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Design of Quinolinedione-Based Geldanamycin Analogues

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Abstract—Quinoline-5,8-dione-based compounds were designed from the structure of the geldanamycin-bound Hsp-90 active site. The active site model predicted that aromatic substituents should be present at the 2-position, to take advantage of a hydrophobic pocket, and amino substituents should be present at the 6- or 7-position. COMPARE analysis revealed that the LC_{50} profile of 2-phenyl-6-(2-chloroethylamino)quinoline-5,8-dione has the highest geldanamycin-like activity (0.74 correlation coefficient).

The term, 'heat shock protein,' comes from the initial observation that this chaperone protein is elevated in the presence of heat stress. Actually, any stress can cause Hsp-90 elevation so as to process signaling proteins important for cell division. The cancer cell is in fact under a great deal of stress due to hypoxia, nutrient deprivation, chemotherapy, and radiation. Thus, various histological cancers types in fact possess elevated levels of Hsp-90 including leukemias, lymphomas, as well as androgen- and estrogen-dependent cancers (prostrate and breast).[1–3] Heat shock protein (Hsp-90) is a relatively new target for cancer chemotherpy and therefore has been the subject of intense drug design efforts.[1,4,5] The prototype inhibitor of Hsp-90 is geldanamycin shown in the inset of Figure 1. This complex natural product has been the subject of total synthesis[6,7] and analogue development.[8] In addition, the synthesis and screening of purine libraries has afforded small molecules capable of geldanamycin-like binding to HSP-90.[9–11] This laboratory approached HSP-90 inhibitor design by molecular modeling and library synthesis. The quinoline quinone analogue shown in Figure 1 was designed from the HSP-90 crystal structure.[12]

The preparation of the target compounds was initiated with the Friedlander quinoline synthesis, Scheme 1. Thus, the reaction of 2-amino-3,6-dimethoxybenzaldehyde[13] with the appropriate ketones afforded substituted-5,8-dimethoxyquinolines. Oxidative demethylation of the substituted 5,8-dimethoxyquinolines, to produce substituted quinoline-5,8-diones, was effected in high yields with ceric ammonium nitrate.[14] Reaction of the quinoline-5,8-diones with aziridine in ethanol[15] produced both 6- and 7-aziridinylquinoline-5,8-diones, with the 6-aziridinyl isomer being produced in much higher yield.[16] Treatment of the quinoline-5,8-diones with aziridine for long periods of time (several hours) could result in diaziridination based of previous reports.[17]

The proton NMRs of the 6- and 7-aziridinyl isomers were indistinguishable and the isomers had to be identified by HMBC and HMQC NMR experiments. The 6-aziridinyl-isomer showed a HMBC 3-bond correlation between both the 4- and 7-protons and the 5-carbon. The 7-aziridinyl-isomer showed a HMBC 3-bond correlation between the 4-proton and the 5-carbon and the 6-proton and the 8-carbon. Therefore, the 6- and 7-isomers can be distinguished by HMBC 3-bond correlation

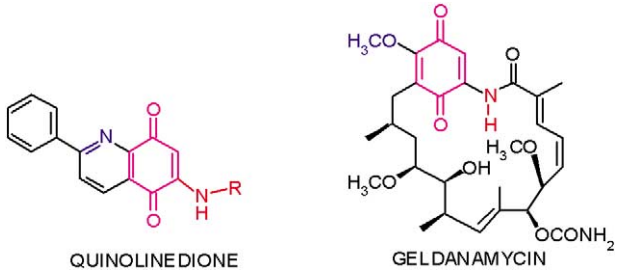

Figure 1. The colors shown in the quinoline structure and the corresponding colors in the geldanamycin structure reflect overlapping binding sites in the active site of Hsp-90.

