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Synthesis, characterization and antineoplastic activity of bis-aziridinyl dimeric naphthoquinone – A novel class of compounds with potent activity against acute myeloid leukemia cells

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ABSTRACT

The synthesis, characterization and antileukemic activity of rationally designed amino dimeric naphthoquinone (BiQ) possessing aziridine as alkylating moiety is described. Bis-aziridinyl BiQ decreased proliferation of acute myeloid leukemia (AML) cell lines and primary cells from patients, and exhibited potent (nanomolar) inhibition of colony formation and overall cell survival in AML cells. Effective production of reactive oxygen species (ROS) and double stranded DNA breaks (DSB) induced by bis-aziridinyl BiQ is reported. Bis-dimethylamine BiQ, as the isostere of bis-aziridinyl BiQ but without the alkylating moiety did not show as potent anti-AML activity. Systemic administration of bis-aziridinyl BiQ was well tolerated in NSG mice.

The clinical outcomes of patients with acute myeloid leukemia (AML) treated with available cytotoxic, targeted, and hematopoietic stem cell transplant therapy remains unsatisfactory with 3–8% survival at 5 years in patients aged 60 years and older and up to 50% in patients younger than 60 years of age.[1–3] This underscores an urgent need for development of novel, efficacious and well-tolerated therapeutic agents and strategies for treatment of AML. AML therapy is challenging because it is an extremely heterogeneous disease with various leukemogenic mutations and cytogenetic abnormalities with poorly understood interplay among them in each patient.[4,5] One solution to this issue may be to target a broader characteristic that is common among all AML cells but is sufficiently different from normal tissues. Growing evidence indicates that AML cells, compared to normal cells and irrespective of their genetic heterogeneity, have an increased susceptibility to the disruption of balance between pro- and anti-oxidant forces.[6]

Quinone moieties such as mitomycin-C and RH1[7] (benzoquinone),[8] daunorubicin, doxorubicin, idarubicin, epirubicin, aclarubicin and mitoxantrone (anthraquinone),[9] and atovaquone,[10] 2,2′-binaphthoquinones[11] and β-lapachone[12] (naphthoquinone[13]) are the most preclinically tested and clinically investigated anticancer agents frequently used for treatment of solid and hematologic neoplasms. The cytotoxic mechanisms of action of these compounds mainly include (i) formation of semiquinone radicals and quinone redox cycling resulting in initiation and propagation of intracellular free oxygen radical chain reactions[14], (ii) nicotinamide adenine dinucleotide phosphate (NADPH) depletion via interference with the enzyme NAD(P)H:Quinone Oxidoreductase 1 (NQO1)[15], (iii) nicotinamide adenine dinucleotide (NAD) depletion via hyperactivation of poly(ADP ribose) polymerase (PARP)[12], and (iv) inhibition of DNA topoisomerase-II.[16] Nevertheless, the tendency of neoplastic cells to become resistant to these agents has led to a constant effort for rational design of novel quinone-based anticancer agents that can overcome drug resistance in malignant cells.

We previously synthesized several dimeric naphthoquinone (BiQ) analogues with potent *anti*-integrase activity against human immunodeficiency virus (HIV),[17] and later reported their cytotoxic activity against prostate[18] and breast[19] cancer cells. Recently, we reported that hydroxylated dimeric, but not monomeric, naphthoquinones could inhibit clonogenicity and induce apoptosis in AML cell lines and primary cells from patients (IC_{50} 3–5 μM) with favorable therapeutic index compared to normal hematopoietic cells.[20]

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To improve the potency and bioavailability of this class of compounds, we decided to incorporate both alkylating agents and amine groups into the quinone cores of each naphthoquinone unit. The quinone moiety can perturb cellular redox balance and its oxidation state would modulate the activity of the alkylating moiety that can form covalent bonds with a different cellular components. To this end, we elected to synthesize a BiQ moiety substituted with aziridine(s) as a bifunctional alkylating compound that can be activated after bioreduction either by one- or two-electron reducing enzymes to form the corresponding aziridinyl hydroBiQ.[21] The electron rich hydroquinone moiety in hydroBiQ increases the electron density at nitrogen and changes the pK of the aziridine ring (s),[22] such that the nitrogen is protonated and is more susceptible to nucleophilic attack under physiological pH. The result is a naphthoquinone-based aziridinium ion that may alkylate DNA and other biomolecules. Here we report the synthesis, characterization and anti-AML activity of rationally designed bis-aziridine BiQ and compare it with bis-dimethylamine BiQ.

