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Synthesis and biological evaluation of novel 1,2-naphthoquinones possessing tetrazolo[1,5-*a*]pyrimidine scaffolds as potent antitumor agents

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ABSTRACT A series of novel 1,2-naphthoquinones possessing tetrazolo[1,5-*a*] pyrimidine scaffolds were synthesized in very good yields using one-pot condensation of 2-hydroxy-1,4- naphthoquinone, aldehydes, and 5-aminotetrazole. All the synthesized compounds were evaluated for their antitumor activity *in vitro* against HCT116 (Human colon cancer) and HepG2 (human hepatoma) cell lines. Among the screened compounds, **4o**, **4k**, **4g**, **4q**, and **4s** howed significant inhibitory activity against the two human cell lines.

Keywords: 2-Hydroxy-1,4-naphthoquinone; Aldehydes; 1,4-Naphthoquinones; Antitumor; Solvent-free

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Introduction

Cancer is one of the most common causes of morbidity and mortality today.[1] Despite the efforts to discover and develop small molecule anticancer drugs in the last decade,[2] the development of new antitumor agents with improved tumor selectivity, efficiency, and safety remains desirable. Recently, naphthoquinones have attracted great attention due to their broad bioactivities, including antioxidant,[3] antifungal,[4] anti-inflammatory,[5] antiviral,[6] and anticancer.[7] Among these active compounds, 1,2-naphthoquinones include Tanshinone IIA,[8] 4-Hydroxysaprothoquinone,[9] β-Lapachone,[10] Mansonones[11] and Salvicine[12] (Figure 1) have been reported to show remarkable antitumor activities by means of inhibiting multiple enzymes. One of these quinones was Salvicine, a novel diterpenoid quinone compound, which possesses potent *in vitro* and *in vivo* activities against malignant tumor cells, especially in some human solid tumor models, and has now entered phase II clinical trials.[13] Salvicine induces apoptosis in various human tumor cell lines and displays prominent activity against multiple-drug resistance.[14] Mechanistic studies have shown that Topo II functions as one of the primary molecular targets of Salvicine.[15] These recent examples highlight the ongoing interest toward new 1,2-naphthoquinone derivatives and have prompted us to investigate this pharmacophore in drug discovery programs aiming at synthesizing novel bioactive molecules.[16]

< **Figure 1** >

On the other hand, tetrazolo[1,5-*a*]pyrimidines, a subtype of purine bioisosteric analogs, have been widely investigated and identified to possess multifaceted pharmacological properties, including anti-proliferative,[17] antimicrobial,[18] antioxidant,[19] anti-HBV,[19] and antifungal activities.[20] In addition, tetrazolopyrimidines are versatile ligands and their coordination compounds can be considered as model systems for metal–ligand interactions observed in biological systems.[21]

There has been significant interest in the combination of two pharmacophores on the same scaffold leading to hybrid molecules.[22] These hybrids combine two active moieties in a single molecule with

the goal of creating a chemical entity that is medically more effective than its individual components. As a result, 1,2-naphthoquinones-fused tetrazolo[1,5-*a*]pyrimidines, which combine two biologically active heterocyclic cores, are expected to be of pharmacological interest, we herein represent the synthesis of a series of novel 1,2-naphthoquinones possessing tetrazolo[1,5-*a*] pyrimidine scaffolds and their antitumor activity against human cancer cell lines *in vitro* (Scheme 1).

< **Scheme 1** >

Results and Discussion

Initially, to achieve suitable conditions for the synthesis of 1,2-naphthoquinones-fused tetrazolo[1,5-*a*]pyrimidines, we tested the three-component reaction of benzaldehyde, 2-hydroxy-1,4-naphthoquinone, and 5-aminotetrazole as a simple model system at 120 °C under solvent-free conditions using various catalysts (Table 1). As could be seen in Table 1, the best result was obtained with 10 % mol of *p*-toluenesulfonic acid as the catalyst at 120 °C under solvent-free conditions (Table1, entry 3). Using less catalyst resulted in lower yields, whereas higher amounts of catalyst did not affect reaction times and yields. When this reaction was carried out without *p*-toluenesulfonic acid the yield of the expected product was low (Table 1, entry 1). In the presence of $InCl_3$, $FeCl_3$, sulfamic acid or H_2SO_4 the product was obtained in moderate yield (Table 1, entries 6-9).

