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Synthesis and anti-proliferative activity evaluation of novel 1,4-naphthoquinones possessing pyrido[2,3-*d*]pyrimidine scaffolds

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ABSTRACT A series of novel 1,4-naphthoquinones possessing pyrido[2,3-*d*]pyrimidine scaffolds were synthesized in very good yields using one-pot condensation of 2-hydroxy-1,4-naphthoquinone, aldehydes, and 2-substituted 4,6-diaminopyrimidine. The *in vitro* anti-proliferative activity of these novel compounds was evaluated in SGC7901 and HepG2 cell lines. Almost all the tested compounds showed manifested potent inhibitory activity against the two tested cancer cell lines.

Keywords: 1,4-naphthoquinone; pyrido[2,3-*d*]pyrimidine; molecular hybridization; anti-proliferative

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Introduction

Multicomponent reactions (MCRs) has become a powerful protocol to access pharmaceutically relevant heterocycles in recent years because of their combined prominent features such as high reaction rate and efficiency, atom economy and selectivity, time and energy savings, target product specificity and minimal environmental impact.[1]

Natural products and pharmaceuticals embedded with 1,4-naphthoquinone units display potential medicinal propertie such as anticancer,[2] antifungal,[3] antibacterial,[4] antiviral,[5] anti-inflammatory,[6] antimalaria,[7] antiplatelet,[5] antithrombotic,[8] and antiallergic, activities.[9] Furthermore, 1,4-naphthoquinones have also been shown to inhibit human DNA topoisomerase.[10] A number of 1,4-naphthoquinone derivatives having nitrogen atom present in them received a great deal of attention for their anticancer activity.[11] Therefore, the development of facile approaches to access these novel targets with structural diversity is highly desirable and valuable for medicinal chemistry and drug discovery.

Nitrogen containing heterocycles constitute an important class of compounds. Among them, pyrido[2,3-*d*]pyrimidine ring system occurs as a principal core skeleton among the drug scaffolds and also play crucial role as an important component in organic synthesis, and medicinal chemistry. Pyrido[2,3-*d*]pyrimidine derivatives gained prominence as they exhibit a wide range of biological and medicinal properties such as analgesic,[12] antiviral,[13] anti-inflammatory,[14] antimicrobial,[15] antifungal,[16] and anticancer activity.[17] Therefore, the synthesis of diverse structures belonging to this class of compounds is very important.

In the design of new drug prototypes, the concept of molecular hybridization is a useful tool and is based on the combination of pharmacophoric moieties of different bioactive substances to produce a new hybrid compound with improved affinity and efficacy. This strategy has resulted incompounds with modified selectivity profile, different and/or dual mode of action and reduced undesired side

effects.[18] Based on the versatile bioactivities of the above mentioned structures, it is promising that the integration of pyrido[2,3-*d*]pyrimidine scaffold with 1,4-naphthoquinone segment might result in the discovery of new drug candidates with unknown or enhanced bioactivities. We herein represent the synthesis of a series of novel 1,4-naphthoquinone and pyrido[2,3-*d*]pyrimidine hybrids and their anti-proliferative activity activity against human cancer cell lines *in vitro* (Scheme 1).

< **Scheme 1** >

Results and Discussion

Our strategy to synthesize 1,4-naphthoquinones-fused pyrido[2,3-*d*]pyrimidines involved three-component reaction of 2-hydroxy-1,4-naphthoquinone, aldehydes, and 2-substituted 4,6-diaminopyrimidine. The initial experiments were performed with 2-hydroxy-1,4-naphthoquinone, benzaldehyde, and 2,4,6-triaminopyrimidine in EtOH at 70 °C for 4 h. This set of conditions led to expected 5-phenyl-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione **4a**, albeit in a low 15 % yield (Table 1, entry 1). Encouraged by this preliminary result, we optimized the reaction conditions further. Solvent effects were first investigated (Table 1, compare entries 1–7). The use of AcOH facilitated the transformation and delivered **4a** in a higher yield of 41 %, whereas THF, $CHCl_3$ and H_2O, as reaction media completely suppressed the reaction. Another two solvents, that is, toluene and DMF, were proven ineffective and gave outcomes inferior to that obtained with AcOH. We then attempted to adjust the temperature to improve the reaction efficiency. Elevating the temperature to 118 °C (reflux) proved more efficient, and expected **4a** was afforded in 65 % yield (Table 1, entry 11).

