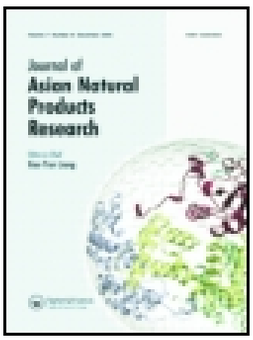

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Re-engineering and synthesis of cytotoxic 2,3:7,8-di(alkylenedioxy)-extended analogs of quaternary sanguinarine chloride

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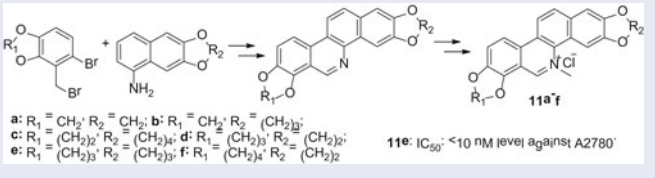
ABSTRACT

A method was developed to synthesize 2,3:7,8-di(alkylenedioxy)-extended analogs of quaternary sanguinarine chloride. 1-Bromo-2-bromomethyl-3,4-alkylenedioxy benzenes and 6,7-alkylenedioxynaphthalen-1-amines were synthesized first. Reactions to construct the target compounds with these two series of synthons involved alterations on a published method for synthesizing 2,3,7,8-tetraoxygenated derivatives of benzo[*c*]phenanthridinium, substituting benzyl bromides for benzoic aldehydes, prolonging the radical annulation time, and conducting *N*-methylation with formic acid and $NaBH_4$. All the target compounds showed the same or better in vitro growth inhibitory activities against cancer cell lines compared with the positive compound. The structure activity relationship relevant to cytotoxicity and lipophilicity of the target compounds was produced.



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1. Introduction

Bioactive natural compounds have been playing great roles as arsenals of clinically applicable drugs or their precursors for human race to treat cancers and other diseases in the history of modern medicine. At present, a shortcut has been set up by medicinal chemists to use bioactive natural compounds to develop innovative drugs through extensive medicinal and chemical studies, the investigated natural compounds being labelled as lead compounds. Of the well investigated lead compounds, alkaloids are an especially brilliant class of compounds getting clinical application, like the famous colchicine, vinblastine, and taxol, and so on. In the process of investigating active natural alkaloids,

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benzo[*c*]phenanthridines, a well-known class of alkaloids belonging to the isoquinoline alkaloids, attracted much attention from medicinal chemists too. Biological activities, such as antimicrobes [1,2], anti-inflammations [3], and anti-tumors [4], and so on, were corroborated in some lab tests on benzo[*c*]phenanthridines from natural resources. Quaternary benzo[*c*]phenanthridin-5-ium is a common existence form of benzo[*c*]phenanthridines in natural world. Some of them, such as quarternary nitidine [5], quaternary fagaronine [6], and quaternary chelerythrine [7], were also reported to display powerful growth inhibitive properties against human cancer cell lines. The well-known example is that the synthetic benzo[*c*]phenanthridine derivative NK-109 with the structure of 7-hydroxy-8-methoxy-5-methyl-2,3-methylenedioxybenzo[*c*]phenanthridin-5-ium hydrogensulfate dihydrate showed significant cytotoxicity against human cancer cell lines. The mechanism was known as inhibiting DNA topoisomerase II through the stabilization of the cleavable complex [8]. Clinical studies on NK-109 confirmed its significant anticancer activities [9]. More importantly, it worked well on several drug-resistant cancer cell lines [10] and was reported to show greater anticancer activity than any known benzo[*c*]phenanthridiniums [11]. Although, somehow, the development of this compound for innovative anticancer drug was interrupted and no further report was found recently, there have still been many studies in this area and several investigators have reported interesting chemical findings and biological effects [12].

Sanguinarine is a natural 2,3:7,8-di(methylenedioxy)-5-methylbenzo[*c*]phenanthridin-5-ium chloride, i.e. quaternary sanguinarine chloride (QSC). This natural compound exhibited both very strong growth inhibitive activities against several human cancer cell lines and anticancer activity pharmacologically [13,14]. In order to seek a new benzo[*c*]phenanthridin-5-ium salt with anticancer property, and because of the similar structure to NK-109 and the documented significant bioactivities of QSC, our group recently engaged in investigations on structural modification of QSC. In our study, the construction of aromatic compounds with alkylenedioxy of middle ring afforded two series of synthons. Reactions to construct the target compounds from these two series of synthons were explored in depth, with the referenced condition for synthesizing similar compounds being altered to a certain extent. Several quaternary 2,3:7,8-di(alkylenedioxy)-5-methylbenzo[*c*]phenanthridin-5-ium chlorides were successfully synthesized. Biological screening of the synthetic compounds shed light on the impact of modifying one, or simultaneously two, of the methylenedioxy moieties into alkylenedioxy of middle ring on the growth inhibitive activities against human cancer cell lines. In addition, it should be indicated that the 2,3:7,8-di(alkylenedioxy)-extended analogs of QSC, and those only modifying one alkylenedioxy moiety, have not yet been isolated from any natural source and therefore can only be obtained by synthesis. This study reports on the applicable synthetic method, the synthesis of involved compounds, and the biological screening of the end compounds for the growth inhibitory activities against five human cancer cell lines.

2. Results and discussion

The quaternary cation of 5,6-imine salt form of sanguinarine (SG^+) was reported to be one of two key determinants for this type of natural products to exhibit anti-proliferative activity [15]. The other is, according to common sense along with published

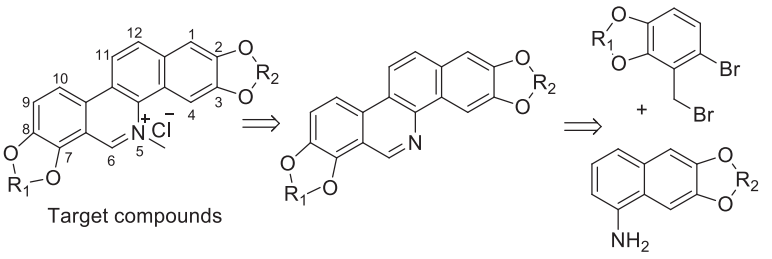
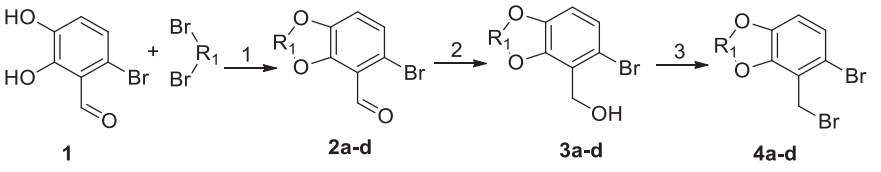


Figure 1. Strategy for synthesizing the 2,3:7,8-di(alkylenedioxy)-extended analogs of QSC.

articles, the solubility [16]. Studies have found that, under the physiological alkaline conditions, there is an equilibrium between the efficacious SG^+ form and the inefficacious pseudobase form (SGOH) of 2,3,7,8-tetraoxygenated 5-methyl-5,6-dihydrobenzo[*c*]phenanthridin-6-ol, with SGOH even being the major form [15]. This equilibrium heavily restricts the in vivo anticancer efficacy. Nowadays, techniques to manage this type of equilibrium were reported. The increasing of solubility was thought of as an effective way to slash the SGOH form in a given physiological conditions from the equilibrium, which increases the SG^+ form [16]. Thus, in this paper, the target derivatives of QSC were designed according to two principles, i.e. both to retain the efficacious quaternary iminium cation form and to increase the solubility, albeit liposolubility.

