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Hetero-annulation reaction between 2-acylnaphthoquinones and 2-aminobenzothiazoles. A new synthetic route to antiproliferative benzo[*g*]benzothiazolo[2,3-*b*]quinazoline-7,12-quinones

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ABSTRACT

A convenient two-step method is developed for the preparation of benzo[*g*]benzothiazolo[2,3-*b*]quinazoline-7,12-quinones from 2-acylnaphthohydroquinones and 2-aminobenzothiazoles. The structure of the heterocyclic quinones is supported by X-ray crystallography. This protocol provides an operationally simple strategy to prepare the title compounds and shows good functional flexibility and easily available starting materials. Evidences are reported on the significant in vitro antiproliferative activities of some of the obtained heterocyclic quinones on prostate, bladder, and breast human-derived tumor cell lines.

Introduction

Quinones are ubiquitous in nature and comprise one of the largest classes of anticancer agents.[1–3] Anticancer quinones are currently the focus of intensive research because of their biological activity and complex modes of action, which differ depending on their particular structure. The biological processes involved with the antitumor activity of quinones are based mainly on DNA intercalation, bioreductive alkylation of biomolecules, and generation of reactive oxygen species (ROS) through redox cycling.[4–8] The DNA intercalative ability of quinonoid antitumor agents, such as daunorubicine, doxorubicine, mitoxantrone, and mitomycin C, is due to their large and planar polycyclic structures, which facilitates the binding between the base pairs through hydrogen bonds and *π-stacking* interactions.[9,10] Our research group has especially focused on biologically active compounds based on quinone cores fused to heterocyclic rings. In this context we have reported the synthesis and antiproliferative activity on cancer cells of a variety of isoquinoline-containing polycyclic quinones.[11] It is worth mentioning that a number of the reported *N*-heterocyclic quinones exhibit inhibition of topoisomerase I and activation of caspase-3 in HL-60 cells.[12] Our synthetic strategy to entry into isoquinoline-containing polycyclic quinones is based on the hetero-annulation reaction of 2-acyl-1,4-quinones with primary acyclic- and endocyclic enaminones, where the electrophilic α,β-unsaturated acyl fragment of the quinone and the ambident nucleophile H_2N—CH═CR— group of the enaminone are involved in the *N*-heterocyclic ring formation.[13–15] Taking into account the similar chemical reactivity of enaminones[16] and 2-aminobenzothiazoles[17] to act as ambident nucleophiles with α,β-unsaturated carbonyl compounds to give heterocycles, we decided to explore the synthesis of benzo[*g*]benzothiazolo[2,3-*b*]quinazoline-7,12-quinones from 2-acylnaphthoquinones and 2-aminobenzothiazoles. To the best of our knowledge, the sole precedent regarding the synthesis of antiproliferative benzo[*g*]benzothiazolo[2,3-*b*]quinazolinequinones is the recent report on amberlyst-15 catalyzed three-component condensation of 2-aminobenzothiazole, aromatic aldehydes, and 2-hydroxy-1,4-naphthoquinone.[18] Herein, we wish to report that benzo[*g*]benzothiazolo[2,3-*b*]quinazoline-7,12-

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quinone derivatives can be conveniently obtained from easily available 2-acyl-1,4-naphthoquinones and 2-aminobenzothiazoles. Preliminary antiproliferative evaluation of some members of the series on cancer cell lines is also described.