<**Table 1**>

Under the above optimized conditions, the substrate scope of this three-component cyclocondensation reaction was examined by using readily available starting materials. As revealed in Table 2, 2, 4, 6-triaminopyrimidine was first subjected to the reaction with 2-hydroxy-1,4-naphthoquinone and different aldehydes in AcOH at reflux without the use of a strong acid or metal catalyst, and expected 1,4-naphthoquinones possessing pyrido[2,3-*d*]pyrimidines scaffolds **4a**–**p** were obtained in good yields. Various aryl aldehydes having substituents at different positions with electron-poor (*e.g.*, fluoro, chloro and nitro), electron-neutral (*e.g.*, H), electron-rich (*e.g.*, methyl and methoxy) groups, aromatic heterocyclic and aliphatic aldehydes were compatible. Next, we selected 2-methylthio-4,6-diaminpyrimidine as representative substrate to expand the synthetic utility of this methodology further. As we expected, these reactions proceeded smoothly to give access to corresponding tetracyclic products **4q**–**s**. Resulting 1,4-naphthoquinones possessing pyrido[2,3-*d*]pyrimidine scaffolds **4** were fully characterized by IR, NMR spectroscopy and HRMS. The IR spectrum of **4a** showed absorptions at 1675 and 1633 cm^{-1} indicating the presence of two C=O bonds. The high resolution mass spectrum of **4a** displayed the quasi-molecular ion ($[M+Na]^+$) peak at *m/z* = 392.1114, which was consistent with the 1:1:1 adduct of 2-hydroxy-1,4-naphthoquinone, benzaldehyde and 2,4,6-diaminopyrimidine with the loss of two water molecule. The ^1H NMR spectrum of **4a** showed four singlet was observed (δ = 5.31, 5.82, 6.23 and 8.68 ppm) for the CH group of C-5 position, NH_2 group of C-2, 4 position and NH group of dihydropyridine respectively. The ^{13}C NMR spectrum of **4a** showed characteristic signals at δ = 34.8 ppm (due to the R^1–CH group), 181.7 and 179.9 ppm (arising from the two nonequivalent carbonyl groups).

<**Table 2**>

A reasonable mechanism is proposed for the formation of 1,4-naphthoquinones possessing pyrido[2,3-*d*]pyrimidine scaffolds **4** (Scheme 2). It is conceivable that 2-hydroxy-1,4-naphthoquinone

initially reacts with aldehyde **2** to form olefin **5**, wich underwent a nucleophilic addition of 2-substituted 4,6-diaminopyrimidine to form the corresponding Michael-type type intermediate **6**. This step is then followed by an intramolecular dehydration to yield to product **4**.

< **Scheme 2** >

To evaluate their anti-proliferative potential, the newly synthesized hybrids **4a**–**s** were subjected to in vitro biological assessment against two human cancer cell lines, SGC7901 and HepG2. The results of the cytotoxicity evaluation, as compared to the anticancer reference compound Doxorubicin, were summarized in Table 3. As evidenced by these results, the majority of the derivatives exhibited at least moderate cytotoxic activity against the SGC7901 and HepG2 cell lines. Six of the new hybrids (**4b**, **4d**, **4n**, **4q**, **4r** and **4s**) even display a considerable activity profile with IC_{50} values below 10 μM against both cell lines. It is worthwhile to note that the majority compounds have lesser cytotoxicity on non-cancerous L02 cells. These results clearly suggest the relevance of this interesting new class of hybrids in the framework of cancer therapy research and medicinal chemistry. The results in Table 3 showed also some important structure-activity relationships (SARs) for this series of derivatives. First, the nature of substituents at the C-2 position have substantial influence on the anti-proliferative activity, introduction of methylmercapto group into the C-2 position was found to be quite favorable for increasing anti-proliferative activity. Among this series, compound **4s** showed the best anti-proliferative activity with IC_{50} values of 4.39 μM and 5.91 μM against SGC7901 and HepG2 cell lines, respectively. Second, the substituents at the C-2 moiety appeared to have an important effect upon cytotoxicity, introduction of aromatic heterocycte group into the C-5 position was found to be quite favorable for increasing anti-proliferative activity.

<Table **3** >

Conclusion

In the present study, a novel hybrids containing 1,4-naphthoquinone and pyrido[2,3-*d*]pyrimidine has been developed by molecular hybridization strategy. Ninteen hybrids were synthesized and evaluated for their anti-proliferative activities against SGC7901 and HepG2. The results showed that most of the new compounds showed good to potent cytotoxic activities. The most potent derivative **4s** displayed significant inhibition of SGC7901 with an IC_{50} of 4.39 μM and an IC_{50} value of 5.91 μM. Therefore, these novel 1,4-naphthoquinones fused with bioactive heterocyclic skeletons may find their pharmaceutical applications after further investigations.