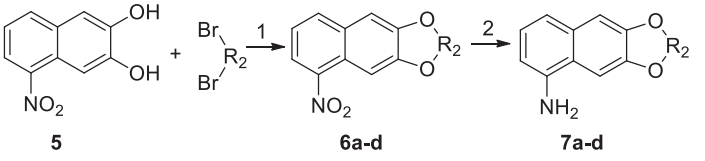
To our knowledge, no research paper of squarely altering the 2,3- and 7,8-methylenedixoy groups of QSC was published up to our investigation. Still, substituting different alkylenedioxy groups of structural diversities for one, or simultaneously two, of the 2,3- and 7,8-methylenedioxy moieties in QSC would definitely cause the differences of molecular planarity among the different products. In some cases, based on the pharmacochemical principles, even a little tortuosity of the molecular structure would lead to great changes of bioactivities and other drug abilities. Thus, a series of QSC analogs which substituted one, or simultaneously two, target alkylenedioxy groups for the native 2,3- and 7,8-methylenedixoy groups of the lead compound were designed, with successful syntheses being achieved in this study. For a systematic goal to screen the growth inhibitory activity against the target cancer cell lines, the considered target alkylenedioxy groups involved 1,2-dimethylenedioxy, 1,3-trimethylenedioxy, and 1,4-tetramethylenedioxy. Apparently, the construction for two series of aryl alkylenedioxy compounds of structural diversity, which was related to the two methylenedioxy groups of QSC, as key synthons of synthesizing the designed compounds was two of the crucial works for this study, although it was proved to be very comfortable (Figure 1 and Schemes 1 and 2). The final route to synthesize the target compounds on these two series of synthons was largely modeled after the reported synthetic route for NK-109 [11], but some synthetic conditions were altered slightly or somewhat significantly to improve the effectiveness and applicability of the method.

As indicated above, the syntheses of 1-bromo-2-bromomethyl-3,4-alkylenedioxy benzenes and 6,7-alkylenedioxy naphthalen-1-amines as two crucial series of synthons to synthesize the designed compounds involved only regular synthetic methods in organic chemistry. The commercially obtainable 6-bromo-2,3-dihydroxybenzaldehyde (**1**) was reacted with 1,ω-dibromo-*n*-alkanes *via* a process of dioxy-de-dibromo



a: $R_1 = CH_2$; **b:** $R_1 = (CH_2)_2$; **c:** $R_1 = (CH_2)_3$; **d:** $R_1 = (CH_2)_4$

Scheme 1. Syntheses of 1-bromo-2-bromomethyl-3,4-alkylenedioxy benzenes (**4a-d**). Reagents and conditions: (1) K_2CO_3, CuO, DMF, 110 °C; (2) $NaBH_4$, MeOH, 0 °C, HCl; (3) PBr_3, Py, DCM, rt.



a: $R_2 = CH_2$; **b:** $R_2 = (CH_2)_2$; **c:** $R = (CH_2)_3$; **d:** $R_2 = (CH_2)_4$

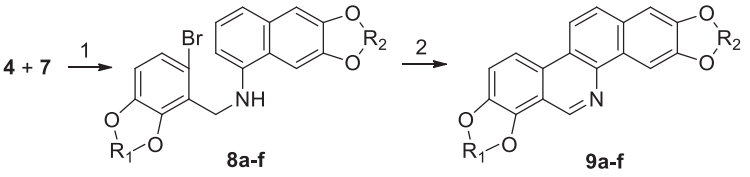
Scheme 2. Syntheses of 6,7-alkylenedioxy naphthalen-1-amines (**7a-d**). Reagents and conditions: (1) K_2CO_3, CuO, DMF, 110 °C; (2) H_2/Pd, MeOH, rt.

nucleophilic substitution under alkaline condition of K_2CO_3 in DMF to afford the 6-bromo-2,3-alkylenedioxybenzaldehydes (**2a-d**) in yields between 51.0% and 83.0%. The reduction of 6-bromo-2,3-alkylenedioxybenzaldehydes (**2**) using $NaBH_4$ in MeOH yielded 6-bromo-2,3-alkylenedioxybenzoic alcohol (**3a-d**) in yields between 78.7% and 86.3%. Substituting bromine for the hydroxyl group using PBr_3 and pyridine as agents in DCM solvent afforded the former series of synthons (**4a-d**) in yields between 83.8% and 92.0% (Scheme 1). The commercially obtainable 5-nitronaphthalene-2,3-diol (**5**) was reacted with 1,ω-dibromo-*n*-alkanes *via* a process similar to the aforementioned synthetic route for **2** to afford the 5-nitro-2,3-alkylenedioxynaphthalenes (**6a-d**) in yields between 39.4% to 66.7%. In the synthetic process, it was noticed that the yield of the desired 5-nitro-2,3-alkylenedioxynaphthalenes was influenced by the relative ratio of 1,ω-dibromo-*n*-alkanes. The open-and-shut example was the synthesis of 8-nitro-2,3,4,5-tetrahydronaphtho[2,3-*b*][1,4]dioxocine (**6d**). When the equiv of 1,4-dibromobutane was increased from 1.5 to 4.0 to promote the reaction balance, an obvious impurity was spotted at the end of the reaction *via* thin-layer chromatography (TLC) monitoring which was identified later as 6,7-bis(4-bromobutoxy)-1-nitronaphthalene. Thus, 1.5 equiv of 1,4-dibromobutane was adopted to synthesize **6d** based on extensive exploration, with the desired compound being obtained as pure compound conveniently. The reduction of **6** using H_2/Pd produced 6,7-alkylenedioxy naphthalen-1-amines (**7a-d**) as the latter series of synthons in yields between 85.2% to 92.0% (Scheme 2).

As phenanthridines make up a well-known class of molecules with established biological properties, there has been many protocols being reported to construct the benzo[*c*]phenanthridine core, such as Bischler-Napieralski route by Ishii [17], Heck route by Harayama [18], intramolecular homolytic aromatic substitution route by Bisai [19], Dual Palladium-catalyzed route by Maestri [12], Benzyne cyclization route by Kessar [20], electron-demand [4 + 2] aza-Diels-Alder cycloaddition of electron-rich

N-aryl imines with arynes route by Castillo [21], and Nakanishi's route for synthesizing NK-109 [11], and so on. These routes were applicable to synthesizing native sanguinarine and other explored natural benzo[*c*]phenanthridines, but none of them was proved tolerant enough of great structural changes in the homologs and relevant target compounds, the aforementioned reports being limited to several kinds of substituent pattern. It is well-known that, sometimes, a little structural change of natural organic molecules, even within homologs, would call for a new synthetic route or a changed route to a considerable degree. The main goal of the current study was to construct the aryl alkylenedioxy compounds of structural diversity and, based on the construction of these synthons, to re-engineer the 2,3:7,8-di(alkylenedioxy) moieties of active QSC in a total synthesis strategy to evaluate the SAR of certain bioactivity. For the building of a synthesized library of compounds to screen bioactivities, tolerance of the synthetic route on structurally diverse homologous compounds or end compounds is quite important. Thus, after an extensive and in-depth exploration on the published routes, including careful test *via* practices as such, the radical annulation route, which Nakanishi adopted to synthesize NK-109 and proved to be quite tolerant of providing kilograms of NK-109 for clinical research, was chosen as a key step to synthesize the end compounds of our study.