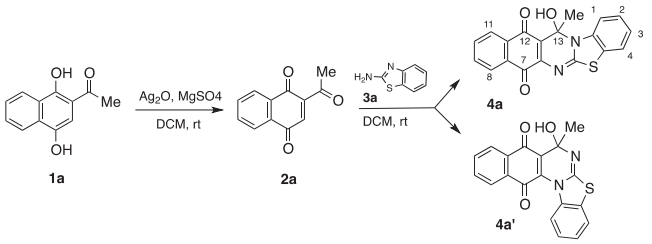
Results and discussion

To explore the possibility of heterocyclic annulation reactions, 2-acetyl-1,4-naphthoquinone **2a** and aminobenzothiazole **3a** were initially chosen. Quinone **2a**, prepared by oxidation of acylhydroquinone **1a**[19] with silver(I) oxide, was reacted with **3a** in dichloromethane at room temperature to give a red solid product. The IR spectrum of the product revealed the presence of O—H and C=O bands at 3421, 1680 and 1644 cm^{-1}. The ^1H NMR spectrum showed hydroxyl and naphthoquinone peri aromatic protons at δ 6.37 (br s), 8.10 (dd) and 8.17 (dd). The ^{13}C NMR spectrum displayed characteristic signals at δ 28.6, 88.5, 182.7, and 185.7 due to a methyl, an aliphatic quaternary carbon, and two carbonyl groups. The mass spectrum showed the molecular ion [M^+] peak at m/z = 348.05649. Based on these data, together with the high electrophilic character of the C-3 in 2-acyl-1,4-naphthoquinones and the behavior of 2-aminobenzothiazole to act as ambident nucleophile with α,β-unsaturated carbonyl compounds, two possible alternative structures derived from benzo[*g*]benzothiazolo[2,3-*b*]quinazoline (**4a**) and benzo[*g*]benzo[4,5]-thiazolo[3,2-*a*]quinazoline (**4a′**), were assigned for the reaction product isolated in 66% yield (Scheme 1).

Encouraged by these results we explored the substrate scope for the synthesis of dibenzothiazoloquinazolinequinones such as **4a** or **4a′**. A variety of 2-acyl-1,4-naphthoquinones **2b**–**2e**, generated by oxidation from their corresponding acylhydroquinones **1b**–**e**[19] with silver(I) oxide, were reacted with different 2-aminobenzothiazoles **3a**–**c**, under standard conditions.[20] The spectral data of the new products **4b**–**4k** were closely similar to those described above for compound **4a** or **4a′**. In order to unequivocally establish the structure of the benzothiazoloquinazolinequinones formed in the reaction of acylquinones **2** with the aminobenzothiazoles **3**, compound **4b** was submitted to X-ray diffraction analysis (Fig. 1).

Based on the X-ray crystallographic data for compound **4b** it may be concluded that the products formed by reaction of 2-acylnaphthoquinones **2** with aminobenzothiazoles **3**, exhibit the benzo[*g*]benzothiazolo[2,3-*b*]quinazoline-7,12-quinone framework (Fig. 1). Table 1 summarizes the results arising from the reaction of acylquinones **2** with aminobenzothiazoles **3** to produce compounds **4**. The possibility to prepare compounds **4** via a one-pot procedure from acylhydroquinone **1**, amine **3,** and silver(I) oxide in dichloromethane was studied using substrates **1a** and **3a**. The assays indicate that this procedure is unfeasible to prepare **4a** due to oxidative decomposition of amine **3a**. A plausible mechanism of this interesting hetero-annulation reaction is shown in Scheme 2 for the formation of compound **4a**.

The reaction seems to proceed via an initial attack of the NH_2 group of **3a** at the 3-position of the activated quinone **2a** to give



Scheme 1. The reaction of 2-acetyl-1,4-naphthoquinone **2a** with **3a**.