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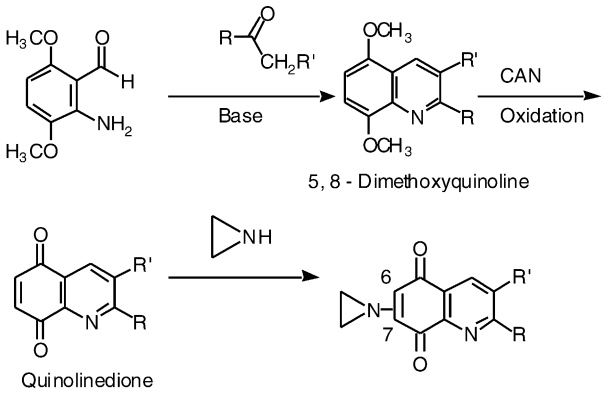

Scheme 1. Preparation of aziridinated quinolinediones.

between the quinone proton of the product and either one carbon (6-isomer) or two carbons (7-isomer).

The aziridinyl group was present as a possible alkylating center of the Hsp-90 active site as well as a starting point for other analogues that could be obtained by ring opening with a variety of nucleophiles. Currently, libraries of a variety of 6- and 7-substituted quinolinedienes are being prepared and screened. In this report the results for two aziridinyl ring opened analogues, 6-(2-hydroxyethyl and 6-(2-chloroethyl), are presented. A list of compounds discussed in this communication are found in Table 1.

Cytotoxic and Cytostatic Evaluation

Provided in Table 2 are the cytostatic/cytotoxic parameters and cancer specificity for the compounds in Table 1. These in vitro data were obtained under the In Vitro Cell Line Screening Project at the National Cancer Institute.[18,19] The cytostatic parameters shown in Table 2 include GI_{50} and TGI, which are the concentrations of drug required for 50% growth inhibition and total growth inhibition, respectively. The cytotoxic parameter is the LC_{50}, which is the concentration required for 50% cell kill. The log mean values for GI_{50}, TGI, and LC_{50} in 60 cell lines are provided in Table 2 along with the log delta value (the maximum sensitivity in excess of the mean) and the log range (the maximum difference between the least sensitive and the most sensitive cell lines). These parameters have provided insights into selectivity and potency of antitumor agents in previous studies.[20] Large values of the delta and range indicate high selectivity for some histological cancers over others. Small negative median values (−5 to −6) along with small delta and range values indicate an inactive compound.

Table 1. Quinolinedione analogues prepared and screened in this study

Position				Compd
2	3	6	7	
Phenyl	H	Aziridinyl	H	**1**
Phenyl	H	H	Aziridinyl	**2**
2-Pyridyl	H	Aziridinyl	H	**3**
2-Naphthyl	H	Aziridinyl	H	**4**
2-Naphthyl	H	H	Aziridinyl	**5**
Phenyl	H	$NHCH_2CH_2OH$	H	**6**
Phenyl	H	$NHCH_2CH_2Cl$	H	**7**

The results shown in Table 2 led to the following requirements for optimal activity: (1) Groups no bigger than phenyl substituted at the 2-position. (2) Minimized structures must have the 2-substituent planar with the quinoline ring. (3) The aziridinyl group should be present at the 6-position for optimal cytostatic activity. The first generalization was made based on comparing the cytostatic/cytotoxic parameters of **1**–**2** with those of **4**–**5**. The increase in size from phenyl to naphthyl results in an increase in the median log GI_{50} and TGI parameters (i.e., a decrease in potency). A decrease in cytostatic and cytotoxic activity accompanies the replacement of the phenyl of **1** with a 2-pyridyl substituent of **3**. A minimized structure of **3** revealed that repulsion between the nitrogen lone pairs twists the 2-pyridyl ring out of the plane of the quinazoline ring. In contrast, the phenyl group of **1** is coplanar with the quinolinedione ring. Finally, the data in Table 2 show that the 6-aziridinyl analogues are consistently more active than 7-aziridinyl analogues.