In the process of applying the published synthetic route to the goal of this study, some conditions were re-optimized considering the findings in practice. First of all, for the tertiary imine formation and reductive amination steps, the expected conversion from the 6-bromo-2,3-alkylenedioxybenzaldehydes (**2a-d**) and 6,7-alkylenedioxynaphthalen-1-amines (**7a-d**) to the relevant compounds was affirmed to be erratic when used the published method without any change. It was supposed that the electron-donating alkylenedioxy substituents on the benzoic aldehydes of **2a-d** resulted in a more drastic reaction situation as compared with the published synthesis using 6-bromo-2-hydroxy-3-methoxybenzaldehyde as substrate, and, as a general principle, the produced unstable imines would take on a complex situation. Thus, the synthetic route of our study was reengineered to substitute bromomethyl for the formyl moiety on the starting materials to explore the applicability of nucleophilic substitution. In the practice, this alteration was affirmed to yield the stable *N*-benzylnaphthalen-1-amines directly (**8a-f**). As such, the reductive step of using expensive and smell-unpleasant dimethylamine-borane as reagent in the published method was successfully avoided which significantly simplify the synthetic route and cut the cost. Secondly, the radical annulation step of the reported route to synthesize NK-109 was found to yield a mixture of two cyclization products in this specific study, i.e. the spontaneously oxidated *N*-demethylated analogs and the *N*-demethylated 5,6-dihydro-type analogs of the end compounds, although without using any oxidizing reagents. This was an interesting phenomenon and, after a research of literatures, it was noticed that this oxidation phenomenon had been observed when synthesizing NK-109 with unprotected phenol. It was hypothesized that this kind of spontaneous oxidation was caused by binding of trialkyltin moiety to hydroxyl oxygen with subsequent releasing of trialkyltin hydride in the process of synthesis, and the resonance energy is probably the driving force of this spontaneous oxidation [22]. Although, based on organic principle, when the hydroxyl and methoxy groups were replaced by



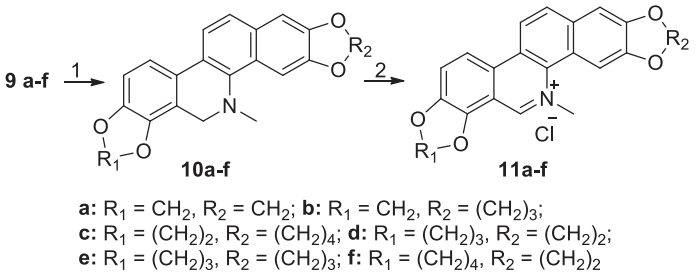
a: $R_1 = CH_2$, $R_2 = CH_2$; **b:** $R_1 = CH_2$, $R_2 = (CH_2)_3$;
c: $R_1 = (CH_2)_2$, $R_2 = (CH_2)_4$; **d:** $R_1 = (CH_2)_3$, $R_2 = (CH_2)_2$;
e: $R_1 = (CH_2)_3$, $R_2 = (CH_2)_3$; **f:** $R_1 = (CH_2)_4$, $R_2 = (CH_2)_2$

Scheme 3. Syntheses of 2,3:7,8-dialkylenedioxybenzo[*c*]phenanthridines (**9a-f**). Reagents and conditions: (1) K_2CO_3, DMF, 110 °C; (2) Bu_3SnH, toluene, N_2, AIBN, 110 °C.

alkylenedioxy groups, the spontaneous oxidation effect would still exist in the synthetic process due to the similar lone pair of electrons on their oxygen atom, the results of the current study only indicated that it was relatively weaker than the case in the literature. So the reaction time of radical annulation had to be elongated, to 16 h in our specific experiment, to fully conduct the spontaneous oxidation, and the *N*-demethylated analogs (**9a-f**) of the target compounds were successfully obtained as lone compounds in each synthesis (Scheme 3). Thirdly, the *N*-methylation step in the published route, i.e. reacting at 110 °C for 35 h under the presence of the custom-made methyl *o*-nitrobenzenesulfonate as reagents to yield the end compounds, made us daunted. Thus, the Ishii's versatile method to use formic acid and $NaBH_4$ as methylation reagents was adopted to complete the methylation reaction within 0.5 h [23], and the altered synthetic route was affirmed to be a significant success (Scheme 4), leading to the syntheses of dihydro-type intermediates (**10a-f**) of the target compounds. Finally, oxidative aromatization of these dihydro-type intermediates using DDQ as reagent, and then salinization reaction using HCl, successfully yielded the target compounds (**11a-f**).

All the synthesized end compounds were characterized using ^{1}H NMR method along with the consideration of reactions. Two sets of aromatic AB-type spin-spin coupling signals in the resonance region of δ_H 8.01–8.82 with ^3J values of around 9.0 Hz assignable to CH(9)=CH(10) and CH(11)=CH(12), respectively, from the two *vic*-tetrasubstituted benzene rings and three isolated aromatic singlets containing one aromatic proton each in the resonance region of δ_H 7.81–10.15 assignable to CH(1), CH(4), and CH(6), respectively, were apparent, confirming the core structure of 2,3:7,8-tetrasubstituted benzo[*c*]phenanthridine core. One singlet of methyl group at δ_H 4.95–5.03 assignable to nitromethyl group linked to *N*-5 established the 5-methylbenzo[*c*]phenanthridin-5-ium structure. All the remaining signals in the ^{1}H NMR spectra of each end compounds were assigned to the modified aryl alkylenedioxy moieties and were all compatible with the relevant structures (see supplementary data for detailed spectra and data). In addition, the recorded ^{13}C NMR spectra for typical representatives of the end compounds and the ESIMS$^+$ data of all the synthesized target compounds conformed to their respective structures (see Experimental section).

The effect of substituting only one, or simultaneously two, alkylenedioxy groups of structural diversities for the two 2,3- and 7,8-methylenedixoy groups of the lead compound on the growth inhibitory activities against human cancer cell lines was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. QSC and the self-synthesized NK-109 were screened in the same batches



Scheme 4. Syntheses of quaternary 2,3:7,8-dialkylenedioxy-5-methylbenzo[*c*]phenanthridin-5-ium chlorides (**11a-f**). Reagents and conditions: (1) HCOOH, $NaBH_4$, 40 °C, NH_4Cl; (2) DDQ, NaOH, HCl.

of experiments as reference substances and positive controls. The explored human cancer cell lines included HCT-116, HepG2, BGC-823, NCI-H1650, and A2780. On evaluation, all target cancer cells were treated successively using every tested compound for 96 h, respectively. The MTT reduction assay procedure was modeled after our earlier study [24]. Results of means of three replicates presented in Table 1 were expressed as the concentration for compounds to inhibit the cell growth by 50%, i.e. the IC_{50} values. All the synthesized 2,3:7,8-di(alkylenedioxy)-extended analogs of QSC (**11b-11f**) displayed significant activities against the five tested human cancer cell lines, with IC_{50} values ranging from <0.01 to 3.54 μM. And the activities of analog **11e**, which contains 2,3:7,8-di(1,3-trimethylenedioxy) groups in its structure, were obviously stronger than that of NK-109 as well as all the other target compounds, with its IC_{50} values for all the five explored cancer cell lines reaching or exceeding the level of 10^{-7} M. Although the IC_{50} values listed in Table 1 demonstrated that no significant SAR involved only in structures was observed amongst the tested target compounds based on the limited alkylenedioxy groups in this study, the aforementioned findings demonstrated another point that extending the size of the two 2,3- and 7,8-alkylenedixoy groups of the lead compound improved or significantly improved the inhibitive activities against the explored cancer cell lines. Thus, more quaternary 2,3:7,8-dialkylenedioxy-5-methylbenzo[*c*]phenanthridin-5-ium chlorides, and other counteranion, will be synthesized in our subsequent planned study to explore the exact SAR as in-depth medicinal chemistry study. Obviously, all the explored alkylenedioxy groups were known for the relatively similar lipophilicity. Therefore, the finding about SAR concerning improving activity by increasing lipophilicity was produced, i.e. the conclusion of the current study is in agreement with previous findings about the influence of lipophilicity. It is worth mentioned that the inhibitive activity of **11e** against the human A2780 cervical cancer cell line reached <10 nM level, which provided us great confidence in our subsequent synthesis and modification works.