< **Table 1** >

Using these optimized reaction conditions, the title compounds **4** were prepared *via* one-pot three-component cyclocondensation reaction between 2-hydroxy-1,4- naphthoquinone, aldehydes, and 5-aminotetrazole at 120 °C under solvent-free conditions containing a catalytic amount of *p*-toluenesulfonic acid. From this approach the new 1,2-naphthoquinones possessing tetrazolo[1,5-*a*]pyrimidine scaffolds **4** were isolated by chromatography and in good yields (65-91 %) as stable crystalline solids (Table 2). In all cases this three-component reaction led regioselectively to

1,2-naphthoquinone derivatives **4** and their structures were characterized by spectroscopic and analytical methods. The IR spectrum of **4e** showed absorptions at 1677 and 1659 cm^{-1} indicating the presence of two C=O bonds. The high resolution mass spectrum of **4e** displayed the quasi-molecular ion ($[M+H]^+$) peak at *m/z* = 398.0865, which was consistent with the 1:1:1 adduct of 2-hydroxy-1,4-naphthoquinone, 4-(trifluoromethyl)benzaldehyde and 5-aminotetrazole with the loss of two water molecule. The ^1H NMR spectrum of **4e** showed two doublets (an AB system: δ =7.78 and 7.73 ppm, *J* = 8.4Hz) arising from the aromatic protons of C-6 position. Two singlet was observed (δ = 7.14, 12.27 ppm) for the CH group of C-6 position and NH group of C-4 position, respectively. The ^{13}C NMR spectrum of **4e** showed characteristic signals at δ = 57.7 ppm (due to the Ar–CH group), 180.9 and 178.8, ppm (arising from the two nonequivalent carbonyl groups).

< **Table 2** >

The formation of isomeric systems **(***ortho*- and *para*-quinone units**)** is possible in the reaction. So, we considered it desirable to obtain independent chemical evidence for the presence of *ortho*- or *para*-quinone units in **4**. To this end, we reacted **4d** with *o*-phenylenediamine for 30 min under solvent-free conditions, affording compound **5** in 57% yield, confirming the *ortho*-quinone structure (Scheme 2). The structure of **5** was fully characterized by spectroscopic data and elemental analysis, The H-13 and H-16 occur as a multiplet at 8.74-9.37 ppm, more downfield than expected of aromatic protons. This is explicable by the close proximity of these protons to the lone pairs of the neighbouring nitrogens and the consequent anisotropic and van de Waals deshielding. The lack of any carbonyl signal and the presence of two imine carbon signals at 153.1 and 142.5 ppm in ^{13} C NMR spectrum of **5**, and the fact that **5** is formed by the reaction of one molecule of **4d** with one molecule of *o*-phenylenediamine clearly support the structure of **5**, which, in turn, further corroborates the structure of **4** and the regiochemistry of its formation.

< **Scheme 2** >

A mechanistic rationalization for this reaction is provided in Scheme 3. The initial step is the formation of a carbocation from the reaction of aldehyde and *p*-toluenesulfonic acid. The higher reactivity of the carbocation compared with the carbonyl species is utilized to facilitate Knoevenagel condensation between aldehyde **2** and 2-hydroxy-1,4-naphthoquinone **1** *via* intermediate **7**, and after dehydration olefin is produced. Subsequent Michael-type addition of 5-aminotetrazole **3** to the olefin followed by intramolecular nucleophilic cyclization, dehydration, and aromatization by autoxidation affords the corresponding products **4**.

< **Scheme 3** >

All target compounds (**4a**-**4s**) were evaluated for their antitumor activity *in vitro* by the MTT method. The IC_{50} values against against HCT116 and HepG2 cell lines are summarized in Table 3. It is clearly observed that all the tested compounds showed moderate to good antiproliferative activities against the tested cancer cell lines. The compound **4o**, **4k**, **4g**, **4q**, and **4s** showed better antitumor activity against all cancer cell line. The results in Table 2 showed also some important structure-activity relationships (SARs) for this series of derivatives. First, the nature of substituents at the C-6 position have substantial influence on the antitumor activity. Substitution of the electron-rich aromatic ring at the C-6 position confered far greater cytotoxicity in comparison with the substitution of the electron-deficient aromatic ring at the C-6 position. Morever, introduction of 3-methoxyphenyl or 3-hydroxyphenyl group into the C-6 position was found to be quite favorable for increasing antitumor activity. Among this series, compound **4q** showed the best anti-tumor activity with IC_{50} values of 0.22 μM and 1.18 μM against HCT116 and HepG2 cell lines, respectively, which was 20 times more potent than Taxol. Second, the *ortho*-quinone moiety appeared to have an important effect upon cytotoxicity, compounds **5** was less potent cytotoxicity, than the corresponding analogues with an *ortho*-quinone

moiety. It is worthwhile to note that the majority compounds have lesser cytotoxicity on non-cancerous L02 cells.

