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Bioorganic & Medicinal Chemistry Letters 16 (2006) 1156–1159
Bioorganic & Medicinal Chemistry Letters

Structure–activity studies of quinuclidinone analogs as anti-proliferative agents in lung cancer cell lines

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Received 4 October 2005; revised 23 November 2005; accepted 28 November 2005
Available online 19 December 2005

Abstract—We have synthesized and tested novel quinuclidinone analogs to assay the effects on H1299 lung cancer cell lines alone or with γ-radiation. We have found two series of quinuclidinone analogs that act as anti-cancer agents. Of these, four interesting analogs significantly decreased cell viability in H1299 lung cancer cell lines. Two derivatives decreased cell proliferation in a dose-dependent fashion alone or in the presence of γ-radiation. Radiosensitization increased when derivative treatment preceded radiation treatment for both derivatives. These preliminary studies show an evidence for both additive and synergistic cytotoxicity for treatment of lung cancer by these novel quinuclidinone analogs.

Tumors are complex collections of cells that respond to therapies differently depending on the organ site and type of cells. Chemotherapeutic agents are developed through empirical screens to identify compounds or molecules that kill cancer cells. Most cytotoxic agents used to treat cancer act by inducing apoptosis.[1,2]

The combination of radiotherapy and chemotherapy is a unique and effective therapy. The role of radiotherapy is to control the primary tumor, while chemotherapy can be used to diminish distant metastases.[3,4] Some chemotherapeutic drugs destroy tumor cells directly by their own cytotoxic action but may also enhance the effects of radiotherapy. Many studies show the advantages of combining radiotherapy with chemotherapy, both in terms of better local control of cancer and metastasis prevention.[5–7]

Given the toxicity, development of resistance, and lack of broad spectrum treatments, there is a continuing need for the development of new chemotherapeutic agents for the treatment of cancer. There has been an effort to develop small molecules that restore function to mutant p53, a tumor suppressor gene frequently mutated in a wide variety of cancers. Several examples of such small molecules have been reported. The pyrimidine derivative **1** is reported to both stabilize wild-type p53, as well as to restore function to mutant p53.[8–10] 2-Methoxyestradiol (**2**) facilitates wild-type p53-mediated apoptosis.[11] More recently, a series of α-methylene ketones (e.g., **3**) were identified as having good anti-proliferative activity against p53 mutant cell types.[12]

The quinuclidinone derivative **4** is also reported to induce apoptosis in a number of cell types.[13,14] This quinuclidinone also restores the active conformation of mutant p53. Original reports suggested **4** induced conformational change in p53. However, our study has indicated that this compound decreases cell proliferation and induces apoptosis in a p53-independent mechanism. The quinuclidinone **4** was identified through the screening of a library of low molecular weight molecules.[14] Consequently, no structural activity studies have been reported for this intriguing molecule.

The quinuclidine ring system itself is a common structural element of a number of pharmacologically active small molecules, especially cholinergic ligands. In general, these ligands lack the 3-keto group seen in **4**.[15,16] This lack of a carbonyl group at C-3 has significant implications for the basicity of the tertiary amine. The pK_a of the conjugate acid of quinuclidine is 11.4, while that of

Keywords: Quinuclidinone; Anti-cancer; Radiation.
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doi:10.1016/j.bmcl.2005.11.085

the conjugate acid of quinuclidinone is only 7.5.[17] These combined differences may account for the differences in activity between the two classes of compounds.

Given the need to develop new chemotherapeutic agents with an improved safety profile, analogs of molecules such as **4** should provide an excellent entry into novel anti-cancer agents. These molecules should have a selective mode of action, they are structurally unique, and yet have a great deal of known chemistry upon which to prepare analogs.

We were particularly interested in molecule **4** as a starting point for the synthesis of novel anti-cancer agents. Given the simple structure of **4**, a large number of analogs could reasonably be proposed. In an initial series of compounds, we chose to ask the following questions: (1) is the diol necessary or could other functional groups suffice, that is, is a H-bond acceptor or H-bond donor (or both) necessary for activity, and (2) is the carbonyl needed, and how might that influence the pK_a of the amine.

