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Synthesis and anticancer properties of new (dihydro)pyranonaphthoquinones and their epoxy analogs

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Abstract

1,4-Dihydroxy-2-naphthoic acid was used as a substrate for a straightforward five-step synthesis of 3-substituted 1*H*-benzo[*g*]isochromene-5,10-diones, with a Michael addition of *N*-acylmethylpyridinium ylides across 2-hydroxymethyl-1,4-naphthoquinone and a subsequent acid-mediated dehydratation of intermediate hemiacetals as the key steps. The obtained benzo[*g*]isochromene-5,10-diones were subsequently deployed for further synthetic elaboration to produce new 3,4-dihydrobenzo[*g*]isochromene-5,10-diones and (3,4-dihydro-)4a,10a-epoxybenzo[*g*]isochromene-5,10-diones. All compounds were screened for their cytotoxic and antimicrobial effects, revealing an interesting cytotoxic activity of 1*H*-benzo[*g*]isochromene-5,10-diones against different cancer cell lines.

Naturally occurring (dihydro)pyranonaphthoquinones can be found in bacteria, fungi and higher plants, pointing to their biochemical relevance in nature.[1] Many of these pyranonaphthoquinone derivatives have indeed been discovered to possess diverse and pronounced biological activities, including antimicrobial, antiparasitic, antiviral and anticancer properties.[2] For example, the dihydropyranonaphthoquinones eleutherin **1** and psychorubrin **2** and the pyranonaphthoquinones pentalongin **3**, dehydroherbarin **4a** and analogs **4b**, **4c** have been shown to exhibit interesting antimicrobial, antiparasitic, phytotoxic and antineoplastic activities (Fig. 1).[3] Furthermore, pyranonaphthoquinone-derived epoxides such as nanaomycin antibiotics **5** (nanaomycin E **5a**, nanaomycin αE **5b** and nanaomycin βE **5c**), isolated from *Streptomyces rosa var. notoensis*,[4] and frenolicin **6**, isolated from *Streptomyces fladiae*,[5] are known to display biological activity against mycoplasmas, fungi and gram-positive bacteria (Fig. 1).[6]

These and many other examples of bioactive synthetic and natural pyranonaphthoquinones have clearly elicited an interest in the preparation of novel analogs of these classes of compounds, and the synthesis of 1*H*-benzo[*g*]isochromene-5,10-diones (also referred to as naphtho[2,3-*c*]pyran-5,10-diones) has thus been the subject of several studies in the chemical literature.

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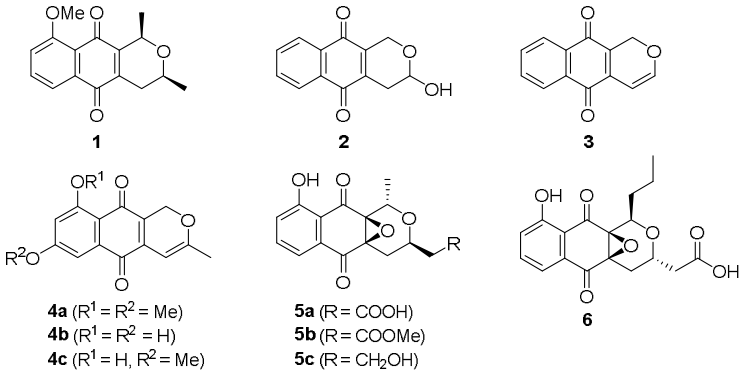


Figure 1. Examples of bioactive (dihydro)pyranonaphthoquinone(-derived) natural products

