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Solid-Phase Combinatorial Synthesis and Cytotoxicity of 3-Aryl-2,4-quinazolindiones

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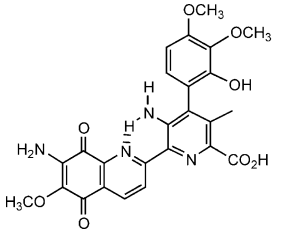
Abstract—A series of 3-aryl-2,4-quinazolinediones with various substitution on aromatic rings has been prepared by solid-phase synthesis. Several compounds showed cytotoxicity on human colon carcinoma (Col2) tested by SRB method.

Introduction

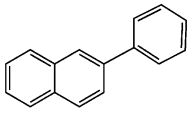
It has been reported that 2-phenylnaphthalene-type structural pattern, which composed of either carbocylic or heterocyclic atoms, was observed among a substantial number of antineoplastic compounds of both natural and synthetic origin.[1] Although not every antineoplastic agent contains this structural pattern, ellipticine, camptothecin, mebarone, streptonigrin, quercetin, genistein, and fagaridine[2] can be the examples of the 2-phenylnaphthalene-type structural pattern. Recently 2-(4-aminophenyl)benzothiazoles were reported to exhibit nanomolar inhibitory activity against a range of human cancer cell lines[3] in vitro and the compounds have the similar structure of two aromatic rings. Also it has been noticed that these compounds often contain a conformation of coplanar of two ring units which was assured either through a condensed ring structure or through hydrogen bond formation.

Extensive structure modifications of camptothecin or streptonigrin have been performed in order to obtain more potent and less toxic anticancer agents, but preparation of analogues needed long-step synthesis. In our search for new cytotoxic compounds with minimal side effects, we focused our efforts in aryl quinazolindiones, since they could be the antineoplastic structural pattern of 2-phenylnaphthalene with heterocyclic atoms even though no cytotoxic quinazolinediones has been known. The quinazolindione moiety is widely found in natural purine bases, alkaloids and many biologically active compounds[4] therefore the toxicity of quinazolinediones could be very low. In addition, the synthesis of quinazolinediones is well studied and recent development in combinatorial chemistry made the preparation of large number of quinazolinediones in a short time possible.

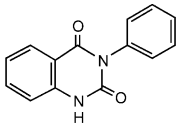
Three different solid-phase combinatorial syntheses of quinazolindiones were reported in recent years, but



Streptonigrin



2-Phenylnaphthalene



Phenylquinazolindione

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most of the compounds prepared by these methods were 3-alkyl substituted quinazolinediones.[5–7] Two methods are limited to preparation of quinazolinedions bearing 7-hydroxy or 3-alkylcarboxylic acid side chain since the phenolic or carboxylic group were used as resin binding site.[5,6] Therefore the method reported by Smith et al.[7] was employed in the preparation of 3-aryl-2,4-quinazolinediones in this study, but the final cyclization step was modified to avoid relatively harsh thermal condition (125 °C, 16 h) (Scheme 1).

Results

Chemistry

A polystyrene resin was reacted with 4-nitrophenyl chloroformate to give the chloroformate functionalized by the polystyrene resin **1**. The resin **1** was reacted individually with substituted anthranillic acid **2**A1–A6 in the presence of DIEA and HOBt to give **3**A1–A6. These resin bound anthranillic acids **3**A1–A6 were coupled with aromatric amines (**4**B1–B7) using PyBOP, HATU or PyBrOP as coupling agent. Urethanes **5**A1B1–A6B7 were reacted with triethylamine in methanol at 60°C for 24 h to give the cyclized product **6**A1B1–A6B7. The structure of building blocks and overall yields for three steps are illustrated in Figure 1 and Table 1, respectively.