The commercially available quinuclidinone **5** was converted to the diol **4** by two successive aldol condensations.[18] With key diol in hand, three modifications to this structure were carried out. The ketone was reduced to the triol **6**, using $LiAlH_4$. The diol was converted to the diacetate **7** in excellent yield using excess acetyl chloride. The diol was also converted to a series of acetals by condensations with the appropriate carbonyl compound. The yields for the acetal formation ranged from 20 to 40%. Significantly, both the initial acetals prepared showed similar or improved activities relative to the parent compound **4**. We thus chose to prepare an additional set of acetals (**8c**–**8f**) using the same methods.[19] Compounds **8a**, **8d**–**8f** appear to be a single isomer at the anomeric carbon by ^{1}H NMR (Fig. 1) (Scheme 1).

Cell viability was measured via the methyl tetrazolium (MTT) bromide mitochondrial activity assay described previously.[20] The synthesized compounds decreased cell survival and cell survival was further decreased if given in conjunction with γ-radiation (Fig. 2).

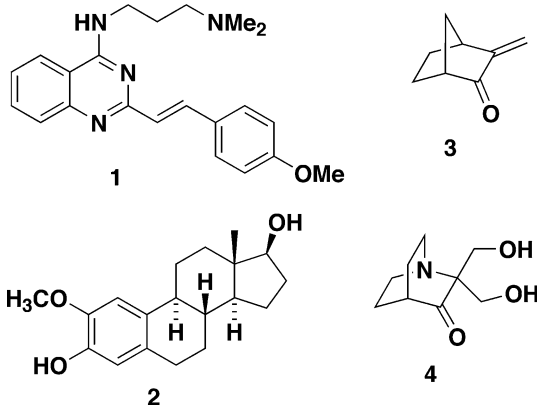
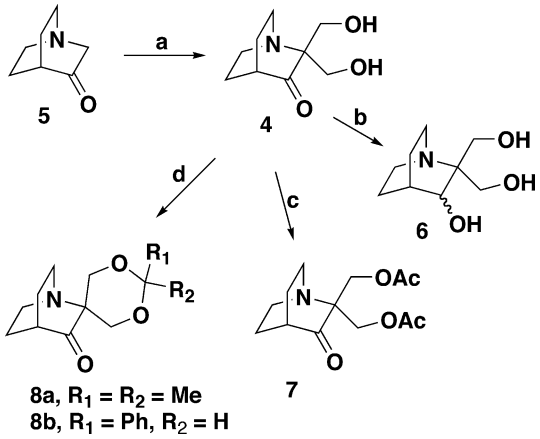


Figure 1. Small molecules reported to restore the function to p53.



Scheme 1. Reagents and conditions: (a) 34% aq HCHO, K_2CO_3, 50 °C, 45%; (b) $LiAlH_4$ (500 mol %), 0 °C, 60%; (c) AcCl, DMAP, pyridine, 96%; (d) **8a**, R_1=H, R_2=Ph, PhCHO, *p*TSA, Δ, 23%; **8b**, R_1=R_2=CH_3, $(H_3C)_2C(OCH_3)_2$, pTSA, Δ, 33%; **8c**, R_1=R_2= $–CH_2(CH_2)_3CH_2–$, cyclohexanone, $BF_3{\cdot}OEt_2$, $HC(OEt)_3$, 20%; **8d**, R_1=H, R_2=*c*-C_6H_{11}, *c*-C_6H_{11}CHO, *p*-TSA, Δ, 34%; **8e**, R_1=H, R_2=CH_2CH_2Ph, $PhCH_2CH_2CHO$, $BF_3{\cdot}OEt_2$, 31%; **8f**, R_1=H, R_2=3,4-Cl_2Ph, 3,4-Cl_2PhCHO, $BF_3{\cdot}OEt_2$, 36%; **8g**, R_1=H, R_2= 4-(OMe)Ph, 4-(OMe)PhCHO, $BF_3{\cdot}OEt_2$, 21%.

