as an inseparable mixture. Subsequent treatment of a mixture of **11** and **12** with NaH and CH_3I at 0 °C provided the desired naphthalene intermediate **7** in good yield.[14] Our modified synthesis led to improved yield of **7** in 42% over 6 steps. Our next task was to selectively install the formyl group at C-6 using formylation reaction. Kozlowski *et al.* has previously reported the Vilsmeier-Haack formylation of a very similar pentamethoxynaphthalene analogue of **7** which yielded the formylated product at C-6.[15]As expected, subjection of **7** to Vilsmeier-Haack formylation using oxalyl chloride and DMF resulted in the formation of naphthaldehyde **13** exclusively in 86% yield.[16] The position of the newly formed formyl group was confirmed by an nOe correlation between aldehyde

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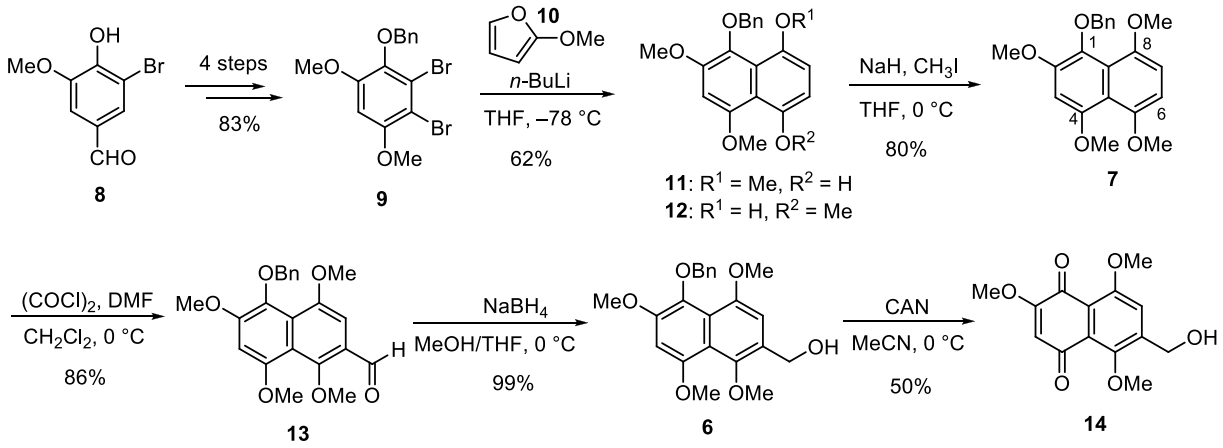
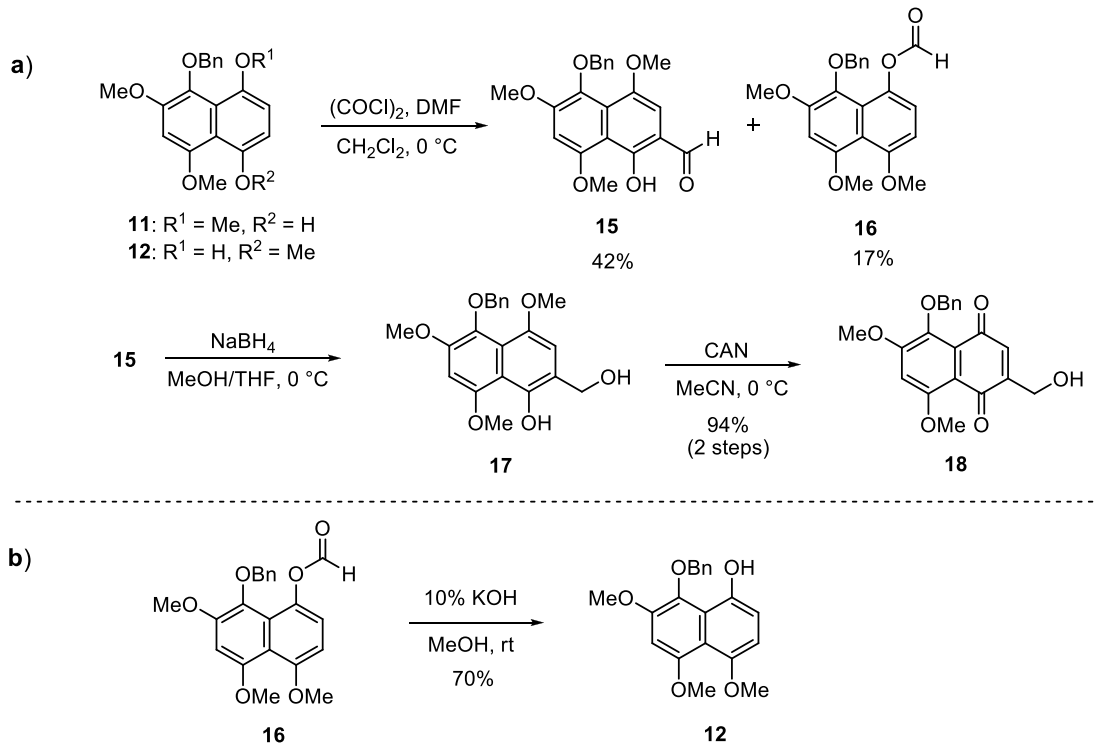
proton (δ 10.49) and C-5 methoxy proton (δ 3.92) signals as well as HMBC correlations. Subsequent $NaBH_4$ reduction of **13** smoothly provided the targeted naphthyl alcohol **6** in quantitative yield. The next challenge was to selectively oxidize the right-hand ring of **6** to naphthoquinone. Electronically, the left-hand ring of **6** would be more likely to be oxidized due to an extra electron-donating methoxy group. Precedents on regioselective oxidation of this type of substrates mediated by cerium ammonium nitrate (CAN) have revealed the exclusive oxidation of the left ring.[18] Unsurprisingly, treatment of **6** with CAN in acetonitrile at 0 °C gave only the undesired product naphthoquinone **14** in 50% yield, which prompted us to revise this step of the synthesis.