Experimental

General

IR spectra were determined on FTS-40 infrared spectrometer. NMR spectra were determined on Bruker AV-400 spectrometer at room temperature using TMS as internal standard. Chemical shifts (d) are given in ppm and coupling constants (*J*) in Hz. High resolution mass spectra were recorded on a bruker micrOTOF-QIII mass spectrometer. Elemental analysis were performed by a Vario-III elemental analyzer. Melting points were determined on a XT-4 binocular microscope and were uncorrected. Commercially available reagents were used throughout without further purification unless otherwise stated

General procedure for the synthesis of compounds 4

To a mixture of 2-hydroxy-1,4-naphthoquinone (1 mmol), aldehyde (1 mmol) and 2-substituted 4,6-diaminopyrimidine (1 mmol), AcOH (10 mL) was added. The mixture was stirred at reflux for an appropriate time (Table 2). After completion of the reaction (TLC), the reaction mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. Then, the crude product was washed sequentially with 20 mL saturated $NaHCO_3$ and 20 mL brine, puried by silica gel column chromatography using $CHCl_3$: ethyl acetate (*v:v* = 10:1) as eluent to afford the pure product **4**.

5-Phenyl-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4a**): reddish-brown power, m.p.168-170 ^{o}C; IR (KBr): *v* 3473, 3383, 3302, 3155, 1675, 1633 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.68 (s, 1H), 8.01-7.99(m, 1H), 7.93-7.91 (m, 1H), 7.84-7.75 (m, 2H), 7.40 (d, 2H, *J* = 7.2 Hz), 7.21 (t, 2H, *J* = 7.6 Hz), 7.13 (d, 1H, *J* = 7.2 Hz), 6.23 (s, 2H), 5.82 (s, 2H), 5.31 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.7, 179.9, 162.6, 162.1, 154.1, 145.9, 139.9, 135.3, 133.5, 132.5, 130.7, 128.5, 128.4, 126.8, 126.3, 126.1, 118.4, 87.4, 34.8; HRMS-ESI (m/z): calc for $C_{21}H_{15}N_5NaO_2$ $[M+Na]^+$: 392.1123, found: 392.1114.

5-(Thien-2-yl)-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4b**): reddish-brown power, m.p. 254-255 ^{o}C; IR (KBr): *v* 3500, 3467, 3387, 3284, 1676, 1623 cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6) δ: 8.86 (s, 1H), 8.00 (d, 2H, *J*=7.6Hz) 7.87-7.77 (m, 2H), 7.23 (d, 1H, *J* = 4.0 Hz), 6.94 (d, 1H, *J* = 0.8 Hz), 6.85-6.83 (m, 1H), 6.40 (s, 2H), 5.85 (m, 2H), 5.70 (s, 1H);^{13}C NMR (100MHz, DMSO-d_6) δ: 181.6, 180.0, 162.7, 162.2, 154.0, 149.4, 139.6, 135.4, 133.6, 132.5, 130.7, 126.9, 126.3, 126.2, 125.0, 124.8, 117.4, 87.0, 30.0; HRMS-ESI (m/z): calc for $C_{19}H_{13}N_5NaO_2S$ $[M+Na]^+$: 398.0688, found: 398.0684.

5-(Fur-2-yl)-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione(**4c**): reddish-brown power, m.p. 297-299 ^{o}C; IR (KBr): *v* 3458, 3392, 3297, 3139, 1676, 1614 cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6) δ: 8.78 (s, 1H), 8.03-7.97 (m, 2H), 7.87-7.78 (m, 2H), 7.40 (s, 1H), 6.39(s, 2H), 6.27-6.23 (m, 2H), 5.84 (m, 2H), 5.51 (m, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ:181.5, 179.8, 162.6, 162.1, 156.0, 154.3, 142.1, 140.8, 135.3, 133.6, 132.5, 130.7, 126.4, 126.2, 114.6, 110.8, 106.2, 84.5, 29.0; HRMS-ESI (m/z): calc for $C_{19}H_{13}N_5NaO_3$ $[M+Na]^+$: 382.0916, found: 382.0909.

5-(3-nitrophenyl)-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4d**): reddish-brown power, m.p. >300 ^{o}C; IR (KBr): *v* 3449, 3317, 3189, 3086, 1673, 1672, 1612 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.90 (s, 1H), 8.36 (t, 1H, J=2.0Hz), 8.01-7.99 (m, 2H), 7.93-7.91 (m,

1H), 7.83-7.77 (m, 3H), 7.53 (t, 1H, J = 8.0 Hz), 6.37 (s, 2H), 5.90 (s, 2H), 5.52 (s, 1H);^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.6, 179.7, 162.6, 162.3, 154.2, 147.9, 147.8, 140.4, 135.3, 135.2, 133.6, 132.4, 130.8, 130.1, 126.3, 126.2, 123.1, 122.0, 117.1, 86.6, 34.8; HRMS-ESI (m/z): calc for $C_{21}H_{15}N_5$ NaO_3 $[M+Na]^+$: 437.0974, found: 437.0974.