As a control, we tested the starting quinuclidinone **5** in the MTT assay.[21] As expected, this compound showed no activity. The initial lead compound **4** showed excellent activity with less than 10% of the cells remaining viable. Compound **6**, in which the carbonyl group has been reduced, showed complete loss of activity. There are two possibilities to account for this observation. The first is that the hydroxyl, while able to engage in similar types of non-covalent contacts, is no longer in an appropriate position. Alternatively, the lower basicity of the quinuclidinones (as compared to quinuclidine) is important for activity.

The diacetate **7** showed similar activity to the lead compound **4**, indicating that the free hydroxyls were not necessary. As part of our study to determine the importance of the hydroxyls, we initially prepared two acetals (**8a**, R_1=H, R_2=Ph, and **8b**, R_1=R_2=CH_3). Both these compounds showed improved activity relative to the parent diol **4**. Based upon these results we prepared five additional acetals as analogs of the benzylidine (**8a**) and acetonide (**8b**) derivatives. The acetal derived from cyclohexanone (**8c**) showed decreased activity relative to both the parent acetonide (**8b**) as well as the diol. This may indicate a lack of tolerance to steric bulk around the acetal ring. We prepared the acetal of cyclohexane carboxaldehyde (**8d**) in order to determine if the aromatic ring of **8a** was essential. We observed a lowering of activity relative to **8a**. Compound **8e** was prepared using 3-phenylpropionaldehyde. Our intention was to determine if the aromatic ring could be moved away from the acetal and yet still retain activity, clearly this compound is somewhat less potent than the benzylidine **8a**, but still as potent as the diol. Compounds **8f** and **8g** were prepared to explore the effects of substitution on the aromatic ring. The 3,4-dichloro derivative lost some potency, but the 4-methoxy derivative lost almost all activity.

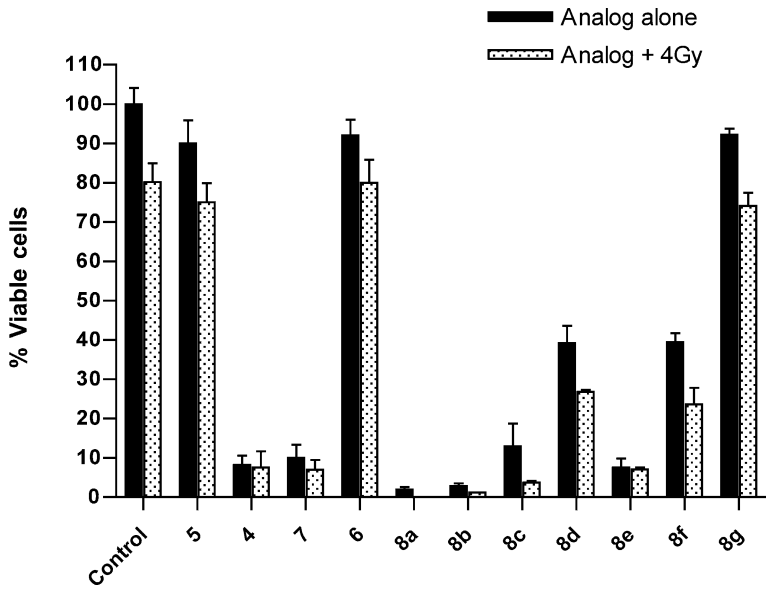


Figure 2. Effect of quinuclidinone derivatives on cell viability of H1299 cells (null for p53) in the presence and absence of 4 Gy of γ-radiation. Each data point is an average of three independent experiments and expressed as $M \pm$ SD. All experiments were carried with a 4 mM concentration of quinuclidinone analogs.