Scheme 2. Synthesis of pentaalkoxynaphthalene **6**

To overcome electronic bias in the oxidation step, we shifted our attention to using a hydroquinone mono ether as an oxidation precursor in order to achieve the selective oxidation of the right-hand ring of **6**. It is well documented that oxidation of hydroquinone mono ethers to 1,4-quinones is a very facile process.[18b,19] In addition, the free hydroxyl group of the hydroquinone mono ether would be a good directing group to induce the incoming formyl group at the requisite *ortho*-like position.[20]Thus, to prove this, we subjected a mixture of naphthols **11** and **12** to the aforementioned formylation conditions (Scheme 3a). Gratifyingly, the desired naphthaldehyde **15** resulting from *C*-formylation was obtained in 42% yield along with *O*-formylation product, naphthyl formate **16** in 17% yield. The position of the formyl group of **15** was confirmed by an nOe correlation between methoxy protons (δ 3.80) and an aromatic proton (δ 7.02) on the right ring. Moreover, the structure of formate **16** was verified by subjection to hydrolysis using 10%KOH to give the naphthol regioisomer **12** in 70% yield (Scheme 3b). The structure of **12** was confirmed by nOe enhancement of the

hydroxy proton (δ 9.66) after irradiation of benzylic protons (δ 5.12) of the Bn group. With the requisite naphthaldehyde **15** in hand, we continued with $NaBH_4$ reduction to obtain the corresponding naphthyl alcohol **17**. Unfortunately, compound **17** is very unstable and can easily decompose in a few hours upon exposure to air. Due to the low stability of alcohol **17**, this compound was immediately taken to the next step without chromatographic purification. To our delight, treatment of naphthol **17** with CAN in MeCN at 0 °C smoothly furnished the desired naphthoquinone **18** in 94% over two steps.



Scheme 3. a) synthesis of naphthoquinone **18** b) basic hydrolysis of formate ester **16**

Although we were successful in the selective oxidation to secure the appropriate oxidation state of the naphthoquinone intermediate, the drawback of this route was the use of a mixture of naphthols **11** and **12** as formylation precursors and only **11** was consumed to the targeted product. The other precursor **12** underwent *O*-formylation to generate the naphthyl formate byproduct, which lowered the total yield of this step. To avoid this limitation, we decided to revisit the first pathway to convert pentaalkoxynaphthalene **6** to the desired naphthoquinone **18** by screening of other oxidizing agents for quinone oxidation (Table 1). Using silver(II) oxide in the presence of 6M nitric acid in dioxane at room temperature[11,21] led to no reaction and the starting compound was recovered (entry 1). Changing the oxidizing