To sum up, a versatile and convenient method for synthesizing the 2,3:7,8-di(alkylenedioxy)-extended analogs of cytotoxic QSC and, as expansion, the aryl alkylenedioxy compounds, were developed. 1-Bromo-2-bromomethyl-3,4-alkylenedioxy benzenes and 6,7-alkylenedioxynaphthalen-1-amines as two crucial series of synthons were synthesized using regular synthetic methods of organic chemistry. These two series of synthons were condensed under the presence of K_2CO_3 as base and DMF as solvent at 110 °C *via* a nucleophilic substitution reaction. Without purification step, the

Table 1. Cancer cells growth inhibitory activity of the target compounds.

			IC_{50} (μM)				
Cpds	R_1	R_2	C1[a]	C2[a]	C3[a]	C4[a]	C5[a]
P[b]	OMe+OH	CH_2	3.94	1.19	1.84	1.75	0.90
QSC	CH_2	CH_2	2.98	2.62	2.16	2.49	2.38
11b	CH_2	$(CH_2)_3$	2.66	3.01	3.39	2.69	2.00
11c	$(CH_2)_2$	$(CH_2)_4$	3.54	3.11	2.74	2.72	2.13
11d	$(CH_2)_3$	$(CH_2)_2$	1.27	1.14	1.75	1.57	0.67
11e	$(CH_2)_3$	$(CH_2)_3$	0.66	0.32	0.98	0.94	<0.01
11f	$(CH_2)_4$	$(CH_2)_2$	2.38	1.95	3.40	1.94	1.40

[a]C1: HCT-116; C2: HepG2; C3: BGC-823; C4: NCI-H1650; C5: A2780.
[b]P: NK109.

crude intermediates in the form of reaction mixture were cyclized through radical annulation reaction under the presence of AIBN and Bu_3SnH as radical initiator, with the tertiary imine-type 2,3:7,8-di(alkylenedioxy)benzo[*c*]phenanthridines, i.e. the *N*-demethylated compounds of the desired target products, being obtained in yields of between 23.7% and 38.5% from 6,7-alkylenedioxynaphthalen-1-amines. The end products of quaternary 2,3:7,8-di(alkylenedioxy)-5-methylbenzo[*c*]phenanthridin-5-ium chlorides were successfully synthesized through *N*-methylation step with formic acid in the presence of $NaBH_4$ as reductive reagents, aromatization step with alkaline DDQ as oxidative reagents, and, finally, salinization reaction step using HCl, in yields ranging from 35.6% to 65.1% from *N*-demethylated compounds. Aryl methylenedioxy group is a common structural moiety in many active natural compounds, such as quaternary coptisine and QSC. To our knowledge, the syntheses of various benzo[*b*][1,4]dioxacycloalkanes and aminonaphtho[2,3-*b*][1,4]dioxacycloalkanes of beyond six-membered ring for the dioxa-rings have not been reported up to this investigation. This investigated method of synthesizing aryl alkylenedioxy compounds of structural diversity, and the 2,3:7,8-di(alkylenedioxy)-extended analogs of QSC, is predicted to be applicable to the expeditious synthesis of relevant compounds arsenals in the field of natural medicinal chemistry. All the synthesized 2,3:7,8-di(alkylenedioxy)-extended analogs of QSC (**11b-11f**) displayed significant activities against the five tested human cancer cell lines with IC_{50} values ranging from <0.01 to 3.54 μM, the end compound **11e** showing the most significant activity against A2780 by the IC_{50} value at <10 nM level.

3. Experimental

3.1. General experimental procedures

Nuclear magnetic resonance (NMR) spectra were recorded on either a Varian Mercury-300 NMR spectrometer or a Varian Mercury-400 NMR spectrometer or a Bruker AV-III-500 NMR spectrometer and reported with tetramethylsilane (TMS) as an internal standard and chloroform-*d* ($CDCl_3$) (D, 99.8% + 0.05% v/v TMS) or dimethyl sulfoxide-d_6 (DMSO-d_6) (D, 99.9% + 0.05% v/v TMS) (Cambridge Isotope Laboratories, Inc., Andover, MA, USA) as solvents. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. $ESIMS^+$ were obtained using an Agilent 1100 series LC/MSD Trap SL mass spectrometer. All the reagents and solvents were reagent grade or were purified by standard procedures

before using. The reaction progress was monitored using TLC on glass plates pre-coated with silica gel GF_{254} (Qindao Haiyang Chemical, Qingdao, China). The spots were visualized under UV light. Column chromatography (CC) was carried out over silica gel (200–300 mesh size; Qingdao Haiyang Chemical, Qingdao, China). The concentration of solution after reactions involved the use of a rotary evaporator operated at a reduced pressure of *ca*. 9.0 mbar.

3.2. Synthetic procedures

3.2.1. Synthesis of 6-bromo-2,3-alkylenedioxybenzaldehydes (2a-d)

3.2.1.1. Synthesis of 5-bromobenzo[d][1,3]dioxole-4-carbaldehyde (2a). To a mixture containing 6-bromo-2,3-dihydroxybenzaldehyde (**1**) (1.85 g, 8.5 mmol), K_2CO_3 (2.35 g, 17.0 mmol), and CuO (0.1 g) in DMF (30 ml) was added dibromomethane (1.19 ml, 17.0 mmol). The reaction mixture was stirred at 110 °C, with the monitoring being carried out using TLC inspection until it was apparent that the reaction was known for completion. The reaction mixture was cooled to rt. and then filtered to remove K_2CO_3 and CuO. After the filtrate was poured into the solvent of H_2O (150 ml), a black precipitate appeared which was collected *via* filtrating. The filter cake was recrystallized using *i*-PrOH as a crystalline solvent, with yellow powder (**2a**, 1.0 g) being obtained in a yield of 51% from **1**. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.11 (s, 1H), 7.18 (d, $J = 8.2$ Hz, 1H), 7.09 (d, $J = 8.2$ Hz, 1H), 6.21 (s, 2H); ESIMS$^+$ *m/z* 228.9 $[M + H]^+$.

3.2.1.2. Synthesis of 6-bromo-2,3-dihydrobenzo[b][1,4]dioxine-5-carbaldehyde (2b). Compound **2b** was obtained as yellow amorphous powder from **1** (1.85 g, 8.5 mmol), K_2CO_3 (2.35 g, 17.0 mmol), CuO (0.1 g), and 1,2-dibromoethane (1.46 ml, 17 mmol) using a similar procedure to that of **2a** in a yield of 53.1% from **1**. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.22 (s, 1H), 7.17 (d, $J = 8.7$ Hz, 1H), 7.06 (d, $J = 8.7$ Hz, 1H), 4.39–4.28 (m, 4H); ESIMS$^+$ *m/z* 244.1 $[M + H]^+$.