5-(4-Chlorophenyl)-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4e**): reddish-brown power, m.p. 165-167 ^oC; IR (KBr): v 3469, 3385, 3309, 3167, 1677, 1617 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.73 (s, 1H), 8.01-7.99 (m, 1H), 7.93-7.91 (m, 1H), 7.84, 7.75 (m, 2H), 7.42 (d, 2H, J = 8.4 Hz), 7.27 (d, 2H, J = 8.4 Hz), 6.28 (s, 2H), 5.85 (s, 2H), 5.34 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.6, 179.8, 162.6, 162.1, 154.1, 144.8, 140.0, 135.3, 133, 132.5, 131.4, 130.7, 130.3, 128.4, 126.3, 126.1, 117.8, 87.0, 34.3; HRMS-ESI (m/z): calc for $C_{21}H_{14}N_5O_2$ $[M+Na]^+$: 426.0734, found: 426.0677。

5-(2,4-Dichlorophenyl)-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4f**): reddish-brown power, m.p. >300 ^oC; IR (KBr): v 3520, 3465, 3398, 3338, 1666, 1643 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.89 (s, 1H), 8.02-7.99 (m, 1H), 788-7.75 (m, 3H), 7.63 (d, 1H, J = 8.4 Hz), 7.48 (d, 1H, J = 2.4 Hz), 7.35-7.32 (m, 1H), 5.92 (s, 2H), 5.82 (s, 2H), 5.51 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ:181.4, 179.7, 162.6, 162.1, 154.0, 141.6, 140.7, 135.4,134.0, 133.6, 132.9, 132.4, 130.6, 130.6, 129.1, 128.0, 126.3, 126.1, 116.2, 86.3, 34.5; HRMS-ESI (m/z): calc for $C_{21}H_{13}C_{l2}N_5NaO_2$ $[M+Na]^+$: 460.0344, found: 460.0330.

5-(4-Florophenyl)-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*] quinoline-6,11-dione (**4g**): reddish-brown power, m.p. 157-159 ^oC; IR (KBr): v 3484, 3377, 3323, 3196, 1674, 1633 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.72 (d, 1H, J = 0.8 Hz), 8.00-7.91 (m, 2H), 7.81-7.76 (m, 2H), 7.45-7.41 (m, 2H), 7.03 (t, 2H, J = 8.8 Hz), 6.29 (s, 2H), 5.87 (s, 2H), 5.34 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.7, 179.8, 162.5, 162.0, 154.0, 142.0, 139.9, 135.3, 133.5, 132.5, 130.7, 130.3,

130.2, 126.3, 126.1, 118.1, 115.3, 115.0, 87.3, 34.1; HRMS-ESI (m/z): calc for $C_{21}H_{14}FN_5NaO_2$ $[M+Na]^+$: 410.1029, found:410.0994.

5-(2-Florophenyl)-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4h**): reddish-brown power, m.p. > 300 °C; IR (KBr): *v* 3495, 3434, 3392, 3301, 1677, 1638 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 8.81 (s, 1H), 8.02-8.00 (m, 1H), 7.89-7.75 (m, 3H), 7.83-7.75 (m, 2H),7.65-7.60 (m, 1H), 7.22-7.16 (m, 1H), 7.10-7.03 (s, 2H), 5.96 (s, 2H), 5.86 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.4, 179.8, 162.5, 162.0, 159.3, 153.9, 140.6, 135.3, 133.5, 132.4, 131.9, 130.6, 129.1, 129.0, 126.3, 126.1, 124.6, 116.2, 86.3, 34.2; HRMS-ESI (m/z): calc for $C_{21}H_{14}FN_5NaO_2$ $[M+Na]^+$: 410.1029, found:410.1018.

5-(4-Methoxyphenyl)-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*] quinoline-6,11-dione (**4i**): reddish-brown power, m.p. 196-198 °C; IR (KBr): *v* 3511, 3397, 3355, 3288, 1626, 1613 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: (8.66s, 1H), 8.00-7.89 (m, 1H) 7.93-7.91 (m, 1H), 7.83-7.74(m, 2H), 7.31 (d, 2H, J = 8.8 Hz), 6.77 (d, 2H, J = 8.8 Hz), 6.24 (s, 2H), 5.86 (s, 2H), 5.25 (s, 1H), 3.65 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ:181.7, 179.8, 162.4, 161.8, 158.3, 153.8, 139.5, 138.0, 135.2, 133.5, 132.5, 130.6, 139.4, 126.2, 126.1, 118.7, 113.9, 87.6, 33.9; HRMS-ESI (m/z): calc for $C_{22}H_{17}N_5NaO_3$ [M+Na]+: 422.1229, found:422.1228.