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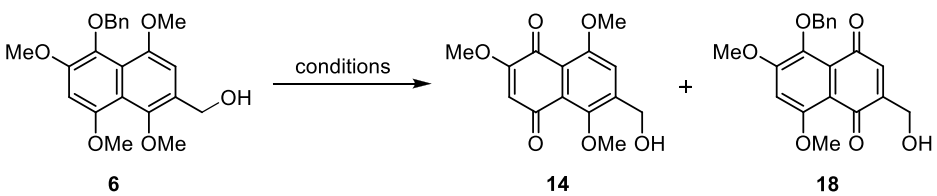
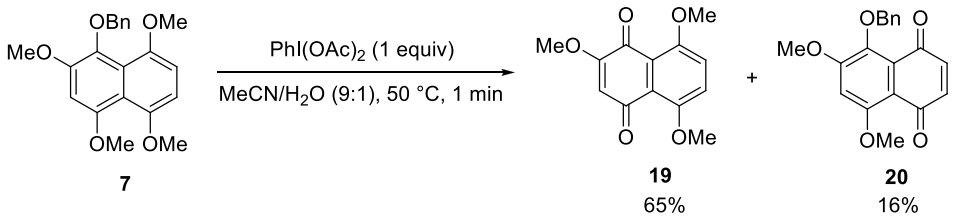
agents to hypervalent iodines gave more satisfactory results. Treating **6** with (bis(trifluoroacetoxy)iodo)benzene ($PhI(OCOCF_3)_2$) in a 9:1 mixture of MeCN and water at 0 °C for 30 minutes yielded the undesired naphthoquinone **14** as a major product in 51% yield and the desired naphthoquinone **18** as a minor product in only 25% yield (entry 2). Increasing the reaction temperature to room temperature slightly improved the yield of **18** to 32% while the undesired **14** was still a major product (entry 3). The more electron-withdrawing ligands of $PhI(OCOCF_3)_2$ might be attributed to oxidation of the more electron-rich left ring of naphthalene **6**.[22] Therefore, the less reactive hypervalent iodine reagent with less electron-withdrawing ability ligand, diacetoxyiodobenzene ($PhI(OAc)_2$) was investigated. Subjection of **6** to $PhI(OAc)_2$ under the same solvent mixture at 0 °C for 30 minutes yielded the desired naphthoquinone **18** as a major product in 58% yield along with **14** in 34% (entry 4). Elevating the reaction temperature to room temperature further enhanced the yield of **18** to 67% (entry 5). Thus, we further increased the reaction temperature to 50 °C under the same conditions for 1 min, which the reaction could be judged complete by the color change from light yellow to dark orange, and found that the requisite naphthoquinone **18** was formed as a sole product in 75% (entry 6). The excellent regioselectivity was attributed to the hydroxymethylene moiety as a directing group in this $PhI(OAc)_2$-mediated oxidation at elevated temperature. To emphasize the importance of the hydroxymethylene directing group in this selective oxidation, pentaalkoxynapthalene **7** absent of hydroxymethylene group was subjected to the same conditions of entry 6 (Scheme 4). It was discovered that oxidation occurred preferentially on the more electron-rich left ring of **7** to give **19** as a major product in 65%, whereas naphthoquinone regioisomer **22** resulting from oxidation of the less electron-rich right-hand ring was obtained as a minor product in only 16% yield. This control experiment strongly suggested that the hydroxymethylene could be exploited as a directing group in hypervalent iodine-mediated quinone oxidation.

Table 1. Screening of oxidation conditions to construct naphthoquinone **18** from naphthalene **6**



6 14 18

entry	reagents	solvent (concentration)	temp	time	yield (%)	
					14	**18**
1	AgO, 6M HNO_3	1,4-dioxane (0.04M)	rt	overnight	no reaction	
2	$PhI(OCOCF_3)_2$	MeCN/H_2O (9:1, 0.1M)	0 °C	30 min	51	25
3	$PhI(OCOCF_3)_2$	MeCN/H_2O (9:1, 0.1M)	rt	30 min	42	32
4	$PhI(OAc)_2$	MeCN/H_2O (9:1, 0.1M)	0 °C	30 min	34	58
5	$PhI(OAc)_2$	MeCN/H_2O (9:1, 0.1M)	rt	30 min	28	67
6	$PhI(OAc)_2$	MeCN/H_2O (9:1, 0.1M)	50 °C	1 min	–	75



Scheme 4. $PhI(OAc)_2$-mediated oxidation of pentaalkoxynaphthalene **7** at 50 °C.

Having established the optimized synthetic sequence to construct the key naphthoquinone core **18**, we proceeded to complete the syntheses of the natural products **3** and **4** (Scheme 5). Although acetonylation of **18** could be performed directly in the presence of free alcohol functionality, the yield and purity of the corresponding product were quite low. Hence, protection of the free alcohol was required. The protecting group of choice was the *tert*-butyldimethylsilyl (TBS) group since it could be removed under acidic conditions which would also induce the intramolecular hemiacetal formation. Therefore, alcohol **18** was protected as silyl ether **21** using TBSCl and imidazole. Introduction of the acetonyl group was accomplished using acetylmethylpyridinium chloride (**22**) in the presence of Et_3N in MeCN to give acetonylnaphthoquinone **23** in quantitative yield.[23] Treatment of **23** with 1M hydrochloric acid in MeCN at room temperature resulted in simultaneous removal of the TBS protecting group and intramolecular acetalization to smoothly furnish pyranonaphthoquinone **24** in 74% yield. Alternatively, **24** could be synthesized in a remarkable 85% via a one-pot fashion from **21** by simply quenching the acetonylation reaction with excess HCl and prolonged stirring without isolation of **23**. Subsequent hydrogenolytic deprotection of the