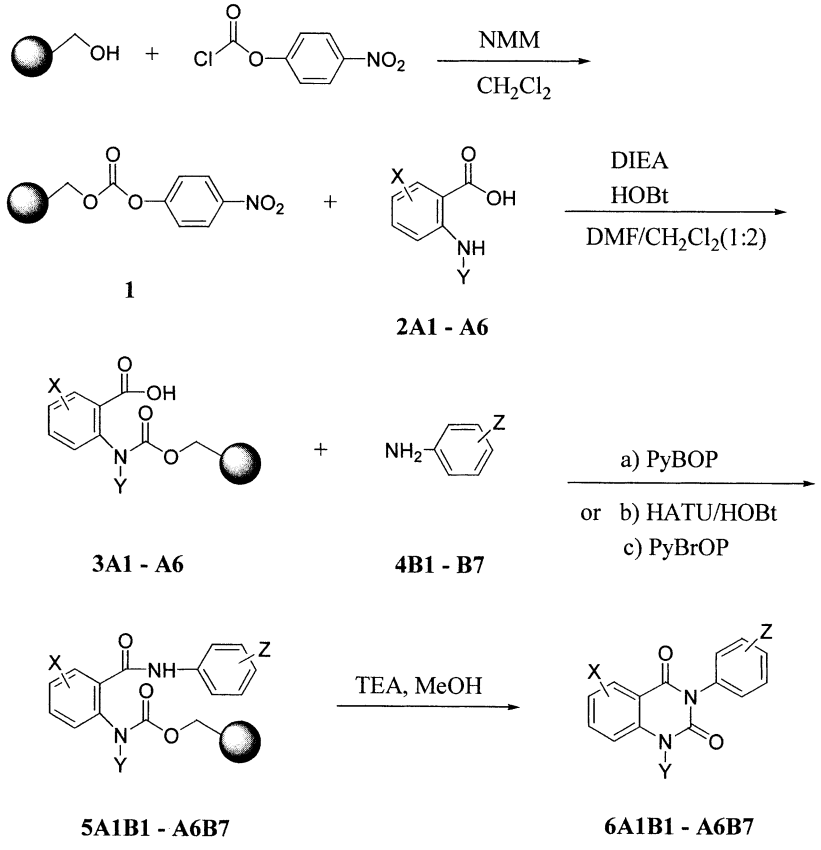
The aromatic amines with electron donating group at *para*-position gave aryl quinazolinediones in good yield, while *meta*-substituted or electron withdrawing group substituted anilines like 3,4-difluoroaniline or 3-chloroaniline gave aryl quinazolinediones in very low yield. Aromatic amines such as 4-chloroaniline, 2-chloroaniline, *p*-aminoacetophenone, 3-aminobenznitrile did not react under this condition. When each reaction was followed by NMR, after cleavage of resin, no coupling product was found from the reaction of anilines with the electron withdrawing group. Various coupling agents such as HATU, PyBrOP, EDCI/4-dimethylaminopyridine failed to give the desired coupling preduct, probably due to the decreased nucleophilicity caused by electron withdrawing substituents. In addition to this electronic effect, a steric effect was also observed. *o*-Anisidine and 2,6-dimethyl aniline did not react in this coupling condition.

The scale up synthesis of compound **6A3B4** to 10 times was performed to demonstrate the usefulness of this method in preparation of quinazolinediones. There was no change in the product yield.

The conventional synthetic approach for the preparation of large quantity of quinazolinediones has been also demonstrated by high yield synthesis of **6A1B2** as shown in Scheme 2.

Biology

Human cancer cell lines of colon (Col2) and lung (A549) were used for in vitro cytotoxicity assay by sul-



Scheme 1. Solid-phase synthesis of 3-aryl-2,4-quinazolinediones.