5-(2,5-Dimethoxyphenyl)- 5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*] quinoline-6,11-dione (4j): reddish-brown power, m.p. 265-266 °C; IR (KBr): *v* 3465, 3385, 3310, 3175, 1669, 1646 cm^{-1}; ^1HNMR (400 MHz, DMSO-d_6) δ: 8.64 (s, 1H), 8.03-8.01 (m, 1H), 7.88-7.76 (m, 3H), 6.94 (d, 1H , J = 8.8 Hz) 6.81 (d, 1H, J = 3.2 Hz), 6.73-6.70 (m, 1H), 6.06 (d, 2H, J = 0.8 Hz), 5.82 (s, 2H), 5.35 (s, 1H), 3.87 (s, 3H), 3.59 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.4, 180.0, 162.4, 161.8, 154.4, 153.6, 149.7, 140.7, 135.8, 135.2, 133.5, 132.5, 130.8, 126.3, 126.0,117.7, 116.5, 113.8, 112.3, 87.5, 57.6, 55.6, 29.9; HRMS-ESI (m/z): calc for $C_{23}H_{19}N_5NaO_4$ $[M+Na]^+$: 430.1515, found:

430.1508.

5-(3,4,5-Trimethoxyphenyl)-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*] quinoline-6,11-dione (**4k**): reddish-brown power, m.p. 276-278 ^{o}C; IR (KBr): *v* 3590, 3450, 3351, 3123, 1663, 1633 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.63 (s, 1H), 8.01-7.93 (m, 2H), 7.84-7.75 (m, 2H), 6.73 (s, 2H) 6.26 (s, 2H), 5.82 (s, 2H), 5.22 (s, 1H), 3.68 (s, 6H), 3.57 (s, 3H), ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.8, 179.9, 162.5, 162.0, 154.0, 152.9, 141.6, 139.8, 136.6, 135.2, 133.5,132.5, 130.8, 126.3, 126.2, 118.1, 106.0, 87.4, 60.3, 56.3, 35.3; HRMS-ESI (m/z): calc for $C_{24}H_{21}N_5NaO_5$ $[M+Na]^+$: 482.1440, found: 482.1362.

5-(3-Bromo-4-methoxyphenyl)-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4l**): reddish-brown power, m.p. 234-236 ^{o}C; IR (KBr): *v* 3459, 3360, 3326, 3092, 1666, 1633 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.79 (s, 1H), 7.95-7.88 (m, 2H), 7.78-7.69 (m, 2H), 7.64 (d, 1H, *J* = 1.2 Hz), 7.31-7.29 (m, 1H), 6.94 (d, 1H, *J* = 8.8 Hz), 6.34 (s, 2H), 5.92 (s, 2H), 5.26, 1H), 3.73 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.7, 179.8, 162.5, 162.0, 154.3, 154. 0, 139.7, 139.6, 135.2, 133.5, 132.7, 130.6, 128.1, 126.2, 126.1, 118.0, 112.9, 110.5, 87.1, 56.5, 33.8; HRMS-ESI (m/z): calc for $C_{22}H_{16}N_5NaO_3$ $[M+Na]^+$: 500.0334, found: 500.0220.

5-Butyl-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4m**) : reddish-brown power, m.p. >300 ^{o}C; IR (KBr): *v* 3466, 3397, 3327, 3200, 1676, 1621 cm^{-1}; ^{1}H NMR (400 MHz,DMSO-d_6) δ: 8.36 (s, 1H), 8.02-7.99 (m, 2H), 7.87-7.79 (m, 2H), 6.34 (s, 2H), 5.75 (s, 2H),4.29 (t, 1H, *J* = 4.8 Hz), 1.57-1.43 (m, 2H), 1.14-1.03 (m, 3H), 0.94-0.88 (s, 1H), 0.74 (t, 3H,*J* = 6.8 Hz) ^{13}C NMR (100 MHz, DMSO-d_6) δ: 182.1, 179.7, 162.6, 161.8, 155.0, 141.3, 135.2, 133.4, 132.7, 130.7, 126.2, 118.0, 85.9, 34.7, 29.1, 23.1, 14.5; HRMS-ESI(m/z): calc for $C_{19}H_{19}N_5NaO_2$ $[M+Na]^+$: 372.1436, found:372.1426.

5-Amyl-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione, (**4n**): reddish-

brown power, m.p. >300 °C; IR (KBr): *v* 3468, 3403, 3327, 3201, 1676, 1620 cm^{-1}; ^1H NMR (400 MHz , DMSO-d_6) δ: 8.37 (s, 1H), 8.00-7.98 (m, 2H), 7.85-7.75 (m, 2H), 6.38 (s, 2H), 5.78 (s, 2H), 4.28 (t, 1H, J = 4.4 Hz), 1.53-1.46 (m, 2H), 1.19-1.09 (m, 5H), 0.89-0.82 (m, 1H), 0.72 (t, 3H, J = 6.8 Hz) ^{13}C NMR (100 MHz, DMSO-d_6) δ:182.1, 179.6, 162.6, 161.8, 155.0, 141. 3, 135.2, 133.4, 132.7, 130.7, 126.2, 118.0, 85.9, 34.8, 29.1, 24.3, 22.5, 14.3; HRMS-ESI (m/z): calc for $C_{19}H_{19}N_5NaO_2$ $[M+Na]^+$: 386.1593, found: 386.1564.