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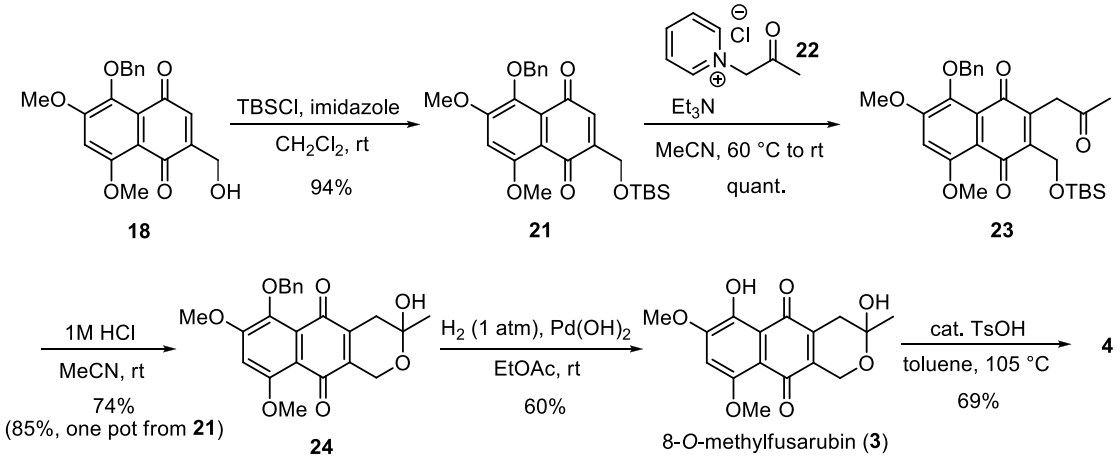
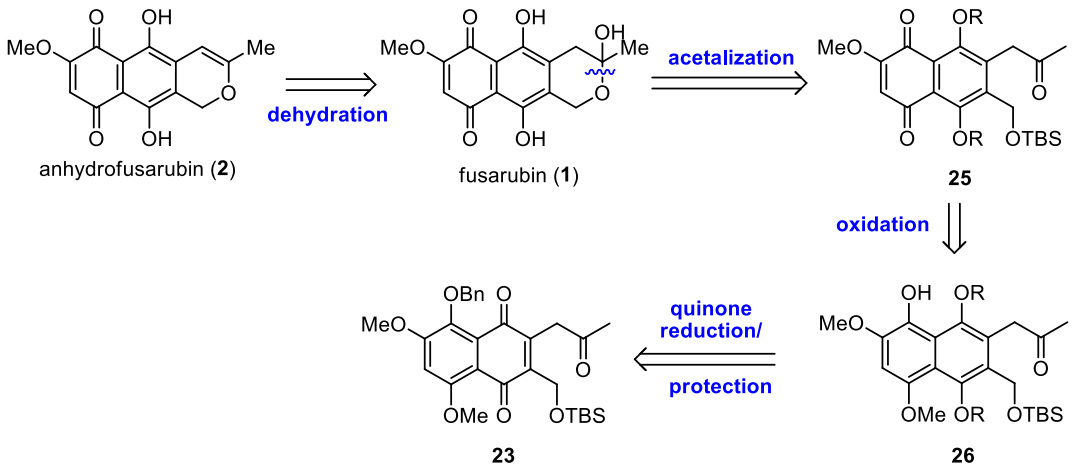
benzyl group using catalytic $Pd(OH)_2$ in ethyl acetate afforded the natural product **3** in 60% yield. It should be noted that hydrogenolysis using typical Pd/C catalyst in alcoholic solvent led to the formation of an unwanted pyran acetal product, which could not be hydrolyzed back to the corresponding pyran hemiacetal. Finally, following a procedure reported by Nguyen *et al.*,[24] compound **3** was subjected to dehydration using catalytic TsOH in toluene at 105 °C to give **4** in 69% yield. The ^{1}H and ^{13}C NMR spectroscopic data of synthetic **3** are in excellent agreement with those previously reported for the natural product.[25] The specific rotation of **3** was observed as $[\alpha]_D^{26}$ = +7.33 (*c* 0.03, acetone), suggesting that synthetic **3** was obtained as a scalemic mixture. Thus, no attempts were made to identify the absolute configuration of synthetic **3**. Although there is one literature precedent by Evidente *et al.* on the determination of the absolute configuration of natural **3** isolated from a grass weed-derived fungus *Rutstroemia capillus-albis*[26], the specific rotation and absolute configuration of **3** isolated from other natural sources particularly *Fusarium* species were not reported. For compound **4**, only the ^{1}H NMR data were reported in the literature.[9] The ^{1}H NMR data of synthetic **4** were identical to those of natural **4**. We further confirmed the identity of synthetic **4** by ^{13}C and 2D NMR as well as HRMS data.