< **Table 3** >

Conclusion

In summary, a series of novel 1, 2-naphthoquinones possessing tetrazolo[1,5-*a*]pyrimidine scaffolds were prepared in very good yields using one-pot condensation of 2-hydroxy-1,4-naphthoquinone, aldehydes, and 5-aminotetrazole. Biological evaluation data revealed that, in general all the tested compounds **4a-4s** possessed good deal of antitumor activity against HCT116 and HepG2 cell lines as compared to the standard drugs. On the whole among all the compounds tested, compound **4q** exhibited showed the best antitumor activity agains the two human cell lines. Therefore, these novel 1,2-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), 5-aminotetrazole (1

mmol), *p*-TsOH (0.1 mmol) was added. The mixture was stirred at 120 °C for an appropriate time (Table 1). After completion of the reaction (TLC), the reaction mixture was treated with water (10 mL) and extracted with CH_2Cl_2 (2 × 10 mL), filtered and the solvent evaporated in vacuo. Solvent was evaporated and the crude product puried by silica gel column chromatography using dichloromethane: ethyl acetate (*v:v* = 5:1) as eluent to afford the pure product **4**.

6-Phenyl-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4a**): Yellow power, m.p. 260-261 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.16 (s, 1H), 8.11 (dd, 1H, J = 4.0, 6.4 Hz), 7.86 (d, 2H, J = 9.2 Hz), 7.50 (d, 3H, J = 6.8 Hz), 7.37-7.32 (m, 3H), 7.00 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 178.8, 148.9, 139.8, 139.5, 135.5, 134.2, 132.0, 130.9, 129.2, 129.0, 128.3, 126.7, 126.2, 113.3, 58.1; IR (KBr): v 1676, 1655, 1593, 1574, 1309, 719 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{18}H_{10}N_5O_2$ [M-H]$^+$: 328.0834, found: 328.0898.

6-(4-Methylphenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4b**): Yellow power, m.p. 298-299 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.13 (s, 1H), 8.11 (dd, 1H, J = 4.0, 6.4 Hz), 7.91-7.85 (m, 3H), 7.37 (d, 2H, J = 8.0 Hz), 7.14 (d, 2H, J = 8.0 Hz), 6.95 (s, 1H), 2.25 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 178.9, 148.9, 139.4, 138.8, 137.1, 135.5, 134.2, 132.0, 130.9, 129.8, 128.2, 126.7, 126.2, 113.4, 57.8, 21.2; IR (KBr): v 1676, 1654, 1591, 1574, 1302, 716 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{19}H_{14}N_5O_2$ [M+H]$^+$: 344.1147, found: 344.1143.

6-(3-Methylphenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4c**): Yellow power, m.p. 247-248 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.13 (s, 1H), 8.11 (dd, 1H, J = 4.0, 6.4 Hz), 7.92-7.85 (m, 3H), 7.31-7.22 (m, 3H), 7.12 (d, 1H, J = 7.6 Hz), 6.94 (s, 1H), 2.26 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 178.9, 148.9, 139.8, 139.5, 138.6, 135.5, 134.2, 132.0, 131.0, 130.0, 129.1, 128.7, 126.7, 126.2, 125.5, 113.2, 58.1, 21.3; IR (KBr): v 1675, 1655, 1589, 1573, 1303, 720 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{19}H_{13}N_5NaO_2$ [M+H]$^+$: 366.0967, found: 366.0979.

6-(2-Methylphenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4d**): Yellow power, m.p. 247-248 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.15 (s, 1H), 8.12-8.10 (m, 1H), 7.89-7.84 (m, 3H), 7.27 (d, 2H, J = 7.6 Hz), 7.21-7.17 (m, 2H), 7.08 (t, 1H, J = 7.6 Hz), 2.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 178.8, 148.8, 139.7, 138.7, 136.4, 135.5, 131.9, 130.9, 129.1, 128.2, 127.3, 126.7, 114.1, 54.5, 19.6; IR (KBr): v 1682, 1647,9, 1591, 1572, 1302, 759, 715 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{19}H_{14}N_5O_2$ $[M+H]^+$: 344.1147, found: 344.1130.

6-(4-trifluoromethylphenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4e**): Yellow power, m.p. 289-290 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.27 (s, 1H), 8.11 (dd, 1H, J = 3.2, 6.0 Hz), 7.91-7.85 (m, 3H), 7.78 (d, 2H, J = 8.4 Hz), 7.73 (d, 2H, J = 8.4 Hz), 7.14 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 178.8, 149.0, 144.0, 140.0, 135.5, 134.3, 131.9, 131.0, 129.5, 129.4, 126.7, 126.2, 123.1, 112.5, 57.7; IR (KBr): v 1677, 1659, 1590, 1575, 1324, 1302, 1117, 1066, 716 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{19}H_{11}F_3N_5O_2$ $[M+H]^+$: 398.0865, found: 398.0876.