5-(3-Hydroxyphenyl)-5,12-dihydro-2,4-amino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4o**) : reddish-brown power, m.p. 292-293 °C; IR (KBr): *v* 3495, 3354, 3215, 3095, 1673, 1607 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6)δ: 9.22(s, 1H), 8.69(s, 1H), 8.00-7.92 (m, 2H), 7.83-7.74 (m, 2H), 6.99 (t, 1H, J = 8.0 Hz), 6.85 (d, 1H, J = 7.6 Hz), 6.76 (s, 1H), 6.52-6.50 (s, 1H), 6.21 (s, 2H), 5.84 (s, 2H), 5.24 (s, 1H); ^{13}CNMR (100 MHz, DMSO-d_6) δ:181.7, 179.9 , 162.6 , 162.0 , 157.6, 154.1, 147.1, 139.8, 135.3, 133.5, 132.5, 130.6, 129.3, 126.2, 126.1, 119.3, 118.4,115.3, 113.9, 87.3, 3.7; HRMS-ESI (m/z): calc for $C_{21}H_{15}N_5\ NaO_3$ $[M+Na]^+$: 408.1073, found: 408.1066.

5-(2-Methylphenyl)-5,12-dihydro-2,4-diamino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4p**): reddish-brown power, m.p.173-175 °C; IR (KBr): *v* 3478, 3375, 3326, 3181, 1672, 1635 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 8.81 (s, 1H), 7.99 (t, 1H, J = 7.6 Hz), 7.87 (t, 1H, J = 6.4 Hz), 7.82-7.74(m, 2H), 7.29(t, 1H, J = 7.2 Hz), 7.07-7.01 (m, 3H), 5.87(s, 2H,), 5.62 (s, 2H), 5.31 (s,1H), 2.63 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ:181.8, 179.9, 169.2, 162.8, 161.8, 153.9, 144.7, 139.9, 135.3, 133.5, 132.5, 128.5, 132.2, 132.0, 126.8, 126.3, 126.1, 118.7, 88.5, 33.3, 19.9; HRMS-ESI(m/z): calc for $C_{22}H_{17}N_5NaO_2$ $[M+Na]^+$: 406.1280, found: 406.1262.

2-Methylthio-5-(4-chlorophenyl)-5,12-dihydro-4-amino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4q**): reddish-brown power, m.p. 157-159 °C; IR (KBr): *v* 3390, 3297, 3196, 1688, 1637 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.53 (s, 1H), 8.51 (d, 1H, J = 8.0 Hz) 7.98-7.96 (m, 1H), 7.84-7.79 (m,

1H), 7.66 (t, 1H, *J* = 7.6 Hz), 7.40 (d, 1H, *J* = 8.4 Hz), 7.28 (d, 2H, *J* = 8.2 Hz), 6.97 (s, 2H), 5.31 (s, 1H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ:179.4, 176.0, 168.5, 161.5, 153.7, 146.3, 143.9, 135.1, 131.7, 131.6, 131.1, 130.2, 129.5, 129.0, 128.5, 125.2, 113.7, 93.3, 33.6, 13.6; HRMS-ESI (m/z): calc for $C_{22}H_{15}ClN_4$ NaO_2S $[M+Na]^+$: 398.0688, found: 398.0684.

2-Methylthio-5-phenyl-5,12-dihydro-4-amino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4r**): reddish-brown power, m.p. 157-159 ^{o}C; IR (KBr): *v* 3455, 3291, 3160, 3082, 1689, 1606 ㎝$^{-1}$; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.51 (s, 1H), 8.51 (d, 1H, *J* = 7.6 Hz) 7.98-7.96 (m, 1H), 7.83-7.79 (m, 1H) 7.67-7.61 (m, 1H), 7.40 (t, 2H, *J* = 1.2 Hz), 7.21 (t, 2H, *J* = 7.2Hz), 7.14-7.10 (s, 1H), 6.94 (s, 2H), 5.29 (s, 1H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 179.5, 176.1, 168.3, 161.6, 153.7, 146.3, 145.0, 135.2, 131.6, 131.1, 130.3, 129.0, 128.6, 128.3, 127.0, 125.2, 114.1, 93.8, 34.1, 13.6; HRMS-ESI (m/z): calc for $C_{22}H_{16}N_4NaO_2S$ $[M+Na]^+$: 423.0892, found: 423.0887.