Scheme 5. Completion of the synthesis of 8-*O*-methylfusarubin (**3**) and 8-*O*-methylanhydrofusarubin (**4**).

As previously mentioned, the other two targets fusarubin (**1**) and anhydrofusarubin (**2**) only differ from **3** and **4** by the position of the 1,4-quinone moiety of the naphthoquinone core. Therefore, we envisioned to synthesize **1** and **2** by exploiting the same strategy and utilizing the common intermediate from the syntheses of **3** and **4** via manipulation of the

oxidation state of the naphthoquinone nucleus. The retrosynthetic analysis of **1** and **2** is shown in Scheme 6. Anhydrofusarubin (**2**) would again be obtained from dehydration of fusarubin (**1**), which in turn would be assembled by intramolecular acetalization of **25**. Naphthoquinone **25** would be accessible from selective oxidation of naphthol **26**. Naphthol **26** would then be derived from selective quinone reduction/protection of naphthoquinone intermediate **23**. The appropriate protecting group should be easily and globally removed under acidic conditions which would be used in the acetalization step as well.



Scheme 6. Retrosynthetic analysis of fusarubin (**1**) and anhydrofusarubin (**2**).

We initially chose the TBS group as a protecting group of hydroquinone for the purpose of global deprotection. Syntheses of **1** and **2** commenced with selective reduction of the quinone moiety of **23** using $Na_2S_2O_4$ in THF at room temperature to furnish the corresponding hydroquinone,[27] which was carried on to the next step without chromatographic purification due to its facile autoxidation upon exposure to air (Scheme 7). Treatment of the hydroquinone with TBSOTf in the presence of 2,6-lutidine in CH_2Cl_2 unexpectedly provided pyran TBS ether **27** in 94% over two steps. The next task was to selectively oxidize the left-hand ring of **27**. This involved hydrogenolytic removal of the benzyl protecting group using Pd/C catalyst to deliver the corresponding unstable naphthol, which was immediately taken to subsequent oxidation with $PhI(OAc)_2$ under previously described conditions to afford pyranonaphthoquinone **28** in moderate yield (57% over 2 steps). Compound **28** was then treated with various Brønsted acids e.g. HCl, TsOH and AcOH or TBAF in order to mediate global deprotection and concomitant formation of the pyran hemiacetal. Disappointingly, only decomposition of the starting material or formation

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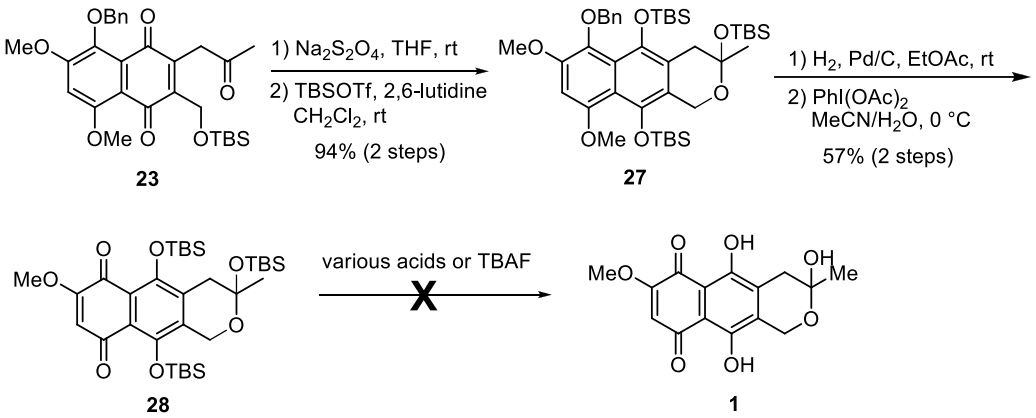
of complex mixtures was observed. Thus, alternative hydroquinone protecting group was sought.



Scheme 7. Attempted synthesis of fusarubin (**1**) via TBS protection.