3.2.1.3. Synthesis of 7-bromo-3,4-dihydro-2H-benzo[b][1,4]dioxepine-6-carbaldehyde (2c). Compound **2c** was obtained as yellow amorphous powder from **1** (1.85 g, 8.5 mmol), K_2CO_3 (2.35 g, 17.0 mmol), CuO (0.1 g), and 1,3-dibromopropane (1.73 ml, 17 mmol) using a similar procedure to that of **2a** in a yield of 83.0% from **1**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 10.31 (s, 1H), 7.17 (d, $J = 8.6$ Hz, 1H), 6.99 (d, $J = 8.6$ Hz, 1H), 4.33 (t, $J = 5.7$ Hz, 2H), 4.25 (t, $J = 5.7$ Hz, 2H), 2.31–2.19 (m, 2H); ESIMS$^+$ *m/z* 258.1 $[M + H]^+$.

3.2.1.4. Synthesis of 8-bromo-2,3,4,5-tetrahydrobenzo[b][1,4]dioxocine-7-carbaldehyde (2d). Compound **2d** was obtained as yellow amorphous powder from **1** (1.85 g, 8.5 mmol), K_2CO_3 (2.35 g, 17.0 mmol), CuO (0.1 g), and 1,4-dibromobutane (2.03 ml, 17 mmol) using a similar procedure to that of **2a** in a yield of 60% from **1**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 10.35 (s, 1H), 7.32 (d, $J = 8.8$ Hz, 1H), 6.93 (d, $J = 8.8$ Hz, 1H), 4.13 (t, $J = 7.5$ Hz, 2H), 4.05 (t, $J = 5.3$ Hz, 2H), 2.25–2.12 (m, 2H), 2.02–1.88 (m, 2H); ESIMS$^+$ *m/z* 272.1 $[M + H]^+$.

3.2.2. Syntheses of 6-bromo-2,3-alkylenedioxybenzoic alcohols (3a-d)

3.2.2.1. Synthesis of (5-bromobenzo[d][1,3]dioxol-4-yl)methanol (3a). To a solution of **2a** (5.0 g, 21.8 mmol) in MeOH (80 ml) at 0 °C was added $NaBH_4$ (0.83 g, 21.8 mmol) batchwise. The reaction mixture was stirred at 0 °C for 0.5 h, with the completion of the reductive reaction being indicated by TLC inspection. The reaction was quenched *via* adding 10% HCl aq. (300 ml) into the reaction mixture. The reaction mixture was extracted three times with EtOAc (100 ml, each time). The organic layer was combined and washed using, first pure H_2O (100 ml) and then brine (100 ml), and dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to yield a crude product, which was purified using CC, eluted using a 10% EtOAc in PE (v/v) isocratic elution to yield a white amorphous powder (**3a**, 4 g) in a yield of 78.7%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.04 (d, $J = 8.3$ Hz, 1H), 6.80 (d, $J = 8.3$ Hz, 1H), 6.06 (s, 2H), 5.07 (t, $J = 4.4$ Hz, 1H), 4.45 (d, $J = 4.4$ Hz, 2H).

3.2.2.2. Synthesis of (6-bromo-2,3-dihydrobenzo[b][1,4]dioxin-5-yl)methanol (3b). Compound **3b** was obtained as white amorphous powder from **2b** (1.5 g, 6.2 mmol) and $NaBH_4$ (0.23 g, 6.2 mmol) using a similar procedure to that of **3a** in a yield of 86.7%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.04 (d, $J = 8.7$ Hz, 1H), 6.73 (d, $J = 8.7$ Hz, 1H), 4.82 (s, 2H), 4.35–4.30 (m, 2H), 4.27–4.24 (m, 2H).

3.2.2.3. Synthesis of (7-bromo-3,4-dihydro-2H-benzo[b][1,4]dioxepin-6-yl)methanol (3c). Compound **3c** was obtained as white amorphous powder from **2c** (5.0 g, 19.4 mmol) and $NaBH_4$ (0.74 g, 19.4 mmol) using a similar procedure to that of **3a** in a yield of 85.1%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.17 (d, $J = 8.7$ Hz, 1H), 6.87 (d, $J = 8.7$ Hz, 1H), 4.83 (s, 2H), 4.31 (t, $J = 5.6$ Hz, 2H), 4.20 (t, $J = 5.5$ Hz, 2H), 2.27–2.18 (m, 2H).

3.2.2.4. Synthesis of (8-bromo-2,3,4,5-tetrahydrobenzo[b][1,4]dioxocin-7-yl)methanol (3d). Compound **3d** was obtained as white amorphous powder from **2d** (7.3 g, 26.9 mmol) and $NaBH_4$ (1.0 g, 26.9 mmol) using a similar procedure to that of **3a** in a yield of 86.3%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.15 (d, $J = 8.7$ Hz, 1H), 6.84 (d, $J = 8.7$ Hz, 1H), 4.80 (s, 2H), 4.46 (t, $J = 5.2$ Hz, 2H), 4.29 (t, $J = 4.8$ Hz, 2H), 1.97–1.87 (m, 4H).

3.2.3. Syntheses of 1-bromo-2-bromomethyl-3,4-alkylenedioxy benzenes (4a-d)

3.2.3.1. Synthesis of 5-bromo-4-bromomethylbenzo[d][1,3]dioxole (4a). To a mixture containing **3a** (1.50 g, 6.5 mmol) and pyridine (439 μl, 5.5 mmol) in DCM (20 ml) at 0 °C was added PBr_3 (514 μl, 5.4 mmol) dropwise. The reaction mixture was stirred at rt. for 1 h, the reaction being known for completion by TLC monitoring. After extra DCM (20 ml) was added, the reaction mixture was washed two times using brine (100 ml, each time) and then dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to yield a crude product which was purified *via* CC, eluted using a 10% EtOAc in PE (v/v) isocratic elution, to yield white amorphous powder (**4a**, 1.6 g) in a yield of 83.8% from **3a**. ^{1}H NMR ($CDCl_3$,

500 MHz) δ: 7.09 (d, $J = 8.3$ Hz, 1H), 6.70 (d, $J = 8.3$ Hz, 1H), 6.11 (s, 2H), 4.61 (s, 2H).

3.2.3.2. Synthesis of 6-bromo-5-bromomethyl-2,3-dihydrobenzo[b][1,4]dioxine (4b).

The synthon **4b** was obtained as white amorphous powder from **3b** (1.30 g, 5.3 mmol), pyridine (359 μl, 4.5 mmol) , and PBr_3 (420 μl, 4.4 mmol) using a similar procedure to that of **4a** in a yield of 92.0%. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.09 (d, $J = 8.8$ Hz, 1H), 6.84 (d, $J = 8.8$ Hz, 1H), 4.61 (s, 2H), 4.44–4.30 (m, 2H), 4.30–4.22 (m, 2H).

3.2.3.3. Synthesis of 7-bromo-6-bromomethyl-3,4-dihydro-2H-benzo[b][1,4]dioxepine (4c).

The synthon **4c** was obtained as white amorphous powder from **3c** (14.10 g, 54.4 mmol), pyridine (3.7 ml, 46.3 mmol), and PBr_3 (4.3 ml, 45.3 mmol) using a similar procedure to that of **4a** in a yield of 87.2%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.14 (d, $J = 8.7$ Hz, 1H), 6.85 (d, $J = 8.7$ Hz, 1H), 4.71 (s, 2H), 4.31 (t, $J = 5.6$ Hz, 3H), 4.20 (t, $J = 5.5$ Hz, 3H), 2.23 (tt, $J = 5.6$ Hz, 5.5 Hz, 2H).