The aziridinyl ring can undergo acid-catalyzed ring opening utilizing either water or chloride present in the buffered solution as nucleophiles.[21] When **1** was screened again ~6 months later by the National Cancer Institute, its cytostatic/cytotoxic parameters changed substantially (new parameters listed under **7** in Table 2). One notable change was the high selectivity toward leukemia cell lines with cytostatic/cytotoxic parameters in the range of $<10^{-8}$ M. This observation prompted screening of the ring opening of the aziridinyl ring opened analogues **6** and **7**. The absence of activity

Table 2. Cytostatic and cytotoxic data obtained from the National Cancer Institute's 60 cancer cell line pane

Compd	GI_{50}			TGI			LC_{50}		
	Median	Delta	Range	Median	Delta	Range	Median	Delta	Range
1	−7.28	0.72	2.18	−6.29	1.71	4.00	−5.39	1.56	2.98
2	−6.58	1.42	2.87	−5.75	1.82	3.27	4.95	1.36	2.02
3	−6.19	1.28	1.87	−5.61	0.88	2.50	−5.01	0.72	1.73
4	−6.69	1.31	2.38	−5.97	1.47	2.22	−5.25	0.88	2.13
5	−5.46	1.34	2.11	−4.97	1.08	2.05	−4.39	0.95	1.34
6	−5.53	2.47	4.00	−4.70	1.49	2.20	−4.22	1.32	1.53
7	−7.56	0.44	2.07	−6.49	1.51	2.47	−5.69	2.31	4.00

observed for the 6-(2-hydroxyethyl) derivative **6** indicates that an alkylating center, either aziridinyl or chloroethylamino, is required for optimal activity.

COMPARE analysis[18] revealed that the LC_{50} profile of **7** correlates well with that of a geldanamycin analogue, NSC-255105, shown in Figure 2 (0.74 correlation coefficient). COMPARE analysis of the LC_{50}, GI_{50}, and TGI profiles of **7** against the 'molecular targets' database revealed good correlations with a variety of tyrosine kinases including cdk2 and cdk5. There was no correlation with levels of the two-electron reducing enzyme DT-diaphorase in the cancer panel indicating reductive activation of the quinone system is not necessary. This enzyme is involved in the reductive activation of aziridinyl quinones as alkylating agents.[20,22] Thus, the structure of NSC-255105 reflects some aspects of the quinolinedione structure–activity relationship outlined above. Thus a planar 'phenyl group' is present in NSC-255105. The COMPARE results merely indicate that both compounds share a common cytotoxic mechanism. The next section addresses the role of Hsp-90 inhibition in quinolinedione cytotoxicity.

Hsp90/Chaperone Assays

The quinolinediones listed in Table 1 were evaluated as Hsp90/chaperone active agents using the following assays: (1) Competition for binding to C-terminal ATP-binding site on Hsp-90 using immobilized novobiocin.[23] (2) Competition for binding to N-terminal ATP-binding site on Hsp-90 using immobilized geldanamycin.[24] 3. Heat shock response in fibroblasts stable transfected with GFP reporter construct.[25] 4. Inhibition of luciferase re-folding in rabbit reticulocyte.[26] None of the compounds shown in Table 1 were active in these assays when tested at concentrations of 20 and 200 μM.

The rational design of Hsp-90 inhibitors using the structure of ATP-active-site-bound geldanamycin afforded two cytostatic/cytotoxic compounds **1** and **7**. Both compounds possess an alkylating center at the 6-position, but do not require reductive activation of the quinone ring for activity. Although COMPARE analysis revealed geldanamycin-like cytostatic and cytotoxic activity, none of the compounds prepared in this study actually interact with Hsp-90 or interfere with protein renaturation. An explanation for this observation is that not all geldanamycin analogues, such as NSC-255105 in Figure 2, target Hsp-90. The molecular targets of **1** and **7** are as yet unknown, but the cdks are the likely targets based on COMPARE studies.

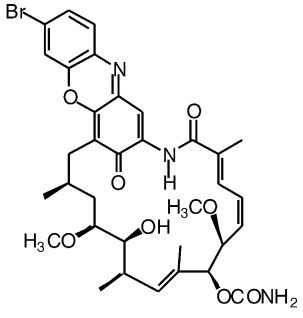

Figure 2. Structure of NSC 255105, a geldanamycin analogue in the National Cancer Institute's archives.