To circumvent the problems encountered with the TBS protecting group, we decided to switch to the ethoxymethyl (EOM) group which should also be easily removed under acidic conditions.[28] The revised synthesis is described in Scheme 8. Selective reduction of naphthoquinone **23** using $Na_2S_2O_4$ in diethyl ether provided the corresponding hydroquinone in 87% yield. We discovered that using Et_2O as a solvent in this step shortened the reaction time and led to higher yield of the hydroquinone product.[29] In addition, despite its facile air oxidation, quick chromatographic purification of the resultant hydroquinone was required because employing the crude hydroquinone directly in the EOM protection step gave the low yield of **29** (22% over two steps). Therefore, after extensive experimentation, subjecting the purified hydroquinone to EOM protection using NaH and EOMCl in DMF at 0 °C delivered the requisite EOM ether **29** in 60% yield (53% over two steps).[30] The benzyl group was then removed under previously described conditions for hydrogenolysis in Scheme 7 to furnish naphthol **30** in 82% yield. Subsequent oxidation of **30** with $PhI(OAc)_2$ under the aforementioned conditions provided the requisite *para*-naphthoquinone **31** in 50% yield along with *ortho*-naphthoquinone byproduct in 15%. However, the *ortho*-quinone byproduct is relatively unstable and decomposes at ambient temperature in a few days. With the requisite naphthoquinone **31** readily available, final global deprotection of **31** using 1M HCl in MeCN and concomitant pyran hemiacetal formation were performed to deliver fusarubin (**1**) in 54% yield along with dehydrated derivative, anhydrofusarubin (**2**) in 22% yield. Compound **1** could be further converted to **2** in 73% yield by treating with catalytic TsOH in toluene at 105 °C. The ^{1}H and ^{13}C NMR spectroscopic data in $CDCl_3$ solvent of synthetic **1**

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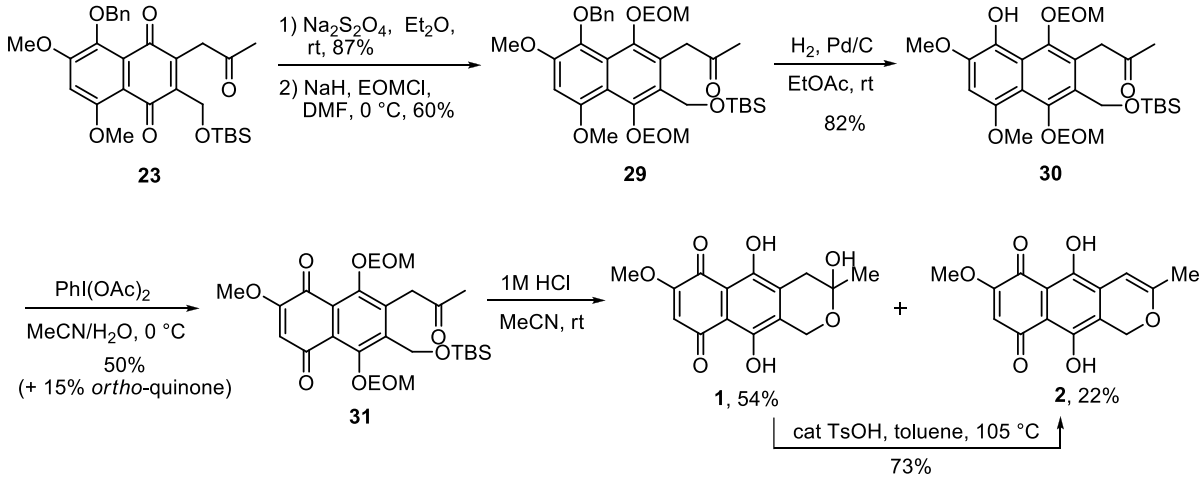
and **2** are identical to those reported in the literature.[11,31] However, due to very low solubility of **1** in $CDCl_3$, we also reported herein the ^1H and ^{13}C NMR spectroscopic data of **1** in DMSO-d_6 solvent. The specific rotation of **1** was observed to be $[\alpha]_D^{26}$ = +4.35 (*c* 0.036, acetone), suggesting that synthetic **1** was also obtained as a scalemic mixture.



Scheme 8. Completion the synthesis of fusarubin (**1**) and anhydrofusarubin (**2**).

The four synthetic naphthoquinones **1**-**4** were evaluated for their *in vitro* cytotoxic activity against the MCF-7 breast cancer cells as well as against noncancerous Vero cells by a colorimetric method using the resazurin microplate assay (REMA, Table 2). Among the compounds tested, 8-*O*-methylfusarubin (**3**) showed the most potent cytotoxic activity against MCF-7 cells with an IC_{50} value of 1.01 μM and no cytotoxic effect to Vero cells (IC_{50} = 47.49 μM). This observation was consistent with the reported data for the natural product (Figure 2). Synthetic **1** and **4** possessed comparably potent cytotoxic effect against MCF-7 cells with IC_{50} values of 3.19 μM and 2.96 μM, respectively. It is worth noting that cytotoxicity of synthetic fusarubin (**1**) is greater compared to the reported data of natural **1**. In addition, contrary to the observed cytotoxicity of natural anhydrofusarubin (**2**) (IC_{50} = 0.9 μM), we found that synthetic **2** displayed the least potent cytotoxic effect against MCF-7 cells with an IC_{50} value of 12.28 μM. Nevertheless, these four synthetic analogues exhibited more potent cytotoxic activity compared to standard drugs doxorubicin and tamoxifen.