3.2.3.4. Synthesis of 8-bromo-7-bromomethyl-2,3,4,5-tetrahydrobenzo[b][1,4]dioxocine (4d).

The synthon **4d** was obtained as white amorphous powder from **3d** (7.50 g, 27.5 mmol), pyridine (1.9 ml, 23.4 mmol), and PBr_3 (2.2 ml, 22.9 mmol) using a similar procedure to that of **4a** in a yield of 87.2%. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.22 (d, $J = 8.7$ Hz, 1H), 6.94 (d, $J = 8.7$ Hz, 1H), 4.66 (s, 2H), 4.38 (t, $J = 5.1$ Hz, 2H), 4.23 (t, $J = 5.1$ Hz, 2H), 2.04–1.65 (m, 4H).

3.2.4. Syntheses of 5-nitro-2,3-alkylenedioxynaphthalenes (6a-d)

3.2.4.1. Synthesis of 5-nitronaphtho[2,3-d][1,3]dioxole (6a).

To a mixture containing 5-nitronaphthalene-2,3-diol (**5**) (2.40 g, 11.7 mmol), K_2CO_3 (3.23 g, 23.4 mmol), and CuO (0.2 g) in DMF (30 ml) was added dibromomethane (1.6 ml, 23.4 mmol) dropwise. The reaction mixture was stirred at 110 °C for 3 h until the reaction was known *via* TLC monitoring for completion. The reaction mixture was cooled to rt. and then filtered to remove K_2CO_3 and CuO. After the filtrate was poured into the solvent of H_2O (150 ml), a black precipitate appeared which was collected *via* filtrating. The filter cake was recrystallized using *i*-PrOH as a crystalline solvent, with pale yellow amorphous powder (**6a**, 1.4 g) being obtained in a yield of 56.0%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.11 (d, $J = 7.8$ Hz, 1H), 7.97 (s, 1H), 7.90 (d, $J = 8.1$ Hz, 1H), 7.37 (dd, $J = 7.8$, 8.1 Hz, 1H), 7.19 (s, 1H), 6.13 (s, 2H); ESIMS$^+$ *m/z* 218.2 $[M + H]^+$.

3.2.4.2. Synthesis of 6-nitro-2,3-dihydronaphtho[2,3-b][1,4]dioxine (6b).

Compound **6b** was obtained as yellow amorphous powder from **5** (2.40 g, 11.7 mmol), K_2CO_3 (3.23 g, 23.4 mmol), CuO (0.2 g), and dibromoethane (2.0 ml, 23.4 mmol) using a similar procedure to that of **6a** in a yield of 66.7%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.25–8.15 (m, 2H), 7.96 (d, $J = 8.1$ Hz, 1H), 7.36 (s, 1H), 7.33 (d, $J = 7.9$ Hz, 1H), 4.52–4.37 (m, 4H); ESIMS$^+$ *m/z* 232.2 $[M + H]^+$.

3.2.4.3. Synthesis of 7-nitro-3,4-dihydro-2H-naphtho[2,3-b][1,4]dioxepine (6c).

Compound **6c** was obtained as yellow amorphous powder from **5** (2.40 g, 11.7 mmol), K_2CO_3 (3.23 g, 23.4 mmol), CuO (0.2 g), and dibromopropane (2.4 ml, 23.4 mmol) using a similar procedure to that of **6a** in a yield of 59.2%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.25 (d, $J = 8.0$ Hz, 1H), 8.22 (d, $J = 8.0$ Hz, 1H), 7.99 (s, 1H), 7.75 (s, 1H), 7.55 (dd, $J = 8.0$, 8.0 Hz, 1H), 4.32 (t, $J = 5.6$, Hz, 2H), 4.30 (t, $J = 5.6$, Hz, 2H), 2.22 (tt, $J = 5.6$, 5.6 Hz, 2H); ESIMS$^+$ m/z 246.2 $[M + H]^+$.

3.2.4.4. Synthesis of 8-nitro-2,3,4,5-tetrahydronaphtho[2,3-b][1,4]dioxocine (6d).

Compound **6d** was obtained as yellow amorphous powder from **5** (2.40 g, 11.7 mmol), K_2CO_3 (3.23 g, 23.4 mmol), CuO (0.2 g), and dibromobutane (2.1 ml, 17.5 mmol) using a similar procedure to that of **6a** in a yield of 39.4%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.21 (d, $J = 8.0$ Hz, 1H), 8.18 (d, $J = 8.0$ Hz, 1H), 7.98 (s, 1H), 7.74 (s, 1H), 7.51 (dd, $J = 8.0$, 8.0 Hz, 1H), 4.42 (t, $J = 4.8$, Hz, 2H), 4.37 (t, $J = 5.2$, Hz, 2H), 1.95–1.78 (m, 4H); ESIMS$^+$ m/z 260.3 $[M + H]^+$.

3.2.5. Syntheses of 6,7-alkylenedioxy naphthalen-1-amines (7a-d)

3.2.5.1. Synthesis of naphtho[2,3-d][1,3]dioxol-5-amine (7a).

To a solution of **6a** (1.0 g, 4.6 mmol) in MeOH (10 ml) at rt. was added 10% Pd (10% on carbon, 0.1 g). The reaction mixture was stirred under the existence of H_2 (1 atm) at rt. for 2 h until the reaction was known for completion *via* TLC inspection. The reaction mixture was filtered through diatomite to remove Pd and the filtrate was evaporated under vacuum to yield a crude product, which was purified using CC, eluted using a 10% EtOAc in PE (v/v) isocratic elution, to yield brown powder (**7a**, 0.73 g) in a yield of 85.0%. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.46 (s, 1H), 7.12 (s, 1H), 7.01 (dd, $J = 7.9$, 7.4 Hz, 1H), 6.92 (br d, $J = 7.9$ Hz, 1H), 6.54 (dd, $J = 7.4$, 1.1 Hz, 1H), 6.06 (s, 2H), 5.43 (s, 2H); ESIMS$^+$ m/z 188.2 $[M + H]^+$.

3.2.5.2. Synthesis of 2,3-dihydronaphtho[2,3-b][1,4]dioxin-6-amine (7b).

The synthon **7b** was obtained as yellow amorphous powder from **6b** (1.0 g, 4.3 mmol) and 10% Pd (0.1 g) using a similar procedure to that of **7a** in a yield of 92.0%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.47 (s, 1H), 7.10 (s, 1H), 6.96 (dd, $J = 8.0$, 7.6 Hz, 1H), 6.86 (d, $J = 8.0$ Hz, 1H), 6.43 (dd, $J = 7.6$, 1.2 Hz, 1H), 5.41 (s, 2H), 4.38–4.20 (m, 4H); ESIMS$^+$ m/z 202.2 $[M + H]^+$.

3.2.5.3. Synthesis of 3,4-dihydro-2H-naphtho[2,3-b][1,4]dioxepin-7-amine (7c).

The synthon **7c** was obtained as yellow amorphous powder from **6c** (1.0 g, 4.1 mmol) and 10% Pd (0.1 g) using a similar procedure to that of **7a** in a yield of 84.4%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.66 (s, 1H), 7.27 (s, 1H), 7.04 (dd, $J = 8.1$, 7.8 Hz, 1H), 6.91 (br d, $J = 8.1$ Hz, 1H), 6.51 (dd, $J = 7.8$, 1.0 Hz, 1H), 5.52 (s, 2H), 4.16–4.13 (m, 4H), 2.11 (tt, $J = 5.6$, 5.6 Hz, 2H); ESIMS$^+$ m/z 216.3 $[M + H]^+$.