6-(4-Methoxyphenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4f**): Yellow power, m.p. 269-270 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.11 (s, 1H), 8.12-8.09 (m, 1H), 7.91-7.85 (m, 3H), 7.42 (d, 2H, J = 8.4 Hz), 6.95 (s, 1H), 6.88 (d, 2H, J = 8.4 Hz), 3.71 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 178.9, 159.9, 148.9, 139.4, 135.5, 134.2, 132.1, 132.0, 130.9, 129.6, 126.7, 126.2, 114.5, 113.4, 57.5, 55.6; IR (KBr): v 1677, 1647, 1590, 1573, 1513, 1300, 1252, 1117, 1031, 719 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{19}H_{14}N_5O_3$ $[M+H]^+$: 360.1096, found: 360.1078.

6-(3-Methoxyphenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4g**): Yellow power, m.p. 225-226 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.13 (s, 1H), 8.08 (dd, 1H, J = 3.6, 6.4 Hz), 7.86-7.82 (m, 3H), 7.26 (t, 1H, J = 8.0 Hz), 7.04 (t, 2H, J = 7.6 Hz), 6.90-6.88 (m, 2H), 3.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 178.8, 159.9, 146.7, 141.2, 139.5, 135.4, 134.2, 131.9, 130.9, 130.4, 126.7, 126.2, 120.4, 114.5, 114.3, 113.1, 57.9, 55.6; IR (KBr): v 1678, 1649, 1586, 1570,

1300, 1280, 1200, 1054, 752, 721 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{19}H_{14}N_5O_3$ $[M+H]^+$: 360.1096, found: 360.1079.

6-(3-Bromophenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4h**): Yellow power, m.p. 206-207 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ: 12.20 (s, 1H), 8.08 (dd, 1H, J = 4.0, 6.4 Hz), 7.92-7.81 (m, 4H), 7.56-7.52 (m, 2H),7.32 (t, 1H, J = 8.0 Hz), 7.03 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 178.7, 148.8, 142.2, 140.0, 135.4, 144.2,132.3, 132.0, 131.4, 131.1, 131.0, 127.7, 126.7, 126.2, 122.5, 112.4, 57.6; IR (KBr): v 1676, 1656, 1590, 1573, 1301, 1071, 728 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{18}H_{11}BrN_5O_2$ $[M+H]^+$: 408.0096, found: 408.0079.

6-(4-Chlorophenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4i**): Yellow power, m.p. 266-267 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ: 12.15 (s, 1H), 8.09 (dd, 1H, J = 4.0, 8.8 Hz), 7.87-7.82 (m, 3H), 7.54 (d, 2H, J = 8.4 Hz), 7.39 (d, 2H, J = 8.4 Hz), 7.01 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 178.8, 148.9, 139.7, 138.8, 135.5, 134.2, 134.0, 131.9, 131.0, 130.4, 129.2, 126.7, 126.2, 112.8, 57.4; IR (KBr): v 1675, 1656, 1590, 1573, 1300, 719 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{18}H_{11}ClN_5O_2$ $[M+H]^+$: 364.0601, found: 364.0586

6-(4-Fluorophenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4j**): Yellow power, m.p. 273-274 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ: 12.18 (s, 1H), 8.11 (dd, 1H, J = 4.0, 6.4 Hz), 7.91-7.85 (m, 3H), 7.59 (dd, 2H, J = 5.4, 8.8 Hz), 7.18 (t, 2H, J = 4.8 Hz), 7.04 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 178.8, 161.3, 148.9, 139.7, 136.2, 135.5, 134.2, 132.0, 131.0, 130.7, 130.6, 126.7, 126.2, 116.2, 116.0, 112.9, 57.4; IR (KBr): v 1676, 1658m 1591, 1574, 1509, 1308, 1240, 718 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{18}H_{11}FN_5O_2$ $[M+H]^+$: 348.0897, found: 348.0892.

6-(4-Hydroxyphenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4k**): Yellow power, m.p. 281-282 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ: 12.33 (s, 1H), 9.60 (s, 1H), 8.11-9.09 (m, 1H), 7.94-7.84 (m, 3H), 7.13 (t, 1H, J = 8.0 Hz), 6.89-6.82 (m, 3H), 6.68 (dd, 1H, J = 1.6, 8.4 Hz); ^{13}C

NMR (100 MHz, DMSO-d_6) δ: 180.7, 179.2, 158.0, 149.4, 141.2, 140.0, 135.5, 134.1, 132.1, 130.9, 130.3, 126.7, 126.2, 118.7, 116.2, 114.9, 113.2, 57.9; IR (KBr): v 1675, 1654, 1591, 1574, 1302, 716 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{18}H_{12}N_5O_3$ $[M+H]^+$: 346.0940, found: 346.0921.