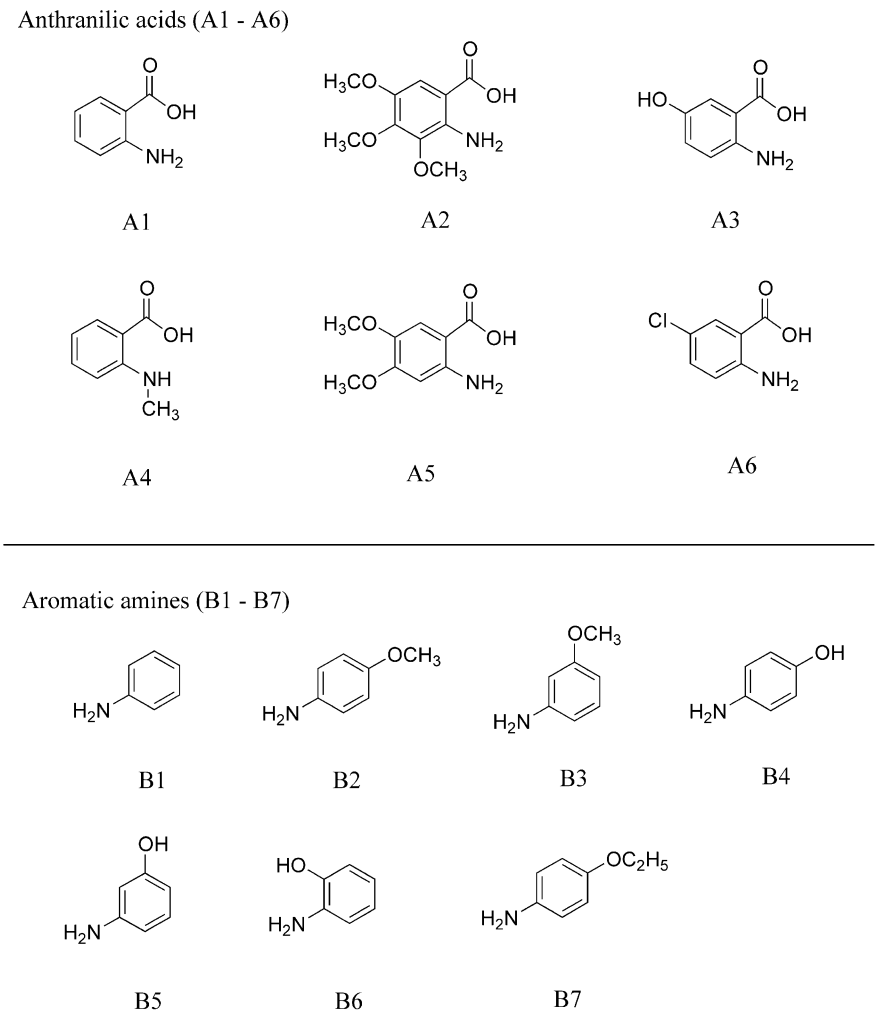


Figure 1. Structures of building blocks.

Table 1. Overall yields of aryl quinazolinediones (%)

	A1	A2	A3	A4	A5	A6
B1	42	2[a]	nr.	34	2	7
B2	100	17	36	31	57	45
B3	7	2	nr.	28	nr.	9[a]
B4	80	22	60	13	36	13[a]
B5	70[a]	22	9	24	2	9[a]
B6	30	27	19	21	23	19[a]
B7	45	15[a]	19	37	8[b]	29

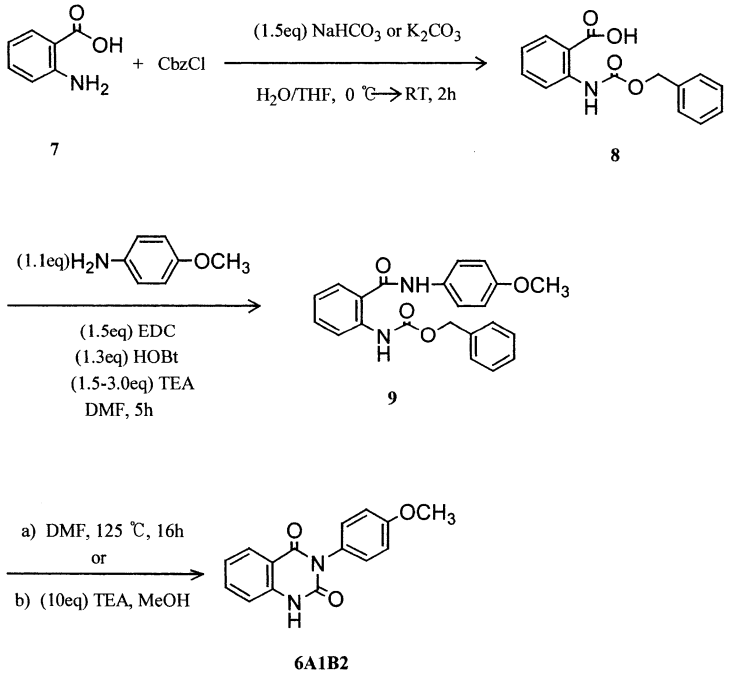
Unmarked: PyBOP as coupling agent; nr, not reacted.
[a]HATU/HOBt as coupling agent.
[b]PyBrOP/DMAP as coupling agent.

forhadamine B (SRB) method.[8,9] Topoisomerase I/II inhibition activity of the prepared compounds was also investigated using camptothecin and etoposide as positive control, respectively.

Several synthesized compounds showed cytotoxicity on colon carcinoma (Col2) but none showed cytotoxic activity on human lung carcinoma (A549) in SRB assay. Among the forty compounds synthesized, 6,7,8-trimethoxy-quinazolinediones (A2 series) and *N*-phenol-substituted compounds (B4–B6 series) showed cytotoxic activity (Table 2). The most active compound was **6A2B5**. However, the cytotoxic potency of quinazolinediones was much weaker than that of anticancer agent ellipticine. The IC_{50} value of **6A2B5** and **6A2B6** was 11.41 and 16.5 μg/mL, respectively whereas that of ellipticine was 0.5 μg/mL. However none of the prepared compounds showed topoisomerase I/II inhibition activity at 100 μM.