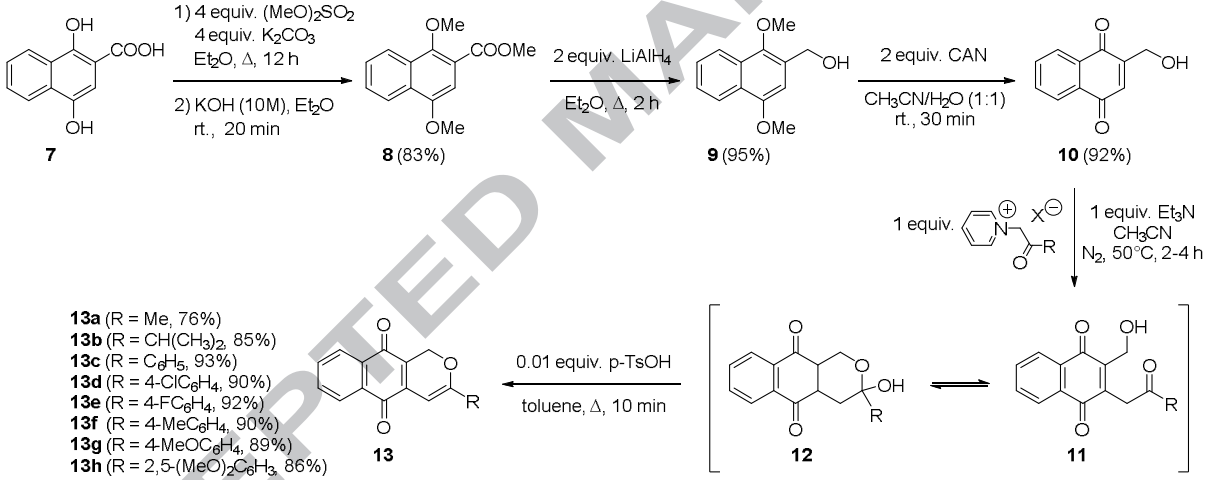
For example, Michael addition of *N*-acylmethylpyridinium ylides to 2-methyl-1,4-naphthoquinone, followed by α-bromination with respect to the keto group, dehydrobromination and hetero-Diels-Alder cyclization has been reported to furnish pyranonaphthoquinones, albeit in rather low yields due to a cumbersome bromination step.[7] An improvement of this synthetic strategy involved the use of 2-(phenoxymethyl)naphthoquinone, allowing for a more straightforward approach to pyranonaphthoquinone structures due to the less reactive (and thus more controllable) nature of the phenoxy moiety as a leaving group.[8] Another variant of this approach was based on the Michael addition of *N*-acylmethylpyridinium ylides to 2-(1-hydroxyethyl)-1,4-naphthoquinone, affording intermediate hemiacetals prone to undergo *p*-toluenesulfonic acid-mediated dehydratation to yield the desired naphtho[2,3-*c*]pyran-5,10-diones.[9] A synthetically different approach comprised transformation of 2-bromo-1,4-naphthoquinone into 3-allyl-2-(1-hydroxyethyl)-1,4-dimethyoxynaphthalene via a number of steps, which then underwent subsequent Lemieux-Johnson oxidation, oxidative demethylation and acid-catalyzed dehydration to produce 1-methylpentalongin.[10] Other routes toward the 1*H*-benzo[*g*]isochromene-5,10-dione scaffold involve for example the reaction of imines with 2-(1-hydroxyalkyl)-1,4-naphthoquinones[11] and an intramolecular Heck reaction of 3-bromo-2-formyl-1,4-dimethoxynaphthalene with 3-hydroxybut-1-ene.[12] Intensive efforts have also been devoted to the synthesis of diverse pyranonaphthoquinone natural products, such as 9-demethoxy(iso)eleutherin,[13] crisamicin A,[14] kalafungin,[15] and many others.

In continuation of our synthetic interest in naphthoquinone derivatives,[16] in the present paper we disclose a straightforward synthesis toward new 1*H*-benzo[*g*]isochromene-5,10-diones and their further transformation into 3,4-dihydro-1H-benzo[*g*]isochromene-5,10-diones and epoxy derivatives. Furthermore, the cytotoxicity with regard to different cancer cell lines and the antimicrobial potential of these compounds will be discussed as well.

The strategy deployed in this work elaborates on our previous study on the Michael addition of *N*-acylmethylpyridinium ylides to 2-(1-hydroxyethyl)-1,4-naphthoquinone and the subsequent acid-mediated dehydratation of intermediate hemiacetals to deliver 3-substituted 1-methylnaphtho[2,3-*c*]pyran-5,10-diones.[9] In the present work, a new variant of this approach is described to provide an entry into 3-functionalized naphtho[2,3-*c*]pyran-5,10-diones bearing no

substituent at the 1-position – as is the case in many natural products. To that end, a 5-step synthetic strategy was designed starting from 1,4-dihydroxy-2-naphthoic acid **7** (Scheme 1).

The first step involved a triple methylation of naphthoic acid **7** with dimethyl sulfate (4 equiv) in Et_2O in the presence of K_2CO_3 (4 equiv) to give methyl 1,4-dimethoxy-2-naphthoate **8** in 83% yield after KOH addition.[17] Reduction of the latter methyl ester **8** was carried out by treatment with lithium aluminium hydride (2 molar equiv) in Et_2O to give rise to 2-hydroxymethyl-1,4-dimethoxynaphthalene **9** in excellent yield (95%).[18] Oxidative demethylation of this 1,4-dimethoxynaphthalene system **9** upon treatment with cerium ammonium nitrate (CAN, 2 equiv) in a CH_3CN/H_2O (1:1) solvent mixture afforded 2-hydroxymethyl-1,4-naphthoquinone **10** in 92% yield.[11] Subsequently, the introduction of acylmethyl groups onto 2-hydroxymethyl-1,4-naphthoquinone **10** was effected by employing *N*-acylmethylpyridinium ylides (1 equiv) in CH_3CN in the presence of triethylamine,[9] providing a clean and high-yielding access to 1*H*-benzo[*g*]isochromene-5,10-diones **13** upon *p*-toluenesulfonic acid-catalyzed dehydratation in toluene.[19] Apparently, the initially formed addition products **11** spontaneously cyclized to give hemiacetals **12**, which underwent an acid-promoted elimination of water to afford 3-substituted pyranonaphthoquinones **13a-h** in excellent yields (Scheme 1).[20]