6-(3-Nitrophenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4l**): Yellow power, m.p. 253-254 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ: 12.29 (s, 1H), 8.46 (d, 1H, J = 2.0 Hz), 8.20-8.01 (m, 3H), 7.89-7.85 (m, 3H), 7.66 (t, 1H, J = 8.0 Hz), 7.26 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 178.7, 148.9, 148.4, 141.7, 140.1, 135.5, 135.1, 134.3, 132.0, 131.0, 130.9, 126.7, 126.2, 124.3, 123.4, 112.2, 57.5; IR (KBr): v 1678, 1654, 1589, 1572, 1526, 1348, 1303, 726 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{18}H_9N_6O_4$ $[M-H]^+$: 373.0686, found: 373.0704.

6-(4-Nitrophenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4m**): Yellow power, m.p. 255-256 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ: 12.13 (s, 1H), 8.20 (d, 1H, J = 8.8 Hz), 8.11 (dd, 1H, J = 3.2, 5.6 Hz), 7.88-7.83 (m, 5H), 7.18 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 178.9, 149.2,, 148.1, 146.4, 140.4, 135.5, 134.3, 131.9, 130.9, 130.0, 126.8, 126.2, 124.4, 112.1; IR (KBr): v 1681, 1652, 1569, 1520, 1348, 1334, 1305, 830, 723 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{18}H_9N_6O_4$ $[M-H]^+$: 373.0791, found: 373.0704.

6-(2,4-Dichlorophenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4n**): Yellow power, m.p. 254-255 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ: 12.31 (s, 1H), 8.12 (dd, 1H, J = 4.0, 6.8 Hz), 7.90-7.86 (m, 3H), 7.72-7.69 (m, 2H), 7.39 (dd, 1H, J = 2.0, 8.4 Hz), 7.31 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.8, 178.8, 149.1, 140.5, 136.1, 135.6, 134.8, 134.3, 134.0, 131.9, 130.8, 1289.1, 128.5, 126.7, 126.2, 119.7, 112.2, 55.4; IR (KBr): v 1681, 1649, 1590, 1575, 1422, 1345, 1300, 796, 724 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{18}H_{10}Cl_2N_5O_2$ $[M+H]^+$: 398.0211, found: 398.0192

6-(2,6-Difluorophenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4o**): Yellow power, m.p. 253-254 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ: 12.33 (s, 1H), 8.12-8.09 (m, 1H),

7.91-7.85 (m, 3H), 7.73-7.67 (m, 1H), 7.33-7.19 (m, 1H), 7.19 (s, 1H), 7.09-7.04 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.6, 179.1, 163.5, 161.8, 149.5, 135.6, 134.2, 132.0, 131.9, 130.8, 126.8, 126.2, 123.5, 112.7, 112.4, 111.8, 104.7, 52.4; IR (KBr): v 1680, 1650, 1588, 1570, 1298, 1097, 1067, 720 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{18}H_8F_2N_5O_2$ $[M-H]^+$: 364.0646, found: 364.0628.

6-(2-Chloro-5-nitrophenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4p**): Yellow power, m.p. 283-284 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.30 (s, 1H), 8.53-8.51 (m, 1H), 8.19 (dd, 1H, J = 2.4, 8.8 Hz), 8.11 (dd, 1H, J = 2.4, 5.2 Hz), 7.85-7.81 (m, 4H), 7.47 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.8, 179.0, 149.6, 147.3, 141.3, 139.7, 138.3, 138.2, 135.6, 134.3, 131.9, 130.9, 126.8, 126.2, 125.8, 111.2, 55.9; IR (KBr): v 1682, 1647, 1593, 1573, 1532, 1300, 1043, 740 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{18}H_{10}ClN_6O_4$ $[M+H]^+$: 409.0452, found: 409.0461.

6-(3,5-Dimethoxyphenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4q**): Yellow power, m.p. 235-236 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.11 (s, 1H), 8.11-8.09 (m, 1H), 7.91-7.85 (m, 3H), 6.89 (s, 1H), 6.63 (d, 2H, J = 2.0 Hz), 6.46 (s, 1H), 3.71 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 178.8, 161.1, 148.9, 141.9, 139.6, 135.4, 134.1, 132.0, 131.0, 126.7, 126.2, 112.9, 106.7, 100.3, 58.0, 55.8; IR (KBr): v 1680, 1649, 1581, 1573, 1298, 1207, 1157, 1053, 740, 716 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{20}H_{16}N_5O_4$ $[M+H]^+$: 390.1202, found: 390.1191.

6-(3-Bromo-4-methoxyphenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4r**): Yellow power, m.p. 261-262 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.14 (s, 1H), 8.11 (dd, 1H, , J = 4.4, 6.4 Hz), 7.91-7.85 (m, 3H), 7.79 (d, 1H, J = 2.4 Hz), 7.51 (dd, 1H, , J = 2.4, 8.4 Hz), 7.06 (d, 1H, J = 8.8 Hz), 6.98 (s, 1H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.8, 178.8, 156.1, 148.8, 139.7, 135.4, 134.2, 133.6, 132.7, 132.0, 131.0, 129.3, 126.7, 126.2, 113.1, 112.7, 111.2, 57.1, 56.8; IR (KBr): v 1676, 1657, 1591, 1574, 1500, 1304, 1283, 1053, 716 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{19}H_{13}BrN_5O_3$ $[M+H]^+$: 438.0202, found: 438.0189.