Discussion

The substituents of quinazolinedione ring part were found to be crucial on cytotoxicity. As shown in Table 2, compounds without any substituent (A1 series) showed no cytotoxicity at all and compounds with 6-hydroxy (A3 series) or 6- chloro (A6 series) showed little activity. Most of the compounds with 6,7,8-trimethoxy-quinazolinediones (A2 series) showed activity. However the 6,7-dihydroxy-quinazolinediones (A5 series) showed little activity. From this result, the trimethoxy group seemed to be critical to cytotoxic activity. The presence of three methoxy groups in cytotoxic compounds can be easily found in structures such as podophyllotoxin or potent antimitotic combleastatin A-4 which showed marked tumor growth suppression against colon 26 murin tumor model.[10,11]



Scheme 2. Conventional synthesis of 3-aryl-2,4-quinazolindone.

Table 2. Growth inhibitory potential of aryl-quinazolinediones on colon carcinoma (Col2)

Compound	Inhibition (%) (at 20 μg/mL)	Compound	Inhibition (%) (at 20 μg/mL)
Ellipticine	91	**A4B1**	<20
A1B1	<20	**A4B2**	<20
A1B2	<20	**A4B3**	<20
A1B3	<20	**A4B4**	<20
A1B4	<20	**A4B5**	33
A1B5	<20	**A4B6**	<20
A1B6	<20	**A4B7**	<20
A1B7	<20	**A5B1**	<20
A2B1	43	**A5B2**	<20
A2B2	<20	**A5B4**	<20
A2B3	32	**A5B5**	30
A2B4	<20	**A5B6**	<20
A2B5	62	**A5B7**	22
A2B6	51	**A6B1**	<20
A2B7	25	**A6B2**	<20
A3B2	<20	**A6B3**	<20
A3B4	<20	**A6B4**	34
A3B5	25	**A6B5**	<20
A3B6	<20	**A6B6**	<20
A3B7	<20	**A6B7**	<20

The substituents on the *N*-phenyl group were also important for the activity. The hydroxy or methoxy group on phenyl (B4–B6 series) was also needed for the cytotoxicity, but the position of these groups on 2 or 3 seemed to be crucial since 4-substituted compounds were not biologically active. We consider further modification of the compounds would promote cytotoxic activity of aryl-quinazolinediones. The mechanism for the cytotoxicity of aryl-quinazolinediones is not known yet.

Conclusion

Forty aryl-quinazolinediones were easily made by solid-phase combinatorial chemistry. The solid-phase synthesis was also useful in the gram sacle preparation of aryl-quinazolinediones. Series of compounds with trimethoxyquinqzolinediones and hydroxy group on 3-aryl showed selective cytotoxicity on colon carcinoma. This result can be applied in the design of the more potent antitumor agents.

Experimental

Materials and methods

Melting points were taken on a Thomas–Hoover melting point apparatus and were uncorrected. ^{1}H NMR spectra were determined with a Varian Gemini 300 instrument. Mass spectra were obtained on a Hewlett-Packard 5988 or JMS AX505WA mass spectrometer. FT-IR spectra were recorded using a Perkin-Elmer 1420 infrared spectrometer.

General procedure for the preparation of quinazolinediones

Quinazolinediones were prepared by the reaction of a chloroformate functionalized polystyrene resin with substituted anthranillic acids, followed by coupling and cyclization. The procedure reported by Smith et al.[6] was employed with slight modification. A chloroformated resin (1 g, 1.03 mmol) was treated individually with various substituted anthranilic acids (5.15 mmol) in the presence of HOBt (0.42 g, 3.09 mmol) in DMF/CH_2Cl_2 (1:2). Hunig's base 0.8 g (6.18 mmol) was added at room temperature and the mixture was stirred for 24 h. After filtration, the resin was washed with MeOH (3 15 mL), CH_2Cl_2 (3×15 mL) MeOH/CH_2Cl_2 (1:1) (3×15 mL),

and MeOH (3×15 mL). The resin was stirred in MeOH/CH_2Cl_2 (1:1) for 1 h and washing with the same sequence of solvents was repeated. Then resin 3 was dried under reduced pressure.

Resin 3 (1 mmol), PyBOP 1.6 g (3.08 mmol), diverse aromatic amines (3.48 mmol), DIEA 1.09 g (8.46 mmol) and DMF 10 mL were added in round bottom flask. The reaction was continued at rt for 24 h with stirring. After filtration, the resin was washed with MeOH (3×15 mL), CH_2Cl_2 (3 15 mL) MeOH:CH_2Cl_2 (1:1) (3×15 mL), and MeOH (3×15 mL). The resin was stirred in MeOH/CH_2Cl_2 (1:1) for 1 h and washing with the same sequence of solvents was repeated. Then the resin 5 was dried under reduced pressure.