2-Methylthio-5-(3-bromo-4-methoxyphenyl)-5,12-dihydro-4-amino-benzo[*g*]pyrimido[4,5-*b*]quinoline-6,11-dione (**4s**): reddish-brown power, m.p. 157-159 ^{o}C; IR (KBr): *v* 3462, 3373, 3304, 3209, 1690, 1600 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.49 (s, 1H), 8.50 (d, 1H, *J* = 7.6 Hz), 7.97 (d, 1H, *J* = 7.6 Hz), 7.83-7.79 (m, 1H) 7.67-7.65 (m, 2H), 7.27-7.25 (m, 1H), 6.96 (d, 3H, *J* = 8.4 Hz), 5.23 (s, 1H), 3.74 (s, 3H), 2.47 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 179.4, 176.1, 168.5, 161.5, 154.4, 153.6, 146.1, 138.7, 135.2, 132.8, 131.6, 131.1, 130.2, 129.0, 128.6, 125.2, 113.9, 113.0, 110.4, 93.4, 56.6, 33.1, 13.6; HRMS-ESI (m/z):calc for $C_{23}H_{17}BrN_4NaO_3S$ $[M+Na]^+$: 531.0102, found: 531.0094.

Anti-proliferative assay

SGC7901, HepG2 and L02 cells were obtained from the Cell Center of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco's modified eagle's media (DMEM, gibco, USA) containing 1% penicillin-streptomycin, supplemented with 10% fetal bovine serum (FBS,

gibco, USA) at 37 °C, 5% CO_2. Cells (3×10^3 cells) were seeded into 96-well plates and incubated at 37 °C in 5%CO_2/95% air condition. Serially two fold diluted test compound solutions of each drug were added 24 h later, and the cells were incubated for the next 48 h. The final concentrations of compounds in the sample wells ranged from 0.103 μM to 50 μM. After 48 h, 20 mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, 5 mg/mL) was added to each well and the cells were incubated for an additional 4 h. Then, 100 μL DMSO were added into each well for dissolving the intracellular formazan crystals. Optical density at 570 nm of each plate was measured with a tunable microplate reader. Each group was in triplicate samples and each drug was divided into at least 5 concentrations. The percentage of absorbance from the sample-treated cells compared to that of the vehicle control (treated with DMSO) was calculated. The resulting cytotoxic acitvities were expressed as IC_{50} values and IC_{50} values were determined by analysis software (Graphpad Prism 6).

Acknowledgements

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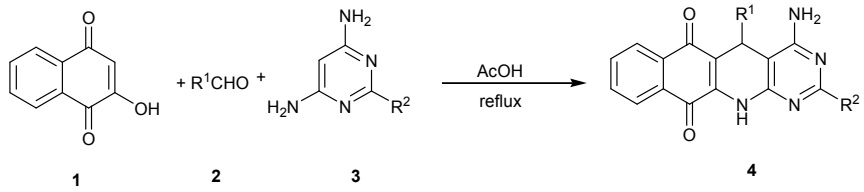


1 2 3 4

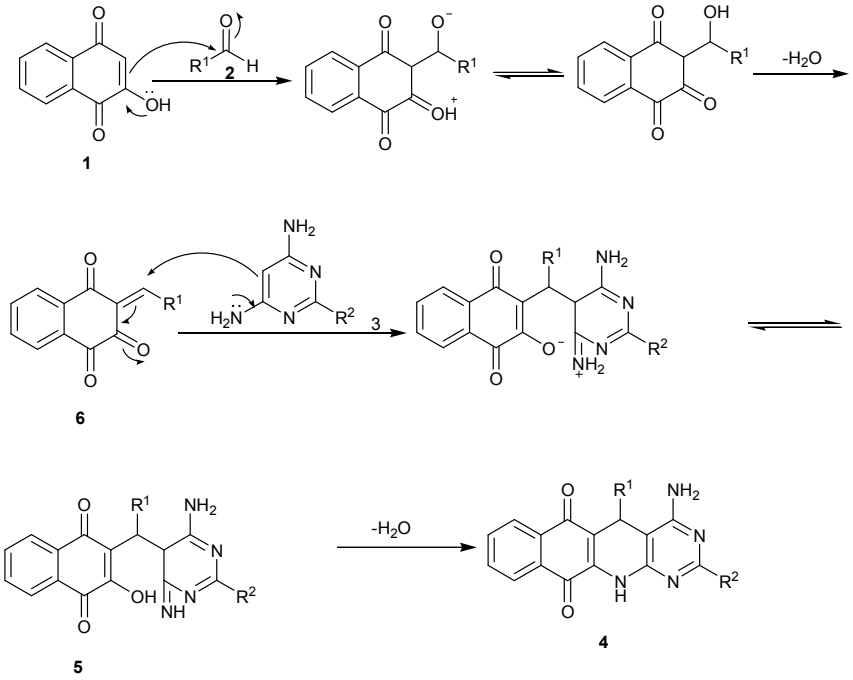
Scheme 1

Table 1. Reaction conditions optimization for the synthesis 5-phenyl-5,12-dihydro- 2,4-diamino-benzo[*g*]pyrimido[4,5-*b*] quinoline-6,11-dione

Entry	Solvent	T/ ^{o}C	Time/h	Yield/%
1	EtOH	70	4	15
2	THF	62	6	6
3	$CHCl_3$	61	6	0
4	H_2O	70	8	0
5	toluene	70	5	10
6	DMF	70	4	39
7	AcOH	70	4	41
8	AcOH	80	4	43
9	AcOH	90	3	49
10	AcOH	100	2	60
11	AcOH	118	2	65

Table 2. Preparation of 1,4-naphthoquinones possessing pyrido[2,3-*d*]pyrimidine scaffolds.