4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (**4s**): Yellow power, m.p. >400 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.79 (s, 1H), 8.05 (dd, 1H, , J = 7.2, 17.6 Hz), 7.93-7.84 (m, 2H), 5.39 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.4, 178.7, 149.6, 139.5, 135.6, 134.2, 132.0, 130.7, 126.8, 126.0, 111.2, 43.5; IR (KBr): v 1672, 1656, 1589, 1573, 1308, 1072, 949, 720 cm^{-1}; HRMS-ESI (*m/z*): calcd for $C_{12}H_8N_5O_2$ $[M+H]^+$: 254.0678, found: 254.0681.

Typical procedure for the synthesis of compounds 5

A mixture of 6-(2-Methylphenyl)-4,6-dihydro-benzo[*h*]tetrazolo[5,1-*b*]quinazoline-7,8-dione (1 mmol) and *o*-phenylenediamine (1.5 mmol) was heated at 110 °C for an appropriate time and monitored by TLC until the final conversion. The reaction mixture was then cooled to room temperature and diluted with cold water (40 mL). The solid product was collected by filtration and was purified by recrystallization from 95% EtOH to afford the desired pure products **5** as a pale orange solid, m.p. >400 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.37 (dd, 1H, , J = 0.8, 8.0 Hz), 8.74 (dd, 1H, J = 0.8, 8.0 Hz), 8.31-8.25 (m, 3H), 8.12 (dd, 1H, J = 4.0, 6.8 Hz), 7.98-7.94 (m, 1H), 7.79-7.77 (m, 2H), 7.19-7.15 (m, 2H), 6.97-6.88 (m, 2H), 6.45 (s, 1H), 3.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 147.0, 142.5, 141.2, 141.0, 134.4, 133.6, 132.0, 130.9, 130.3, 130.0, 129.7, 129.6, 129.1, 129.0, 128.9, 126.9, 126.6, 126.5, 126.1, 125.3, 122.7, 116.2, 59.6, 29.7; IR (KBr): v 2921, 2851, 1957, 1289, 1202, 1047, 757, 713 cm^{-1}; Anal. Calc. for $C_{25}H_{17}N_{7:}$ C 69.37, H 3.03, N 19.41; found: C 69.43, H 3.08, N 19.57. 148.8, 139.7, 138.7136.4, 135.5, 131.9, 130.9,

Antitumor assay

HCT116 cells and HepG2 cells were maintained in DMEM (Gibco, Invitrogen Corporation, NY, USA) medium supplemented with 10% FBS, streptomycin (100 μg/mL) and penicillin (100 units/mL) and incubated at 37 ^{o}C, 5% CO_2. HCT116 cells (5000 cells/well) and HepG2 cells (10000 cells/well) were seeded into 96-well plates and incubated at 37 ^{o}C in 5%CO_2/95% air condition. Serially twofold

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).

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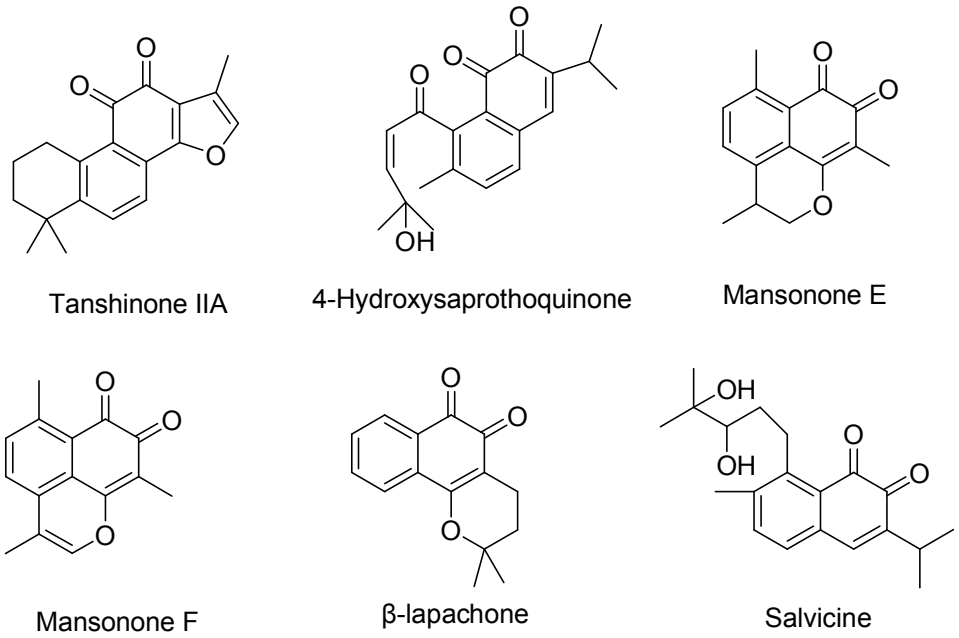
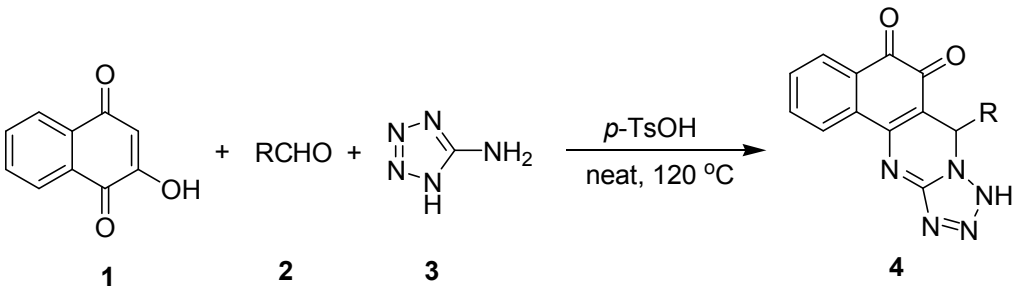