3-Phenyl-2,4-quinazolinedione (A1B1). Yield 20 mg (42%), mp 282–283 °, IR (NaCl, cm^{-1}): 3211 (w), 2349 (w), 1734 (m), 1649(s). ^1H NMR ($CDCl_3/CD_3OD$): δ 8.19–8.16 (d, 1H), 7.75 (t, 1H), 7.61–7.56 (m, 3H), 7.38–7.27 (m, 4H). MS (*m*/*z*): 238 (M^+).

3-(4-Methoxyphenyl)-2,4-quinazolinedione (A1B2). Yield 54 mg (100%), mp 299–300 °C, IR (NaCl, cm^{-1}): 2355 (m), 1728 (m), 1646 (s, multiple), 1514 (m, multiple), 1272 (m, doublet). ^1H NMR ($CDCl_3/CD_3OD$): δ 8.15–8.11 (d, 1H), 7.63–7.60 (t, 1H), 7.27–7.11 (m, 4H), 7.05–7.01 (m, 2H), 3.86 (s, 3H). MS (*m*/*z*): 268 (M^+).

3-(4-Hydroxyphenyl)-2,4-quinazolinedione (A1B4). Yield 8 mg (80%), mp over 300 °C, IR (NaCl, cm^{-1}): 3211 (w), 2355 (w), 1727 (m), 1648 (s), 1270 (m). ^1H NMR ($CDCl_3/CD_3OD$): δ 8.12–8.08 (d, 1H), 7.66–7.63 (t, 1H), 7.28–7.18 (q, 2H), 7.11–7.07 (d, 2H), 6.96–6.93 (d, 2H). MS (*m*/*z*): 254 (M^+).

3-(4-Methoxyphenyl)-6,7,8-trimethoxy-2,4-quinazolinedione(A2B2). Yield 8 mg (17%), mp 297–300 °C, IR (NaCl, cm^{-1}): 3046 (w, multiple), 2355 (w), 1719 (m), 1654 (s), 1512 (m). ^1H NMR ($CDCl_3/CD_3OD$): δ 8.30 (s, 1H), 7.33 (s, 1H), 7.23–7.17 (d, 2H), 7.06–7.01 (d, 2H), 4.00 (s, 6H), 3.91 (s, 3H), 3.85 (s, 3H). MS (*m*/*z*, FAB): 359($[M+H]^+$).

3-(4-Hydroxyphenyl)-6,7,8-trimethoxy-2,4-quinazolinedione (A2B4). Yield 10 mg (22%), mp over 300 °C, IR (NaCl, cm^{-1}): 2349 (w), 1719 (m), 1651 (s), 1514 (m, doublet), 1360 (m). ^1H NMR ($CDCl_3/CD_3OD$): δ 7.28 (s, 1H), 7.04–7.00 (d, 2H), 6.91–6.87 (d, 2H), 3.96 (s, 6H), 3.86 (s, 3H). MS (*m*/*z*, FAB): 345 ($[M+H]^+$).

3-(3-Hydroxyphenyl)-6,7,8-trimethoxy-2,4-quinazolinedione (A2B5). Yield 10 mg (22%), mp 293–294 °C, ^1H NMR ($CDCl_3/CD_3OD$): δ 7.30–7.27 (d, 1H), 7.16–7.13 (d, 1H), 7.02–6.94 (m, 2H), 4.01 (s, 6H), 3.91 (s, 3H). MS (*m*/*z*, FAB): 345 ($[M+H]^+$).

3-(2-Hydroxyphenyl)-6,7,8-trimethoxy-2,4-quinazolinedione (A2B6). Yield 12 mg (27%), mp 288–289 °C, ^1H NMR ($CDCl_3$): δ 8.21(1H), 7.54–7.43 (m, 3H), 7.34–7.30 (m, 2H), 4.06–3.96 (d, 6H), 3.91–3.86 (s, 3H). MS (*m*/*z*, FAB) : 345 ($[M+H]^+$).

3-(4-Ethoxyphenyl)-6,7,8-trimethoxy-2,4-quinazolinedione (A2B7). Yield 7 mg (15%), mp 263–264 °C, IR (NaCl, cm^{-1}): 3050 (w, multiple), 2361 (w), 1719 (m), 1656 (s), 1513 (m, doublet), 1415 (m), 1362 (m). ^1H NMR ($CDCl_3$): δ 8.27 (s, 1H), 7.33 (s, 1H), 7.21–7.14 (d, 2H), 7.04–6.99 (d, 2H), 4.12–4.03 (q, 2H), 1.46–1.41 (t, 3H), MS (*m*/*z*, FAB): 373($[M+H]^+$).