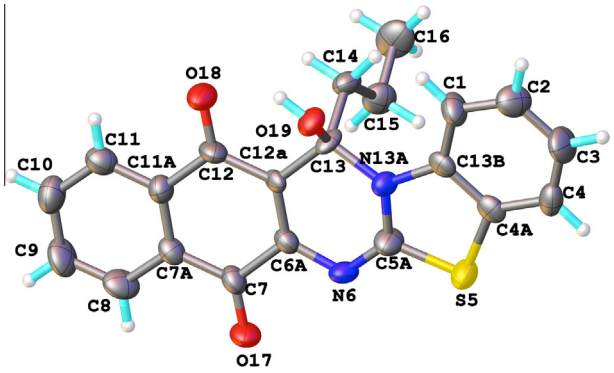
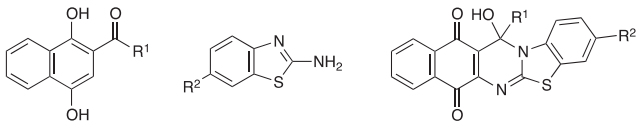


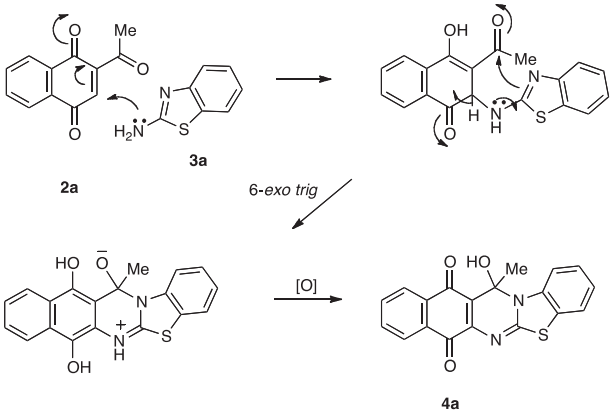
Figure 1. X-ray crystal structure of **4b**.

Table 1
Synthesis and yields of compounds **4a**–**k**[a]



Substrates		Quinone	R^1	R^2	Yield[b] (%)
1a	**3a**	**4a**	Methyl	H	67
1b	**3a**	**4b**	Propyl	H	78
1c	**3a**	**4c**	Pentyl	H	47
1d	**3a**	**4d**	Heptyl	H	41
1a	**3b**	**4e**	Methyl	Me	61
1b	**3b**	**4f**	Propyl	Me	68
1d	**3b**	**4g**	Heptyl	Me	84
1a	**3c**	**4h**	Methyl	OMe	43
1c	**3c**	**4i**	Pentyl	OMe	75
1d	**3c**	**4j**	Heptyl	OMe	60
1e	**3a**	**4k**	Furyl	H	55

[a] Reagents: (1) acylhydroquinone **1a**–**e** (1 equiv), Ag_2O (5 equiv); (2) aminobenzothiazole **3a**–**c** (1.1 equiv).
[b] Isolated yield after column chromatography and referred to **1a**–**e**.



Scheme 2. Plausible mechanism for the formation of quinone **4a**.

a Michael intermediate adduct, which by a further 6-*exo trig* ring closure, followed by aerobic oxidation, yields the heteropentacyclic quinone **4a**.

Quinones **4b**, **4e**, **4f,** and **4h** were evaluated for their in vitro antiproliferative activity on a panel of three human-derived tumor cell lines, using the conventional MTT (microculture tetrazolium reduction) assay.[21] The data in Table 2 show that compounds **4e**,

Table 2
In vitro antiproliferative activity of **4b**, **4e**, **4f,** and **4h** on T24 (bladder), DU-145 (prostate) and MCF7 (breast) cancer cell lines

Compound	IC_{50} ± SEM[a] (μM)		
	T-24	DU-145	MCF7
4b	4.86 ± 0.50	11.58 ± 2.05	4.25 ± 0.50
4e	0.22 ± 0.06	0.11 ± 0.03	2.98 ± 0.52
4f	1.36 ± 0.15	0.85 ± 0.10	1.77 ± 0.26
4h	1.32 ± 0.11	0.79 ± 0.11	2.72 ± 0.18
DOX[b]	0.65 ± 0.07	0.42 ± 0.03	0.33 ± 0.05
MIT[c]	42.2 ± 5.8	14.3 ± 2.6	16.8 ± 2.9
TF[d]	32.9 ± 1.5	28.3 ± 1.7	23.6 ± 1.4

[a] Data represent IC_{50} mean values ± SEM of at least three different experiments.
[b] DOX: doxorubicin.
[c] MIT: mitomycin C.
[d] TF: tamoxifen.