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Table 2. Cytotoxic activity of synthetic compounds **1-4** against MCF-7 cancer and Vero cells using the resazurin microplate assay.

compound	cytotoxicity, IC_{50} (μM)	
	MCF-7 cells	Vero cells
1	3.19	19.36
2	12.28	77.54
3	1.01	47.49
4	2.96	20.54
doxorubicin	15.25	–
tamoxifen	17.23	–
ellipticine	–	3.97

To further verify the results of cytotoxic activity against MCF-7 cancer cells using REMA, synthetic compounds **1-4** were subjected to *in vitro* cytotoxic activity evaluation against MCF-7 cancer cells using MTT colorimetric assay (Table 3). We observed the similar trend in potency of compounds **1-4** against MCF-7 cells compared to REMA i.e. compound **3** displayed the most potent cytotoxicity with an IC_{50} value of 2.85 μM. Importantly, compounds **1-4** were tested for MCF-7 cancer cell viability in 3D cancer spheroid models via detection of live and dead cells using high-content imaging system (Figure 3). In this assay, compound **3** still showed the highest potency in a long-term effect (day 3). These data suggested that 8-*O*-methylfusarubin (**3**) is the most potent analogue of this series of pyranonaphthoquinones against MCF-7 breast cancer cells and could be a very promising lead compound for anti-breast cancer drug discovery.

Table 3. Cytotoxic activity of synthetic compounds **1-4** against MCF-7 cancer cells using the MTT and 3D spheroid assays.

compound	cytotoxicity, IC_{50} (μM)			
	MTT assay	3D cancer spheroid assay		
		day 1	day 2	day 3
1	11.31	18.70	12.50	13.14
2	20.88	57.09	39.67	31.46
3	2.85	4.44	1.83	2.29
4	8.63	25.49	16.42	5.79
doxorubicin	28.94	–	–	–

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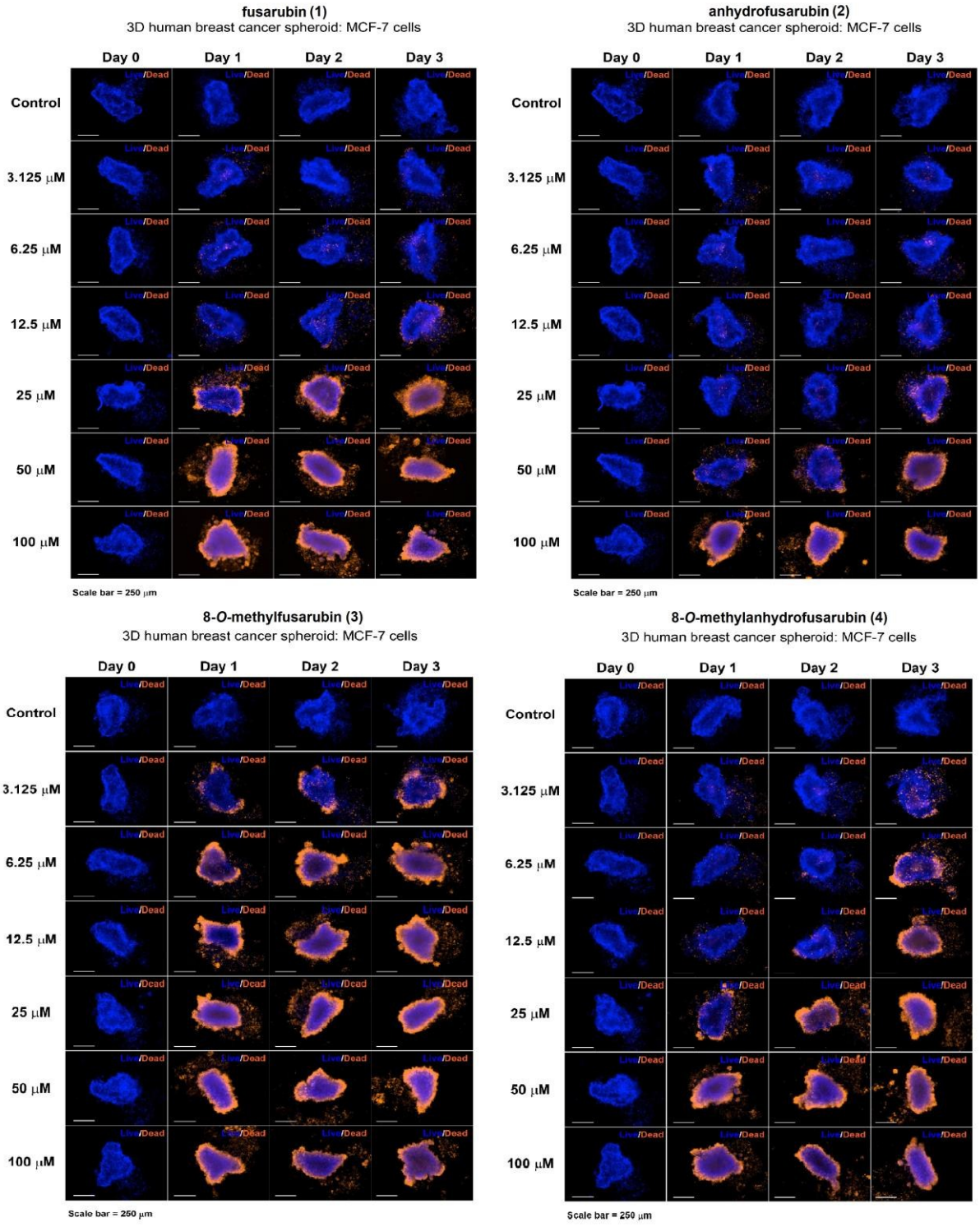
Figure 3. High-content imaging of synthetic **1-4** on 3D MCF-7 breast cancer spheroids.