The compounds that showed the greatest effect on cell viability, **8a** and **8b**, were chosen for further analysis. Concentrations of **8a** and **8b**, ranging from 20 to 1000 μM, were prepared and dissolved in complete Dulbecco's modified essential media. H1299 cells that have a deletion of the p53 gene were treated with the compound alone, or in combination with 4 Gy of γ-radiation (IR) were assayed for cell survival. Compounds **8a** and **8b** decreased the percent of cell survival in a dose-dependent fashion both in the absence and presence of γ-radiation (Fig. 3). The GI_{50} for all compounds were relatively similar. Compounds **8a** and **8b** alone had GI_{50}s of 17.26 μM and 19.21 μM. In the presence of γ-radiation, the GI_{50}s decreased with compound **8b** + 4Gy being slightly more potent (4.74 μM) than **8a** + 4Gy (4.49 μM). Similar results were obtained from dose–response curves of the same concentrations of **8a** and **8b** in the presence of ultraviolet radiation (10 J/m^2) (data not shown).

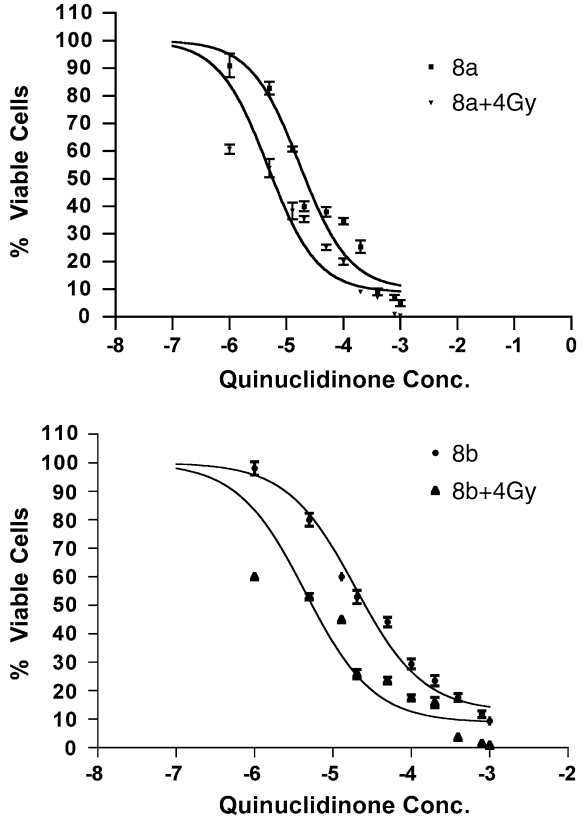


Figure 3. Dose–response curves of **8a** and **8b** in H1299 cells alone or in the presence of 4 Gy γ-radiation. Each data point is an average of three independent experiments and expressed as $M \pm$ SD.

γ-irradiation (IR) is a local treatment, focusing on a specific area of the body, while chemotherapy is systemic, attacking cancer by treating the whole body. There continues to exist a need for new anti-cancer agents with improved safety profiles, specifically those that have a reduced liability for resistance and unwanted side effects. In this study, we synthesized new acetals and di-acetates that were potent in decreasing cell viability. These analogs will enable us to examine important structural determinants of small molecule macromolecular interactions for anti-cancer activity. The discovery of a class of highly selective and potent quinuclidinones will lead to development of new anti-cancer drugs. Additional experiments are required to determine the mechanism of action of these new quinuclidinone derivatives as well as to fully elucidate the structure–activity relationships of this class of molecules.

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21. *Cell culture and drug treatment*: H1299 cells that have a deletion of the p53 gene were derived from a human large cell lung carcinoma. Cells were maintained in Dulbecco's modified essential media (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a 5% CO_2 atmosphere.
Cell proliferation (*MTT assay*): 4000–5000 cells/well in 100 μL of medium were seeded in a 96-well plate for 24 h prior to drug treatment. The media were then changed to media with analogs, and cells were treated with either γ- or UV-radiation. At the end of the incubation (24 h), 10 μL of 5 mg/mL MTT reagent (ATCC) was added to each well for 4 h. After incubation, 100 μL of detergent reagent was added to each well to dissolve the formazan crystals. The absorbance was determined at 570 nm. Assays were performed in triplicate and standard error determined. GI_{50} values were obtained by averaging values generated from non-linear regression analyses (Prism, GraphPad, San Diego, CA) of individual concentration–response curves.