Figure 1. Structures of potent anticancer 1,2-naphthoquinone analogues.



1 2 3 4

Scheme 1

Table 1. Catalyst optimization for the synthesis **4a**

Entry	Catalyst	Mol%	Time/ h	Yield/ %[a]
1	-	-	10	19
2	*p*-Toluenesulfonic acid	5	4	72
3	*p*-Toluenesulfonic acid	10	2	84
4	*p*-Toluenesulfonic acid	15	2	83
5	*p*-Toluenesulfonic acid	20	2	84
6	$InCl_3$	10	3	57
7	$FeCl_3$	10	3	49
8	Sulfamic acid	10	3	72
9	H_2SO_4	10	3	58

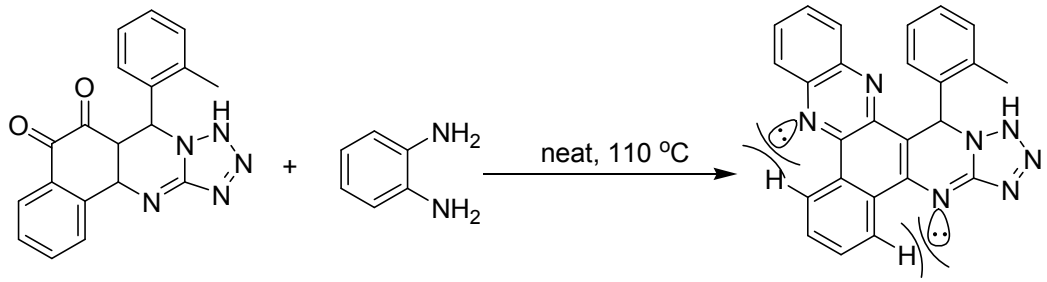
[a] Isolated yield.

Table 2. Preparation of 1,2-naphthoquinones possessing tetrazolo[1,5-*a*]pyrimidine scaffolds.

Entry	R	Time/ h	Product	Yield/ %[a]
1	C_6H_5	2	**4a**	84
2	4-Me-C_6H_4	2	**4b**	83
3	3-Me-C_6H_4	2.5	**4c**	81
4	3-Me-C_6H_4	2.5	**4d**	83
5	4-CF_3-C_6H_4	2	**4e**	83
6	4-MeO-C_6H_4	1.5	**4f**	91
7	3-MeO-C_6H_4	2	**4g**	85
8	3-Br-C_6H_4	2	**4h**	82
9	4-Cl-C_6H_4	2	**4i**	90
10	4-F-C_6H_4	2	**4j**	76
11	3-OH-C_6H_4	4	**4k**	68
12	3-NO_2-C_6H_4	2	**4l**	82
13	4-NO_2-C_6H_4	3	**4m**	75
14	2,4-Cl_2-C_6H_3	1.5	**4n**	83
15	2,6-$(F)_2$--C_6H_3	3	**4o**	70
16	2-Cl-5-NO_2-C_6H_3	3	**4p**	73
17	3,5-$(MeO)_2$-C_6H_3	2	**4q**	80
18	4-MeO-3-Br-C_6H_3	3	**4r**	79
19[b]	H	4	**4t**	65