3.2.5.4. Synthesis of 2,3,4,5-tetrahydronaphtho[2,3-b][1,4]dioxocin-8-amine (7d).

The synthon **7d** was obtained as yellow amorphous powder from **6d** (1.0 g, 3.9 mmol) and 10% Pd (0.1 g) using a similar procedure to that of **7a** in a yield of 85.2%. ^{1}H

NMR (DMSO-d_6, 500 MHz) δ: 7.66 (s, 1H), 7.27 (s, 1H), 7.03 (dd, $J = 8.1$, 7.6 Hz, 1H), 6.91 (d, $J = 8.1$ Hz, 1H), 6.50 (d, $J = 7.6$ Hz, 1H), 5.51 (s, 2H), 4.34–3.14 (m, 4H), 1.92–1.72 (m, 4H); ESIMS$^+$ m/z 230.3 $[M + H]^+$.

3.2.6. Syntheses of 2,3:7,8-dialkylenedioxybenzo[c]phenanthridines (9a-f)

3.2.6.1. Synthesis of 2,3:7,8-di(methylenedioxy)benzo[c]phenanthridine (9a). To a mixture containing **4a** (744 mg, 2.5 mmol) and **7a** (425 mg, 2.3 mmol) in DMF (10 ml) at rt. was added K_2CO_3 (470 mg, 3.4 mmol) batchwise. The reaction mixture was heated to 110 °C and stirred successively until the substitutive reaction was known for completion on TLC monitoring. The reaction mixture was cooled to rt. and then poured into a solvent of H_2O (25 ml) and filtered. The filter cake was resolved in DCM and dried over anhydrous Na_2SO_4 and filtered. Evaporating of the filtrate under vacuum yielded a white amorphous powder (436 mg). This white amorphous powder (1.1 mmol), along with Bu_3SnH (885 μl, 3.3 mmol), was added into a solvent of toluene (48 ml). The solution was heated to 90 °C under the N_2 atmosphere and, then, AIBN (361 mg, 2.2 mmol) was added in, and the reaction mixture was stirred at 110 °C for 16 h until the reaction was known for completion *via* TLC monitoring. The reaction mixture was cooled to rt. and then poured into a solvent of H_2O (50 ml). The solution was extracted two times with EtOAc (25 ml, each time). The organic solution was combined and washed using brine (50 ml), and then dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to yield a crude product which was purified *via* CC, eluted using a 10% EtOAc in PE (v/v) isocratic elution, to yield yellow amorphous powder of precursor [1,4]dioxolo[4′,5′:4,5]benzo[1,2-*c*][1,3]dioxolo[4,5-i]phenanthridine (**9a**, 255 mg) in a yield of 35.5%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.40 (s, 1H), 8.55 (d, $J = 9.0$ Hz, 1H), 8.50 (s, 1H), 8.40 (d, $J = 8.7$ Hz, 1H), 7.99 (d, $J = 8.9$ Hz, 1H), 7.69 (d, $J = 8.7$ Hz, 1H), 7.52 (s, 1H), 6.38 (s, 2H), 6.21 (s, 2H).

3.2.6.2. Synthesis of 2,3-(1,3-trimethylenedioxy)-7,8-methylenedioxybenzo[c]phenanthridine (9b). Precursor **9b** was obtained as yellow amorphous powder from **4a** (744 mg, 2.5 mmol) and **7c** (502 mg, 2.3 mmol) using a similar procedure to that of **9a** in a yield of 23.7%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 9.67 (s, 1H), 8.64 (s, 1H), 8.59 (d, $J = 9.0$ Hz, 1H), 8.47 (d, $J = 9.0$ Hz, 1H), 8.04 (d, $J = 8.9$ Hz, 1H), 7.68 (d, $J = 8.9$ Hz, 1H), 7.56 (s, 1H), 6.25 (s, 2H), 4.51 (t, $J = 5.3$ Hz, 2H), 4.36 (t, $J = 5.3$ Hz, 2H), 2.36–2.26 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 149.0, 148.9, 148.6, 147.2, 145.7, 130.3, 130.0, 128.7, 128.6, 128.4, 126.9, 126.8, 120.7, 119.3, 118.2, 105.2, 102.3, 101.3, 72.1, 71.3, 31.6.

3.2.6.3. Synthesis of 2,3-(1,4-tetramethylenedioxy)-7,8-(1,2-dimethylenedioxy)benzo[c]phenanthridine (9c). Precursor **9c** was obtained as yellow amorphous powder from **4b** (2.13 g, 6.9 mmol) and **7d** (1.45 g, 6.3 mmol) using a similar procedure to that of **9a** in a yield of 38.5%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 9.59 (s, 1H), 8.76 (s, 1H), 8.58 (d, $J = 9.0$ Hz, 1H), 8.38 (d, $J = 9.0$ Hz, 1H), 8.01 (d, $J = 8.9$ Hz, 1H), 7.69 (s, 1H), 7.61 (d, $J = 8.9$ Hz, 1H), 4.60–4.56 (m, 2H), 4.50–4.46 (m, 2H), 4.44–4.40 (m, 4H), 1.93–1.85 (m, 4H).

3.2.6.4. Synthesis of 2,3-(1,2-dimethylenedioxy)-7,8-(1,3-trimethylenedioxy)benzo[c]phenanthridine (9d). Precursor **9d** was obtained as yellow amorphous powder from **4c** (1.06 g, 3.3 mmol) and **7b** (604 mg, 3.0 mmol) using a similar procedure to that of **9a** in a yield of 34.6%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 9.71 (s, 1H), 8.70 (s, 1H), 8.58 (d, J = 9.0 Hz, 1H), 8.51 (d, J = 9.0 Hz, 1H), 8.04 (d, J = 8.9 Hz, 1H), 7.72 (d, J = 8.9 Hz, 1H), 7.61 (s, 1H), 4.53 (t, J = 5.2 Hz, 2H), 4.50–4.43 (m, 4H), 4.39 (t, J = 5.2 Hz, 2H), 2.40–2.31 (m, 2H).

3.2.6.5. Synthesis of 2,3:7,8-di(1,3-trimethylenedioxy)benzo[c]phenanthridine (9e). Precursor **9e** was obtained as yellow amorphous powder from **4c** (612 mg, 1.9 mmol) and **7c** (372 mg, 1.7 mmol) using a similar procedure to that of **9a** in a yield of 24.2%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.78 (s, 1H), 8.93 (s, 1H), 8.29 (d, J = 8.9 Hz, 1H), 8.14 (d, J = 9.0 Hz, 1H), 7.80 (d, J = 9.0 Hz, 1H), 7.51 (s, 1H), 7.48 (d, J = 8.9 Hz, 1H), 4.51 (t, J = 5.5 Hz, 2H), 4.40–4.32 (m, 6H), 2.42–2.33 (m, 2H), 2.33–2.25 (m, 2H).

3.2.6.6. Synthesis of 2,3-(1,2-dimethylenedioxy)-7,8-(1,4-tetramethylenedioxy)benzo[c]phenanthridine (9f). Precursor **9f** was obtained as yellow amorphous powder from **4d** (906 mg, 2.7 mmol) and **7b** (493 mg, 2.4 mmol) using a similar procedure to that of **9a** in a yield of 28.7%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 9.67 (s, 1H), 8.67 (s, 1H), 8.57 (d, J = 9.0 Hz, 1H), 8.52 (d, J = 9.0 Hz, 1H), 8.02 (d, J = 8.8 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.60 (s, 1H), 4.67–4.59 (m, 2H), 4.59–4.53 (m, 2H), 4.49–4.43 (m, 4H), 2.08–1.95 (m, 4H).