Additionally, synthetic analogues **1-4** were further assessed for cytotoxic activities by MTT assay against five other human cancer cell lines including three cervical carcinoma (C33A, HeLa and SiHa), one colorectal carcinoma (HCT116) and one hepatoma (HepG2)

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cells as well as noncancerous Vero cells (Table 4). The four synthetic compounds showed antiproliferative effect against the five cancer cell lines tested with IC_{50} ranges of 4.73 – >22.5 μM albeit in lower extent compared to a standard drug doxorubicin. Notably, compounds **1**-**4** exhibited significant and comparable cytotoxic effect against the HCT116 colorectal carninoma cells (IC_{50} = 4.73–6.08 μM) suggesting that the naphthoquinone core might play an important role in this inhibitory effect. Nevertheless, in this MTT assay, synthetic compounds **1**-**4** displayed less pronounced selectivity on cancer cells over Vero cells compared to REMA. This might be attributed to sensitivity of different cancer and Vero cells to the compounds tested as well as the different assays used.

Table 4. Cytotoxic activity of synthetic **1**-**4** against five human cancer cell lines and Vero cells.

cell lines	cytotoxicity, IC_{50} (μM)					
	1	**2**	**3**	**4**	doxorubicin	cisplatin
C33A	10.47±3.21	>22.5	7.3±1.08	13.15±0.35	0.14±0.03	7.44±0.77
HeLa	6.98±1.72	14.12±1.86	13.4 ±5.03	14.6±1.14	0.16±0.01	12.73±6.41
SiHa	6.05±1.74	14.75±3.36	19.8±0.54	6.87±0.33	0.185±0.02	15.33±4.28
HCT116	6.08±1.47	4.87±0.33	5.42±1.43	4.73±2.10	0.23±0.03	>25
HepG2	5.33±0.09	4.87±0.53	12.65±2.87	15.54±2.24	0.66±0.06	>25
Vero	9±0.67	17.92±5.46	17.83±4.73	13.63±2.21	>1	20.13±2.44

Conclusion

We herein reported a simple and unified synthesis of four related pyranonaphthoquinone natural products **1**-**4**. We relied on the precedented Diels-Alder cycloaddition to assemble the naphthalene core. Selective installation of the formyl and acetonyl groups was used to install the three carbons of the pyran rings, which were in turn constructed by intramolecular acetalization. Our work also highlighted an unprecedented directing effect of the hydroxymethylene group in the selective hypervalent iodine-mediated quinone oxidation. Employing this strategy, 8-*O*-methylfusarubin (**3**) and 8-*O*-methylanhydrofusarubin (**4**) have been synthesized from Green's intermediate **7** in 5 and 6 steps in 41% and 28% overall yields, respectively. By manipulation of the oxidation state of the naphthoquinone nuclei from the syntheses of **3** and **4**, fusarubin (**1**) and anhydrofusarubin (**2**) could be synthesized from **7** in 8 steps in 9% and 4% overall yields, respectively. The four synthetic compounds were evaluated for cytotoxic activity against six human cancer cells

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using the resazurin microplate and MTT assays. 8-*O*-Methylfusarubin (**3**) is the most potent analogue and displays excellent cytotoxic activity against MCF-7 breast cancer cells with an IC_{50} value of 1.01 μM with no cytotoxic effect to noncancerous Vero cells determined by REMA. The four synthetic compounds also showed antiproliferative effect against other five cancer cell lines tested with IC_{50} ranges of 4.73 – >22.5 μM as determined by MTT assay.

Conflicts of interest

There are no conflicts of interest to declare.

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