3-(4-Methoxyphenyl)-6-hydroxy-2,4-quinazolinedione (A3B2). Yield 16 mg (36%), mp 233–234 °C, IR (NaCl, cm^{-1}): 3421 (s, broad), 2361 (w), 1717 (w), 1649 (s), 1511 (w). ^1H NMR($CDCl_3/CD_3OD$): δ 7.45–7.44 (d, 1H), 7.20–7.16 (m, 3H), 7.04–7.01 (d, 3H). MS (*m*/*z*, FAB): 285($[M+H]^+$).

3-(4-Hydroxyphenyl)-6-hydroxy-2,4-quinazolinedione (A3B4). Yield 26 mg (60%), mp over 300 °C, IR (NaCl, cm^{-1}): 2360 (s, doublet), 1714 (m), 1654 (m), 1516 (m), 1465 (m). ^1H NMR($CDCl_3/CD_3OD$): δ 7.44–7.43 (d, 1H), 7.22–7.17 (d, 1H), 7.11–7.06 (m, 3H), 6.95–6.90 (m, 2H). MS (*m*/*z*, FAB): 271 ($[M+H]^+$).

3-(3-hydroxyphenyl)-6-hydroxy-2,4-quinazolinedione (A3B5). Yield 4 mg (9%), oil, ^1H NMR($CDCl_3/CD_3OD$): δ 7.45–7.44 (d, 1H), 7.33–7.30 (t, 1H), 7.23–7.18 (d, 1H), 7.08–7.05 (d, 1H), 6.94–6.93 (d, 1H), 6.76–6.71 (m, 2H). MS(*m*/*z*, FAB): 271 ($[M+H]^+$).

3-(2-Hydroxyphenyl)-6-hydroxy-2,4-quinazolinedione (A3B6). Yield 8 mg (19%), mp over 300 °C, IR (NaCl, cm^{-11}): 3225 (w), 2372 (w), 1711 (m), 1658 (s), 1499 (m). ^1H NMR ($CDCl_3/CD_3OD$): δ 7.45–7.44 (d, 1H), 7.28–7.25 (t, 1H), 7.21–7.08 (m, 3H), 7.01–6.94 (m, 2H). MS (*m*/*z*, FAB): 271 ($[M+H]^+$).

1-Methyl-3-phenyl-2,4-quinazolinedione (A4B1). Yield 12 mg (34%), mp 225–227 °C, IR (NaCl, cm^{-1}): 3067(w), 2363 (w), 1708 (s), 1671 (s, doublet), 1611 (m), 1488 (m). ^1H NMR ($CDCl_3/CD_3OD$): δ 8.26–8.258 (d, 1H), 7.75–7.74 (t, 1H), 7.53–7.45 (m, 3H), 7.33–7.26 (m, 2H). MS (*m*/*z*, FAB): 253 ($[M+H]^+$).

1-Methyl-3-(4-methoxyphenyl)-2,4-quinazolinedione (A4B2). Yield 12 mg (31%), mp 191–192 °C, IR (NaCl, cm^{-1}): 2918 (w), 2362 (s, doublet), 1711 (m), 1660 (m), 1608 (m), 1484 (m), 1391 (m). ^1H NMR ($CDCl_3$): δ 8.28–8.26 (m, 1H), 8.25–7.68 (d, 1H), 7.33–7.26 (m, 2H), 7.21–7.17 (d, 2H), 7.04–7.01 (d, 2H), 3.85 (s, 3H), 3.65 (s, 3H). MS(*m*/*z*, FAB): 283($[M+H]^+$).

1-Methyl-3-(3-methoxyphenyl)-2,4-quinazolinedione (A4B3). Yield 11 mg (28%), mp 217–219 °C, IR (NaCl, cm^{-1}): 2941 (w, multiple), 2361 (w), 1709 (s), 1671 (s), 1609 (s), 1409 (s), 1428 (m), 1390 (s). ^1H NMR($CDCl_3$): δ 8.28–8.25 (d, 1H), 7.74 (t, 1H), 7.33–7.26 (m, 2H), 7.02–7.01 (d, 1H), 6.88–6.80 (m, 2H), 3.82 (s, 3H), 3.65 (s, 3H). MS (*m*/*z*, FAB): 283 ($[M+H]^+$).

1-Methyl-3-(3-hydroxyphenyl)-2,4-quinazolinedione (A4B5). Yield 9 mg (24%), mp 300–301 °C, ^1H NMR ($CDCl_3/CD_3OD$): δ 8.23–8.20 (d, 1H), 7.82 (t, 1H), 7.44–7.31(m, 3H), 6.95–6.91 (d, 1H), 6.77–6.73 (m, 2H), 3.67 (s, 3H). MS (*m*/*z*, FAB): 269($[M+H]^+$).