Acknowledgements

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14. To a stirred solution of 2-substituted-5,8-dimethoxyquinoline (2.83 mmol) in acetonitrile (50 mL) was added drop wise a solution ceric ammonium nitrate (12.0 mmol) in water (50 mL). After 45 min the reaction mixture was extracted with dichloromethane (3×50 mL). The combined organic layers were washed with water (50 mL), dried (Na_2SO_4) and the solvent removed in vacuo to yield a yellow solid.
15. To a stirred slurry ofthe quinolinedione (0.85 mmol) in methanol (10 mL) was added aziridine (2.54 mmol). After 2 h the solvent was removed in vacuo and the resulting solid was purified by flash chromatography (SiO_2, chloroform) to yield a yellow solid. This mixture of the two isomers was separated by prep-TLC (SiO_2, chloroform).
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26. **Physical properties**. **6-Aziridinyl-2-phenylquinoline-5, 8-dione (1)**: mp 183 °C dec.; TLC (chloroform/methanol [19:1]) R_f 0.68; IR (KBr disc) 1676, 1654, 1578, 1468, 1445, 1382, 1309, 1273, 1133, 1094 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.46 (d, $J=8.5$ Hz, 1H), 8.17 (m, 2H), 8.05 (d, $J=8.5$ Hz, 1H), 7.52 (m, 3H), 6.47 (s, 1H), 2.34 (s, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.01, 181.15, 162.04, 157.33, 147.89, 137.47, 135.33, 130.68, 128.97, 127.80, 126.93, 123.32, 120.11, 27.76; MS 276 (M^+), 249, 221, 193, 182, 153, 102. Anal. calcd ($C_{17}H_{12}N_2O_2$): C, 73.90; H, 4.38; N, 10.14. Found: C, 73.61; H, 4.46; N, 10.09.

7-Aziridinyl-2-phenylquinoline-5,8-dione (2): mp 185 °C dec.; IR (KBr disc) 1676, 1654, 1578, 1309, 1273, 1133, 1094 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.44 (d, $J=8.2$ Hz, 1H), 8.18 (m, 2H), 8.08 (d, $J=8.2$ Hz, 1H), 7.52 (m, 3H), 6.36 (s, 1H), 2.36 (s, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.67, 179.89, 161.34, 158.38, 147.32, 137.47, 135.09, 130.57, 129.01, 127.67, 127.63, 124.09, 118.67, 27.89; MS 276 (M^+), 275, 248, 193, 102. Anal. calcd ($C_{17}H_{12}N_2O_20.25H_2O$): C, 72.72; H, 4.49; N, 9.98. Found : C, 72.48; H, 4.40; N, 9.88.

6-Aziridinyl-2-(2-pyridyl)quinoline-5,8-dion (3). (130 mg, 38%); mp 202–204 °C dec.; TLC (chloroform/methanol [19:1]) R_f 0.61; IR (KBr disc) 1672, 1602, 1582, 1318, 1264, 1131 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.79 (d, $J=8.2$ Hz, 1H), 8.71 (m, 2H), 8.53 (d, $J=8.2$ Hz, 1H), 7.88 (dt, 1H), 7.40 (m, 1H), 6.49 (s, 1H), 2.35 (s, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) 182.97, 181.15, 160.55, 157.43, 154.20, 149.36, 147.55, 137.18, 135.48, 128.20, 125.12, 124.15, 122.95, 120.13, 27.79; MS 277 (M^+), 248, 222, 194, 179. Anal. calcd ($C_{16}H_{11}N_3O_2·0.1H_2O$): C, 68.86; H, 4.04; N, 15.06. Found: C, 68.72; H, 3.99; N, 15.07.