4f, and **4h** exhibit significant antiproliferative activity compared to doxorubicin, mitomycin C, and tamoxifen, thus supporting our approach to entry into new potentially antiproliferative agents, based on the benzo[*g*]benzothiazolo[2,3-*b*]quinazoline chromophore.

In conclusion, a novel and straightforward way for the construction of benzo[*g*]benzothiazolo[2,3-*b*]quinazoline **4a**–**k** from 2-acylnaphthohydroquinones **1a**–**e** and 2-aminobenzothiazoles **3a**–**c** has been found. The present method accommodates diverse 2-acylnaphthohydroquinones, where the acyl group ranges from C_2 to C_8, and easily accessible substituted 2-aminobenzothiazoles. These remarkable advantages make this approach very suitable for an easy preparation of these highly annulated antiproliferative heterocyclic quinones. In progress in our laboratory are the scope of the hetero-annulation reaction between 2-acylnaphthoquinones and 2-aminobenzothiazoles, directed to the synthesis of a broad variety of new members of this class of heterocyclic quinones, and their biological evaluation on representative cancer cell lines.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.07.034.

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20. *Synthesis of 13-hydroxy-13-(1-propyl)-7H-benzo[g]benzo[4,5]thiazolo[2,3-b]quinazoline-7,12-quinone* (**4b**): Typical Procedure. A suspension of acylhydroquinone (**1b**; 215 mg, 0.93 mmol), Ag_2O (1.1 g, 4.65 mmol), anhydrous $MgSO_4$ (200 mg), and dichloromethane (15 mL) was left at room temperature with stirring at rt for 1 h. The mixture was filtered and, over the solution, 2-aminobenzothiazole (**3a**; 165.22 mg, 1.02 mmol) was added, and the resulting solution was left with stirring at room temperature after completion of the reaction, as indicated by TLC (24 h). The solvent was removed under reduced pressure to give crude quinone **4b**. Further column chromatography of the crude product over silica gel (petroleum ether/CH_2Cl_2/EtOAc, 15:5:20) yielded pure **4b** (273 mg, 0.73 mmol, 78%) as a red solid; mp 203.5–204.0 °C; IR (KBr, cm^{-1}): 3444 (O–H), 1664, 1641 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76 (t, 3H, *J* = 7.2 Hz, CH_3), 0.99 (m, 1H, C**H**H), 1.35 (m, 1H, CH**H**), 2.41 (dt, 1H, *J* = 13.3, 4.2 Hz, C**H**H), 2.72 (dt, 1H, *J* = 13.3, 4.7 Hz, CH**H**), 6.37 (br s, 1H, OH), 7.29 (t, 1H, *J* = 8.0 Hz, 2- or 3-H), 7.41 (t, 1H, *J* = 8.0 Hz, 3- or 2-H), 7.56 (d, 1H, *J* = 7.8 Hz, 4-H), 7.71 (t, 1H, *J* = 8.0 Hz, 9- or 10-H), 7.75 (t, 1H, *J* = 8.0 Hz, 10- or 9-H), 8.07 (d, 1H, *J* = 7.4 Hz, 8- or 11-H), 8.15 (d, 1H, *J* = 7.3 Hz, 11- or 8-H), 8.22 (d, 1H, *J* = 8.4 Hz, 1-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.9, 18.2, 42.4, 91.7, 115.0, 118.1, 122.6, 125.0, 125.4, 126.3, 127.2, 127.5, 131.7, 133.3, 133.7, 134.5, 137.7, 146.7, 167.5, 182.4, 185.7; HRMS (APCI) calcd for $C_{21}H_{16}N_2O_3S$: 376.08816 $[M+H]^+$; found: 376.09454.
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