3-(4-Methoxyphenyl)-6,7-dimethoxy-2,4-quinazolinedione (A5B2). Yield 26 mg (57%), mp over 300 °C, IR (NaCl, cm^{-1}): 3655 (w, multiple), 2344 (w), 1717 (m, doublet), 1662 (s), 1614 (m, multiple), 1407 (m, multiple). ^1H NMR($CDCl_3/CD_3OD$): δ 7.48 (s, 1H), 7.21–7.17 (d, 2H), 7.05–7.01 (d, 2H), 6.66–6.61 (s, 1H), 3.99–3.96 (s, 3H), 3.93 (s, 3H), 3.86–3.82 (s, 3H). MS (*m*/*z*, FAB): 329 ($[M+H]^+$).

3-(4-Hydroxyphenyl)-6,7-dimethoxy-2,4-quinazolinedione (A5B4). Yield 16 mg (36%), mp over 300 °C. IR(NaCl, cm^{-1}): 3371 (w), 2361 (w), 1709 (m), 1653 (s), 1512 (m), 1431 (m, multiple), 1371 (w), 1294 (m). ^1H NMR ($CDCl_3/CD_3OD$): δ 7.47–7.45 (m, 1H), 7.12–7.07 (d, 2H), 6.97–6.92 (d, 2H), 6.70 (s, 1H), 3.99 (s, 3H), 3.93 (s, 3H). MS (*m*/*z*, FAB): 315($[M+H]^+$).

3-(2-Hydroxyphenyl)-6,7-dimethoxy-2,4-quinazolinedione (A5B6). Yield 10 mg (23%), mp 301–302 °C, IR (NaCl, cm^{-1}): 2361(w), 1709 (m), 1650 (s), 1514 (m), 1427 (m). ^1H NMR ($CDCl_3/CD_3OD$): δ 7.49 (s, 1H), 7.32–7.26 (t, 1H), 7.17–7.13 (d, 1H), 7.02–6.97 (m, 2H), 6.66 (s, 1H), 3.99 (s, 3H), 3.93 (s, 3H). MS (*m*/*z*, FAB): 315($[M+H]^+$).

3 - (4 - Methoxyphenyl) - 6 - chloro - 2,4 - quinazolinedione (A6B2). Yield 15 mg (45%), mp 287–288, IR (NaCl, cm^{-1}): 3442 (s, broad), 2355 (w), 1731 (m), 1651 (s), 1511(m). ^1H NMR($CDCl_3/CD_3OD$): δ 8.09–8.08 (s, 1H), 7.60–7.56 (d, 1H), 7.21–7.01 (m, 5H), 3.86 (s, 3H). MS (*m*/*z*, FAB): 303 ($[M+H]^+$).

3 - (3 - Hydroxyphenyl) - 6 - chloro - 2,4 - quinazolinedione (A6B5). Yield 3 mg (9%), oil, ^1H NMR ($CDCl_3/CD_3OD$): δ 8.03–8.02 (s, 1H), 7.67–7.63 (d, 1H), 7.33–7.30 (t, 1H), 7.23–7.20 (d, 2H), 6.93–6.89 (d, 1H), 6.76–6.73 (m, 2H). MS (*m*/*z*, FAB): 289 ($[M+H]^+$).

3 - (2 - Hydroxyphenyl) - 6 - chloro - 2,4 - quinazolinedione (A6B6). Yield 6 mg (19%), mp over 300 °C, IR (NaCl, cm^{-1}): 2363 (w), 1715 (m), 1661 (s), 1434 (m), 1374 (w), 1276 (m). ^1H NMR ($CDCl_3/CD_3OD$): δ 8.06–8.05 (s, 1H), 7.64–7.58 (m, 1H), 7.30–7.27 (t, 1H), 7.21–7.13 (m, 2H), 7.02–6.94 (m, 2H). MS (*m*/*z*, FAB): 289 ($[M+H]^+$).

3-(4-Ethoxyphenyl)-6-chloro-2,4-quinazolinedione (A6B7). Yield 10 mg (29%), mp 297–299 °C, IR (NaCl, cm^{-1}): 2918 (w), 2361 (m), 1727 (m), 1669 (m), 1476 (w, multiple). ^1H NMR ($CDCl_3/CD_3OD$): δ 8.13–8.12 (s, 1H), 7.59–7.54 (d, 1H), 7.20–7.16 (m, 2H), 7.04–6.94 (m, 3H), 4.12–4.04 (q, 2H), 1.47–1.42 (t, 3H). MS (*m*/*z*, FAB): 317($[M+H]^+$).