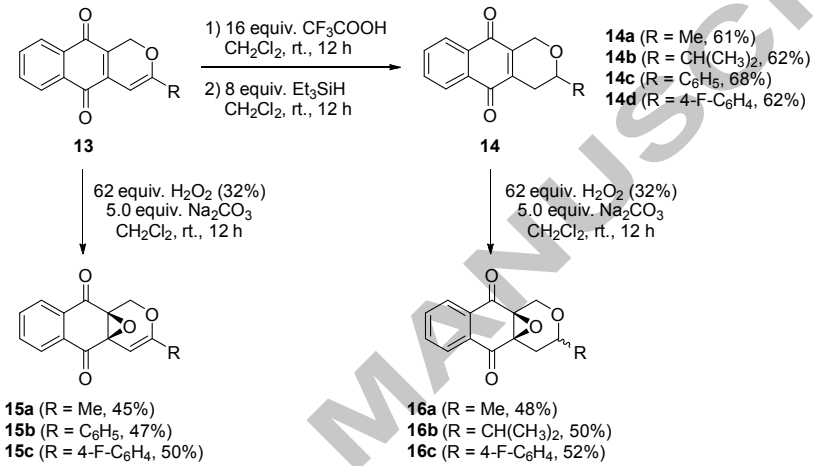


Scheme 1. Five-step synthesis of 1*H*-benzo[*g*]isochromene-5,10-diones **13** starting from 1,4-dihydroxy-2-naphthoic acid **7**

In order to expand our synthetic approach toward the saturated counterparts of tricycles **13**, the reduction of the olefinic moiety in the oxacyclic ring was tackled. Thus, treatment of benzo[*g*]isochromene-5,10-diones **13** with a combination of CF_3COOH (16 equiv) and Et_3SiH (8 equiv) in CH_2Cl_2 provided the desired 3,4-dihydro-1*H*-benzo[*g*]isochromene-5,10-diones **14a-d**[21] in good yields (Scheme 2).[22] This reduction is believed to proceed via initial protonation of the vinyl ether moiety in compounds **13** toward oxonium intermediates, which are then trapped by hydride addition across the C-O double bond.

Given the occurrence of biologically interesting pyranonaphthoquinone-derived epoxides in nature (e.g. nanaomycin antibiotics **5**), efforts were also made toward the epoxidation of (dihydro)benzo[*g*]isochromene-5,10-diones **13**/**14**. This goal was realized by reaction of both

substrates with H_2O_2 (32% aqueous solution, 62 equiv) in CH_2Cl_2 in the presence of Na_2CO_3 (5 equiv), as an alternative for the *tert*-butyl hydrogen peroxide-based procedure described in the literature,[23] affording novel epoxybenzo[*g*]isochromene-5,10-diones **15a-c** and 3,4-dihydro-epoxybenzo[*g*]isochromene-5,10-diones **16a-c** in acceptable yields (Scheme 2). These epoxidation reactions are presumed to involve initial Michael addition of the hydroperoxide anion across the quinone system followed by enolate-induced cyclization with concomitant hydroxide displacement. The epoxidation of dihydropyrane compounds **14** produced structures **16** as single isomers after purification. All attempts to assign the relative stereochemistry of these compounds (e.g. by means of X-ray analysis), however, failed.



Scheme 2. Synthesis of 3,4-dihydrobenzo[g]isochromene-5,10-diones **14**, epoxybenzo[*g*]isochromene-5,10-diones **15** and 3,4-dihydro-epoxybenzo[*g*]isochromene-5,10-diones **16**

With this set of compounds in hand, the biological activity spectrum of these molecules was investigated in the next part. In particular, (dihydro)pyranonaphthoquinones **13**, **14**, **15** and **16** were screened for their cytotoxicity against four cancer cell lines (KB, Hep-G2, MCF7 and Lu) and for their antimicrobial properties against two gram-positive bacteria (*B. subtilis* and *S. aureus*), two gram-negative bacteria (*E. coli* and *P. aeruginosa*) and one yeast strain (*C. albicans*).