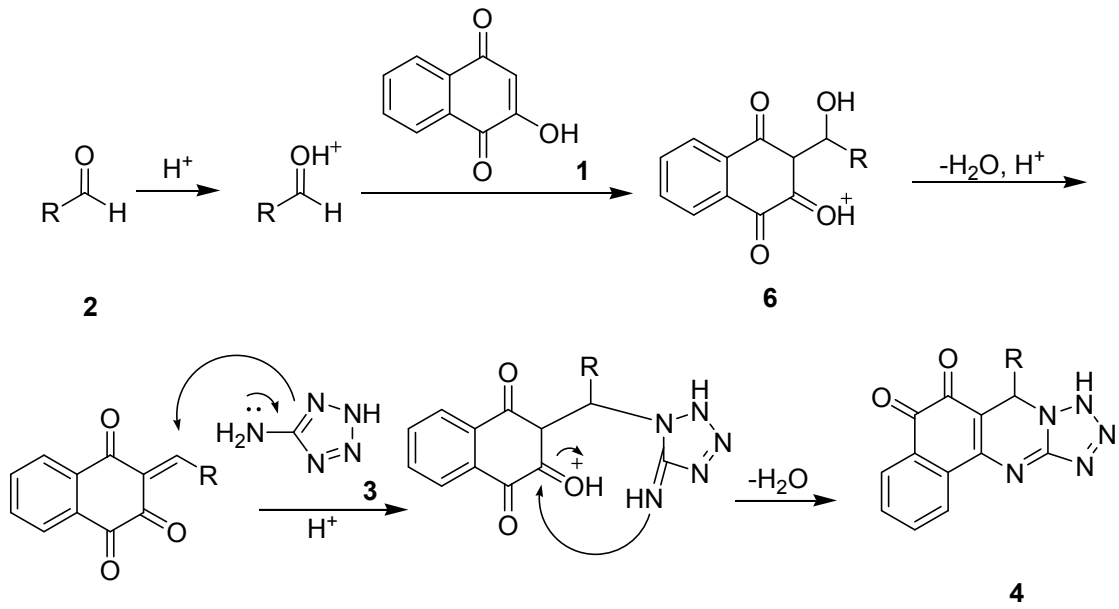
[a] Isolated yield.

[b] Aldehyde is polyoxymethylene in the reaction.



4d **5**

Scheme 2



Scheme 3

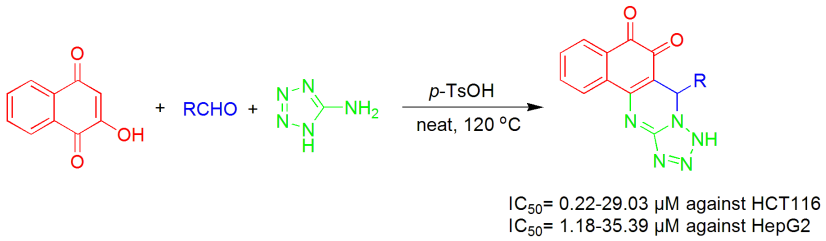
Table 3. Antitumor activities of 1,2-naphthoquinones possessing tetrazolo[1,5-*a*]pyrimidine scaffolds.

Compounds	IC_{50} (μM)[a]		
	HCT116	HepG2	L02
4a	12.06±0.886	9.50±0.442	14.05±0.992
4b	4.73±2.150	7.60±0.675	9.20±0.768
4c	5.35±0.232	15.77±1.328	20.10±2.003
4d	8.03±0.631	5.22±0.043	10.41±0.167
4e	25.43±1.524	35.39±2.651	32.34±1.170
4f	10.64±1.113	9.92±1.802	12.34±1.321
4g	1.23±0.236	4.59±0.451	6.94±0.687
4h	3.24±0.684	13.62±2.369	20.52±2.690
4i	14.31±1.120	11.27±1.101	13.47±0.956
4j	7.76±2.368	10.01±0.575	10.31±0.865
4k	0.84±0.064	2.07±0.234	5.13±0.435
4l	5.26±1.69	5.43±0.059	9.58±0.145
4m	7.16±1.237	7.54±0.133	10.67±0.245
4n	29.03±2.337	19.12±3.086	20.56±2.578
4o	1.02±0.381	2.54±2.556	3.92±1.987
4p	20.19±0.425	14.16±0.618	20.46±0.887
4q	0.22±0.045	1.18±0.077	1.96±0.109
4r	12.78±0.720	10.64±0.568	15.42±0.778
4s	3.17±0.650	2.56±0.210	4.67±0.348
5	>200	>200	>200

Taxol	3.82±0.534	20.92±2.376	22.76±2.682

[a] The means of triplicates ± SD

Graphical Abstract



IC_{50}= 0.22-29.03 μM against HCT116
IC_{50}= 1.18-35.39 μM against HepG2

A series of novel 1,2-naphthoquinones possessing tetrazolo[1,5-*a*] pyrimidine scaffolds were synthesized and all compounds exhibited excellent antitumor activities.