Entry	R^1	R^2	Time/h	Product	M.p/℃	Yield/%
1	C_6H_5	NH_2	2	**4a**	168-170	65
2	2-thienyl	NH_2	2	**4b**	254-255	69
3	2-furanyl	NH_2	3	**4c**	297-299	59
4	3-NO_2-C_6H_4	NH_2	3	**4d**	>300	53
5	4-Cl-C_6H_4	NH_2	3	**4e**	165-167	57
6	2,4-Cl-C_6H_3	NH_2	1.5	**4f**	>300	72
7	4-F-C_6H_4	NH_2	3	**4g**	157-159	55
8	2-F-C_6H_4	NH_2	1.5	**4h**	>300	71
9	4-MeO-C_6H_4	NH_2	1	**4i**	196-198	79
10	2,5-MeO-C_6H_3	NH_2	2	**4j**	265-266	63
11	3,4,5-MeO-C_6H_2	NH_2	3	**4k**	276-278	60
12	3-Br-4-MeO-C_6H_3	NH_2	3	**4l**	234-236	58
13	C_4H_9	NH_2	4	**4m**	192-194	50
14	C_5H_{11}	NH_2	4	**4n**	193-195	51
15	3-OH-C_6H_4	NH_2	2.5	**4o**	292-293	57
16	2-CH_3-C_6H_4	NH_2	3	**4p**	173-175	66
17	4-Cl-C_6H_4	SCH_3	3	**4q**	258-260	52
18	C_6H_5	SCH_3	3	**4r**	237-239	53
19	3-Br-4-OCH_3-C_6H_3	SCH_3	3	**4s**	>300	55



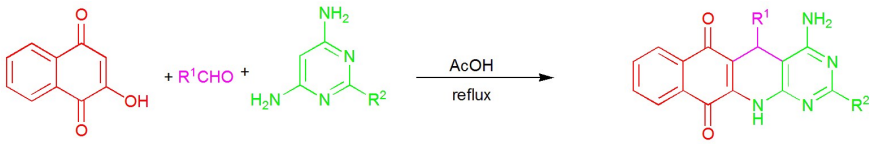
Scheme 2

Table 3. Anti-proliferative activities of 1,4-naphthoquinones possessing pyrido[2,3-*d*]pyrimidine scaffolds.

Compound	IC_{50} (μM)		
	SGC7901	HepG2	L02
4a	38.86 ± 2.38	29.59 ± 1.43	80.26 ± 3.23
4b	5.88 ± 0.15	9.54 ± 0.51	24.54 ± 0.98
4c	10.15 ± 1.01	18.52 ±1.26	45.23 ±2.53
4d	9.79 ± 1.12	9.98 ± 1.21	25.31 ± 1.20
4e	31.00± 2.78	42.21 ± 1.98	70.21 ± 2.32
4f	>100	79.64 ± 8.42	>100
4g	30.34 ± 1.67	28.16 ± 3.01	63.25 ± 2.99
4h	36.34 ±1.25	33.88 ± 5.21	>100
4i	10.02 ± 0.89	16.28 ±1.59	24.29 ±1.72
4j	33.74 ± 1.26	53.02 ± 2.87	>100
4k	26.45 ± 1.36	21.55 ± 1.53	50.78 ± 2.42
4l	43.63 ± 4.07	67.95 ± 9.01	>100
4m	26.48 ± 1.29	56.81 ± 9.56	>100
4n	9.91 ± 1.01	8.25 ± 0.84	17.53 ± 0.76
4o	25.54 ± 1.04	22.20 ± 1.07	41.20 ± 1.75
4p	51.05 ± 1.32	39.57± 1.87	>100
4q	5.61 ± 0.43	9.15 ± 0.85	19.52 ± 0.99
4r	9.46 ± 0.55	9.99 ± 0.98	18.45 ± 1.32
4s	4.39 ± 0.19	5.91 ± 0.73	11.26 ± 1.05

Doxorubicin	3.18 ± 0.54	2.25 ± 0.42	6.22 ± 0.95

Graphical Abstract



Have moderate anti-proliferative activity against the SGC7901 and HepG2

4s: Ic_{50}=4.39 μM against SGC7901; 5.91 μM against HepG2

A series of novel 1,4-naphthoquinones possessing pyrido[2,3-*d*]pyrimidine scaffolds were synthesized and most of compounds exhibited excellent anti-proliferative activities.