3.2.7. Syntheses of 2,3:7,8-dialkylenedioxy-5-methylbenzo[c]phenanthridin-5-ium chloride (11a-f)

3.2.7.1. Synthesis of QSC (11a). To a solution of **9a** (200 mg, 0.6 mmol) in formic acid (20 ml) at rt. was added $NaBH_4$ (3.6 g, 95 mmol) batchwise. The reaction mixture was stirred at 40 °C until the reaction was known for completion *via* TLC monitoring. After adding aq. 10% NH_4Cl (50 ml) into the reaction mixture, the mixture was stirred at 25 °C for 10 min, and then extracted two times using EtOAc (20 ml, each time). The organic phase was combined and washed with brine (50 ml), and then dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to yield a crude residue. This crude residue was added into a solvent of toluene (3 ml) containing aq. 5% NaOH (1.5 ml) and DDQ (286 mg, 1.2 mmol). After stirred at rt. for 1 h, the reaction mixture was concentrated to dryness, the residue was dissolved in $CHCl_3$ (10 ml), the solution was cooled to 0 °C. With aq. 10% HCl (1ml) being added in dropwise, a red precipitation was crushed out, which was collected *via* filtration and recrystallized using 1,4-dioxane as solvent to yield QSC (**11a**) as red powder in a yield of 80.4%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 10.13 (s, 1H), 8.78 (d, J = 9.0 Hz, 1H), 8.64 (d, J = 8.7 Hz, 1H), 8.30 (d, J = 9.0 Hz, 1H), 8.27 (s, 1H), 8.12 (d, J = 8.7 Hz, 1H), 7.77 (s, 1H), 6.60 (br s, 2H), 6.34 (br s, 2H), 4.91 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 150.0, 148.8 (×2), 147.6, 146.3, 132.2, 131.5, 131.4, 127.2, 125.7, 120.3, 120.0, 118.9, 117.4, 109.5, 105.8, 105.0, 104.3, 102.9, 52.2, ESIMS$^+$ *m/z* 332.1 [M-Cl]$^+$.

3.2.7.2. Synthesis of 2,3-(1,3-trimethylenedioxy)-7,8-methylenedioxy-5-methyl-benzo[c]phenanthridin-5-ium chloride (11b). The target compound **11b** was obtained as yellow amorphous powder from **9b** (120 mg, 0.35 mmol) using a similar procedure to that of **11a** in a yield of 32.3%. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.15 (s, 1H), 8.78 (d, J = 9.0 Hz, 1H), 8.66 (d, J = 8.9 Hz, 1H), 8.41 (s, 1H), 8.30 (d, J = 8.9 Hz, 1H), 8.13 (d, J = 8.8 Hz, 1H), 7.88 (s, 1H), 6.60 (s, 2H), 4.95 (s, 3H), 4.38 (t, J = 6.0 Hz, 2H), 4.36 (t, J = 6.0 Hz, 2H), 2.27 (tt, J = 6.0 Hz, 6.0 Hz, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 152.6, 151.6, 149.8, 148.1, 146.6, 132.1, 131.6, 131.4, 127.6, 126.7, 120.8, 120.5, 119.8 (×2), 118.8, 118.1, 110.2, 105.4, 71.2, 71.1, 52.5, 31.0; ESIMS$^+$ *m/z* 360.4 [M-Cl]$^+$.

3.2.7.3. Synthesis of 2,3-(1,4-tetramethylenedioxy)-7,8-(1,2-dimethylenedioxy)-5-methyl-benzo[c]phenanthridin-5-ium chloride (11c). The target compound **11c** was obtained as yellow amorphous powder from **9c** (350 mg, 0.94 mmol) using a similar procedure to that of **11a** in a yield of 32.3%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.06 (s, 1H), 8.80 (d, J = 9.0 Hz, 1H), 8.62 (d, J = 9.0 Hz, 1H), 8.50 (s, 1H), 8.30 (d, J = 9.0 Hz, 1H), 8.00 (d, J = 9.0 Hz, 1H), 7.89 (s, 1H), 5.02 (s, 3H), 4.70–4.65 (m, 2H), 4.60–4.51 (m, 4H), 4.48–4.41 (m, 2H), 1.98–1.84 (m, 4H); ESIMS$^+$ *m/z* 388.1 [M-Cl]$^+$.

3.2.7.4. Synthesis of 2,3-(1,2-dimethylenedioxy)-7,8-(1,3-trimethylenedioxy)-5-methyl-benzo[c]phenanthridin-5-ium chloride (11d). The target compound **11d** was obtained as yellow amorphous powder from **9d** (120 mg, 0.33 mmol) using a similar procedure to that of **11a** in a yield of 35.3%. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.05 (s, 1H), 8.79 (d, J = 9.1 Hz, 1H), 8.61 (d, J = 9.1 Hz, 1H), 8.45 (s, 1H), 8.31 (d, J = 9.0 Hz, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.90 (s, 1H), 5.00 (s, 3H), 4.79–4.62 (m, 2H), 4.62–4.52 (m, 2H), 4.48–4.27 (m, 4H), 2.27 (tt, J = 5.4, 5.4 Hz, 2H); ESIMS$^+$ *m/z* 374.1 [M-Cl]$^+$.

3.2.7.5. Synthesis of 2,3:7,8-di(1,3-trimethylenedioxy)-5-methyl-benzo[c]phenanthridin-5-ium chloride (11e). The target compound **11e** was obtained as yellow amorphous powder from **9e** (151 mg, 0.40 mmol) using a similar procedure to that of **11a** in a yield of 32.3%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 10.14 (s, 1H), 8.82 (d, J = 9.0 Hz, 1H), 8.71 (d, J = 9.0 Hz, 1H), 8.48 (s, 1H), 8.33 (d, J = 9.0 Hz, 1H), 8.04 (d, J = 9.0 Hz, 1H), 7.92 (s, 1H), 5.03 (s, 3H), 4.59 (t, J = 5.3 Hz, 2H), 4.45 (t, J = 5.3 Hz, 2H), 4.40 (t, J = 5.3 Hz, 2H), 4.38 (t, J = 5.3 Hz, 2H), 2.38 (tt, J = 5.3, 5.3 Hz, 2H), 2.29 (tt, J = 5.3, 5.3 Hz, 2H); ESIMS$^+$ *m/z* 388.1 [M-Cl]$^+$.

3.2.7.6. Synthesis of 2,3-(1,2-dimethylenedioxy)-7,8-(1,4-tetramethylenedioxy)-5-methyl-benzo[c]phenanthridin-5-ium chloride (11f). The target compound **11f** was obtained as yellow amorphous powder from **9f** (250 mg, 0.67 mmol) using a similar procedure to that of **11a** in a yield of 65.8%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.05 (s, 1H), 8.74 (d, J = 9.2 Hz, 1H), 8.69 (d, J = 9.2 Hz, 1H), 8.35 (s, 1H), 8.28 (d, J = 9.2 Hz, 1H), 8.01 (d, J = 9.2 Hz, 1H), 7.81 (s, 1H), 5.00 (s, 3H), 4.74–4.69 (m, 2H), 4.56–4.51 (m, 2H), 4.51–4.44 (m, 4H), 2.03–1.93 (m, 4H); ^{13}C NMR (DMSO-d_6,

100 MHz) δ: 149.3, 147.5, 145.5, 145.0, 144.3, 134.3, 131.9, 130.845, 130.837, 130.8, 125.3, 119.82, 119.80, 118.8, 118.5, 114.5, 113.8, 74.3, 72.6, 64.6, 64.4, 52.2, 26.3, 25.8; $ESIMS^+$ m/z 388.1 $[M\text{-}Cl]^+$.

3.3. Growth inhibitory activity assay against human cancer cell lines

The growth inhibitory activity of all the target compounds against the human HCT-116, HepG2, BGC-823, NCI-H1650, and A2780 cell lines were examined using a published method [24].

Disclosure statement

No potential conflict of interest was reported by the authors.

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