Conventional synthesis of 6A1B2

To anthranilic acid (500 mg, 3.65 mmol) in H_2O/THF (30:7 v/v) 37 mL K_2CO_3 (757 mg, 5.48 mmol) was added. The reaction mixture was cooled to 0 °C and carbobenzoxy chloride (0.677 mL, 4.74 mmol) was added and the mixture was stirred at rt for 2 h. After extraction with ether at pH 6, the water layer was acidified to pH 1 with 1 N HCl and extracted with ethyl acetate. Evaporation of solvent in vacuo gave compound **7** in 61% (600 mg). IR (NaCl, cm^{-1}) 3300–2500, 1700. ^1H NMR δ 10.33 (s, 1H), 8.5 (d, 1H), 8.10 (q, 1H), 7.59–7.07 (m, 9H), 5.24 (s, 2H).

TEA (1.23 mL, 3.0 mmol) was added to a solution of compound **7** (500 mg, 1.85 mmol), *p*-anisidine (250 mg, 2.04 mmol), HOBt (516 mg, 1.3 mmol) and EDCI (846 mg, 1.5 mmol) in DMF (150 mL). The reaction mixture was stirred at rt for 5 h and the solvent was evaporated to 5 mL in vacuo. Water (pH 4–5) and ethyl acetate (15 mL each) were added. The ethyl acetate layer was washed with saturated sodium bicarbonate and purified by chromatography (silica gel, *n*-hexane/ethyl acetate 2:1) to give compound **8** in 97% yield (790 mg). IR (NaCl, cm^{-1}) 3320, 1734. ^1H NMR δ 10.26 (s, 1H), 8.35 (d, 1H), 7.84–6.73 (m, 13H), 5.18 (s, 2H), 3.81 (s, 3H).

A solution of amide **8** (60 mg, 0.16 mmol) in 60 mL methanol was added TEA (0.4 mL, 1.6 mmol) and the mixture was heated to 60 °C for 24 h. The solvent was evaporated in vacuo and the residue was purified by chromatography (silica gel, *n*-hexane/ethyl acetate). **6A1B2** was obtained in 94% yield (40 mg). The cyclized product was also obtained by heating: amide **8** (50 mh, 0.13 mmol) in DMF (5 mL) was refluxed for 16 h. The ethyl acetate and 1N NaOH were added. The organic layer was washed with water, dried and evaporated. The residue was purified by column chromatography (silica gel, CH_2Cl_2/CH_3OH) to give **6A1B2** in 95% yield.

Sulforhodamine B(SRB) assay[8,9]

Human lung cancer cell line (A 549) and colon carcinoma cells (Col2) were obtained from University of Illinois, Department of Surgical Oncology. The cultured cells were harvested, counted, and diluted to 5×10^4 cells/well with fresh medium, and added to 96-well microtiter plates containing various concentrations of test materials (10 μl in 10% aqueous DMSO). Test plates were incubated for 3 days at 37 °C in a CO_2 incubator. The zero-day control was incubated at 37 °C for 30 min. All treatments were performed in triplicate. After the incubation periods, cells were fixed by addition of 50 μl of cold 50% aqueous trichloroacetic acid, washed 4-5 times with tap water, and air-dried. The fixed cells were stained for 1 h with 0.4% SRB dissolved in 1% acetic acid. After solubilizing the bound dye with 10 mM tris-base, the absorbance at 515 nm was measured with ELISA reader.

Topoisomerase I/II inhibition test[12]

Purified human topoisomerase I and topoisomerase II were obtained from TopoGEN, Inc. (Columbus, OH, USA). Camptothecin and etoposide were used as positive control for topoisomerase I and II, respectively. The cleavage reactions contained 0.3 μg of pBS and and clevage buffer (30 mM Tris–HCl, pH 7.6, 60 mM KCl, 8 mM $MgCl_2$, 15 mM 2-mercaptoethanol, 3 mM ATP, 30 μg/mL bovine serum albumin) in a total volume of 20 μL. Test samples (100 μM) were added and the reac-

tions were initiated by adding 8 units of purified human topoisomerase I or II. After incubation for 30 min at 37 °C, the cleavage complexes were trapped by addition of 2 μL of 10% SDS followed by topoisomerase digestion with protein kinase for 30 min at 56 °C. The reaction products were purified with phenol/chloroform extraction and electrophoresed on 1.2% agarose gel containing 0.5 μg/mL ethidium bromide for 2 h at 0.25 V/cm. The amount of DNA product was quantified by densitometric analysis using Eagle Eye II (Stratagene).

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