Pyranonaphthoquinones are known to act as bioreductive DNA alkylating agents via quinone methide intermediates, resulting in cross-linking of DNA strands and thus interference with the cell replication process.[24] The cytotoxicity test data clearly show that also benzo[*g*]isochromene-5,10-diones **13** and, to a lesser extent, 3,4-dihydrobenzo[g]isochromene-5,10-diones **14** exhibit reasonable anticancer effects (Table 1). On the other hand, (3,4-dihydro-)epoxybenzo[*g*]isochromene-5,10-diones **15** and **16** do not display any cytotoxic effect at all, pointing to the conclusion that the presence of a quinone moiety is probably crucial to convey cytotoxicity to these molecules. Within the series of benzo[*g*]isochromene-5,10-diones **13**, isopropyl derivative **13b** and 4-chlorophenyl derivative **13d** seem to display a considerable cytotoxic effect against all four cell lines, and compound **13d** exhibits a particularly strong anticancer effect against KB cells with an IC_{50}-value (1.5 µM) similar to that of ellipticine (1.3 µM). Saturation of the C-C double bond in compounds **13** to form dihydro analogs **14** appears to result

in slightly less, albeit still reasonably, active compounds, which might be attributable to the higher sp^3 fraction (and thus lower intercalation potential) of the latter systems. The inactivity of the epoxy analogs **15b** and **16**, lacking the quinone system and having a distinct 3D structure, corroborates the assumption that biological activity in this case is probably associated with flat molecules bearing a quinone motif.

Table.1. Cytotoxicity evaluation of (dihydro)pyranonaphthoquinones **13**, **14**, **15** and **16**[a]

Entry	Compound	IC_{50} (µM) KB	IC_{50} (µM) Hep-G2	IC_{50} (µM) MCF7	IC_{50} (µM) Lu
1	**13a**	5.6	6.7	29.5	13.1
2	**13b**	4.1	10.7	6.7	14.9
3	**13c**	7.5	12.2	>34.7	>34.7
4	**13d**	1.5	3.6	12.9	11.5
5	**14a**	25.3	22.5	22.1	23.2
6	**14b**	17.5	19.8	26.2	>39.0
7	**14c**	16.1	18.7	27.2	> 34.4
8	**14d**	20.6	23.4	>32.4	22.1
9	**15a**	>41.3	>41.3	>41.3	>41.3
10	**15b**	>32.9	>32.9	>32.9	>32.9
11	**15c**	>31.0	>31.0	>31.0	>31.0
12	**16a**	>41.0	>41.0	>41.0	>41.0
13	**16b**	>32.6	>32.6	>32.6	>32.6
14	**16c**	>30.8	>30.8	>30.8	>30.8
15	Ellipticine	1.3	1.3	1.8	2.2

[a] Purity of all compounds >95% based on NMR

The antimicrobial assessment of the same compounds, however, revealed a poor biological activity profile, and only benzo[*g*]isochromene-5,10-diones **13a** and **13b** showed moderate anti-*Staphylococcus aureus* activity.

In conclusion, a series of pynanonaphthoquinones was conveniently and selectively synthesized in excellent yields via introduction of acylmethyl groups onto 2-hydroxymethyl-1,4-naphthoquinone by using *N*-acylmethylpyridinium ylides followed by intramolecular cyclization and acid-mediated dehydratation. These pynanonaphthoquinones were further synthetically exploited to generate the corresponding new dihydro analogs and their epoxy derivatives. Biological assessment of all these compounds demonstrated a significant cytotoxic effect of benzo[*g*]isochromene-5,10-diones against different cancer cell lines, pointing to the biological relevance of these scaffolds. However, the mechanism of action may be promiscuous given the fact that the quinone fragment often occur in the scaffold of pan-assay interference compounds (PAINS)[25] and hence a more rigid study is required to demonstrate target selectivity and true anti-cancer potential.

Acknowledgments

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[19] As an example, the synthesis of pyranonaphthoquinone **13d** is described here. To a solution of 2-hydroxymethyl-1,4-naphthoquinone **10** (100 mg, 0.53 mmol) and 1-[2-(4-chlorophenyl)-2-oxoethyl]pyridinium bromide (245 mg, 0.79 mmol) in acetonitrile (10 mL) under N_2 at 50°C was added dropwise a solution of Et_3N (80 mg, 0.79 mmol) in acetonitrile (5 mL). This mixture was stirred for 4 hours at room temperature. Afterwards the solvent was evaporated *in vacuo* and the residue was quenched with water and extracted with CH_2Cl_2. The extract was then washed with 1 M HCl, water and dried ($MgSO_4$). The solvent was evaporated *in vacuo* to give the crude intermediate reaction product **11d**/**12d**. This product was dissolved in toluene (15 mL), and *p*-TsOH (0.005 mmol) was added. The reaction mixture was heated under reflux for 10 min, after which the mixture was poured into water and extracted with EtOAc. The extract was washed with water and dried ($MgSO_4$). The solvent was evaporated *in vacuo* to give the crude product, which was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate 9/1) to afford 154 mg of the pure product **13d** (90%).

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Graphical Abstract