6-Aziridinyl-2-(2-naphthyl)quinoline-5,8-dione (4): mp 179–180 °C dec.; TLC (ethyl acetate/hexanes [1:1]) R_f 0.25; IR (KBr disc) 1638, 1598, 1578, 1305, 1142, 1006 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.47 (d, $J=8.5$ Hz, 1H), 8.31 (dd, 1H), 8.23 (d, $J=8$ Hz, 1H), 7.99 (m, 2H), 7.89 (m, 1H), 7.56 (m, 2H), 6.38 (s, 1H), 2.37 (s, 4H); MS 326 (M^+), 325, 298, 241, 152. Anal. calcd ($C_{21}H_{14}N_2O_2$): C, 77.29; H, 4.32; N, 8.58. Found: C, 77.04; H, 4.27; N, 8.45.

7-Aziridinyl-2-(2-naphthyl)quinoline-5,8-dione (5): mp 206–207 °C dec.; TLC (ethyl acetate/hexanes [1:1]) R_f 0.18; IR (KBr disc) 1676, 1578, 1310, 1264, 1133, 1099 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.51 (d, $J=8$ Hz, 1H), 8.30 (dd, 1H), 8.21 (d, $J=8.5$ Hz, 1H), 8.01 (m, 1H), 7.98 (d, $J=9$ Hz, 1H), 7.89 (m, 1H), 7.55 (m, 2H), 6.50 (s, 1H), 2.36 (s, 4H); MS 326 (M^+), 299, 271, 242, 227, 203, 152. Anal. calcd ($C_{21}H_{14}N_2O_2$): C, 77.29; H, 4.32; N, 8.58. Found: C, 77.17; H, 4.26; N, 8.42.

6-(2-Hydroxyethylamino)-2-phenylquinoline-5,8-dione (6) was prepared by treating the 2-phenylquinoline-5,8-dione with ethanolamine in ethanol: mp 218–219 °C dec.; TLC (chloroform/methanol [9:1]) R_f 0.26; IR (KBr disc) 3405, 3333, 1677, 1607, 1573, 1524, 1338, 1251, 1129, 1066 cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 8.39 (d, $J=8.4$ Hz, 1H), 8.29 (d, $J=8.4$ Hz, 1H), 8.26 (m, 3H), 7.56 (m, 2H), 7.42 (t, $J=5.4$ Hz, 1H), 5.90 (s, 1H), 4.88 (t, $J=5.7$ Hz, 1H), 3.62 (m, 2H), 3.28 (m, 2H); MS (MALDI TOF) 295 ($M+H^+$). Anal. calcd ($C_{17}H_{14}N_2O_3·0.25H_2O$): C, 68.33; H, 4.89; N, 9.37. Found: C, 68.42; H, 4.74; N, 9.39.

6-(2-chloroethylamino)-2-phenylquinoline-5,8-dione (7). To a solution of 6-aziridinyl-2-phenylquinoline-5,8-dione (20 mg, 0.072 mmol) in ethanol (10 mL) was added four drops of 4 M HCl. The solution was sonicated for one min and then stirred for 10 min. The solution was then filtered and the solid washed with ice-cold ethanol. The solid was then crystallized from chloroform/hexanes to yield dark red crystals: (16 mg, 71%); mp 197–198 °C dec.; TLC (chloroform/ethyl acetate [6:4]) R_f 0.38; IR (KBr disc) 3195, 3018, 1675, 1608, 1577, 1512, 1458, 1344, 1292, 1246, 1116 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.43 (d, $J=8.2$ Hz, 1H), 8.20 (m, 2H), 8.02 (d, $J=8.2$ Hz, 1H), 7.52 (m, 3H), 6.17 (t, $J=6$ Hz, 1H), 5.98 (s, 1H), 3.78 (t, $J=5.7$ Hz, 2H), 3.62 (q, $J=6$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$); MS (MALDI TOF) 313, 315 ($M+H^+$). Anal. calcd ($C_{17}H_{13}ClN_2O_2$): C, 65.29; H, 4.19; N, 8.96. Found: C, 65.25; H, 4.16; N, 8.94.