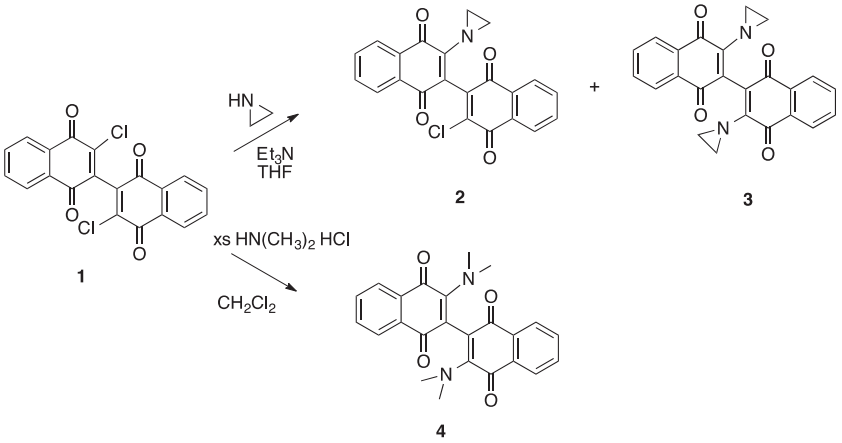
Amination of dichloro BiQ **1** was accomplished through treatment with limiting and excess amounts of aziridine to afford mono- and bis-aziridinyl (**2** and **3**) BiQ, or excess dimethylamine to afford bis-dimethylamino BiQ (**4**) (Scheme 1). Compound **2** (*3-(Aziridin-1-yl)-3′-chloro-[2,2′-binaphthalene]-1,1′,4,4′-tetraone*) and **3** (*3,3′-Di(aziridin-1-yl)-[2,2′-binaphthalene]-1,1′,4,4′-tetraone*) were isolated in 49% and 44% yield, respectively, by dropwise addition of aziridine to a solution of *3,3′-dichloro-[2,2′-binaphthalene]-1,1′,4,4′-tetraone* (**1**) in anhydrous tetrahydrofuran (THF) at room temperature (RT) and stirring for 16–20 h. Initial gentle warming (35 °C) was required to complete dissolution of **1**. The dichloro BiQ **1** was synthesized according to the literature.[23] Aziridine was used at 2 and 6 equivalents concentration of **1** for synthesis of mono- (**2**) and bis- (**3**) aziridinyl BiQ, respectively. The next day, the reaction mixture was partitioned between CH_2Cl_2 and water. The organic layer was collected, washed with water (×2) and brine, dried (Na_2SO_4), filtered and concentrated. The residue was adsorbed onto silica gel in the cold and chromatographed over silica gel using a Biotage Isolera running with a gradient of ethyl acetate (EtOAc) in hexane to provide the title compound as an orange red solid.

To demonstrate the importance of presence of aziridine moiety on the naphthoquinone core for antileukemic activity, we synthesized **4** (*3,3′-bis(dimethylamino)-[2,2′-binaphthalene]-1,1′,4,4′-tetrone*) that, in theory, cannot effectively undergo a nucleophilic attack by DNA. Compound **4** is the isostere of bis-aziridine BiQ with similar electronics and size characteristics but without the alkylating moiety. As shown in Scheme 1, in a pressure tube and in presence of *N,N-diisopropylethylamine*, dimethylamine hydrochloride was added to **1**, and the solution was stirred in dichloromethane for 48 h. After removing all volatile materials in the reaction solution in vacuo, the crude solid was preabsorbed onto silica gel and chromatographed in 75% EtOAc/hexanes to 100% EtOAc. The purified product was triturated with hexanes and ether (1:1, 5 mL), and the final dark red product was collected by filtration in 48% yield.

To investigate antiproliferative activity of amino-BiQs against AML cells, we first performed an MTT-like cell proliferation assay in the AML cell lines using a reagent called WST-1 (Promega, Wisconsin). We observed a concentration-dependent decrease in metabolic activity of all cell lines after exposure to compound **3**, with IC_{50} values of 0.18 ± 0.06 μM for MOLM-14, 1.05 ± 0.05 μM for MV4-11, and 0.65 ± 0.30 μM for THP-1 cells (see Supplementary data Fig. S1). Compared to **3**, compounds **2** and **4** demonstrated less potent anti-AML activities. IC_{50} values of **2** were 3.9 ± 1.0 μM for MOLM-14 and 7.7 ± 1.3 μM for THP-1 cells. Compound **4** showed IC_{50}s of 2.9 ± 0.9 and 2.4 ± 0.3 against MOLM-14 and MV4-11 cells, respectively (See Supplementary data, Methods and Materials).

Because compound **3** demonstrated a superior potency, it was selected for testing against primary leukemia cells from patients and the remaining experiments including mechanistic assays. Table 1 summarizes the genetic characteristics of primary leukemia cells as well as their sensitivity to compound **3**. To measure the selectivity of **3** against neoplastic cells, we treated normal hematopoietic bone marrow cells as well. Interestingly, IC_{50} of bis-aziridinyl BiQ **3** for normal bone marrow cells was 3.37 ± 1.27 μM, which was approximately five to eighteen times higher than those for AML cell lines and 1.5–2 times higher for primary leukemia cells, suggesting a favorable therapeutic index of this agent.

We next determined the effect of bis-aziridinyl-BiQ **3** on AML cell survival and viability, as well as on clonogenic activity, which is an *in vitro* assay to test the ability of every leukemic cell in the population to produce a colony by demonstrating neoplasticity



Scheme 1. Synthesis of mono- and bis-aziridinyl (**2** and **3**) and bis-dimethylamino dimeric naphthoquinones. (**2**) Aziridine (21 μL, 0.4 mmol, 2 equiv), **1** (76 mg, 0.2 mmol, 1 equiv), THF, RT, 12 h, 49%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28–8.26 (m, 1H, Ar), 8.18–8.15 (m, 2H, Ar), 8.11–8.09 (m, 1H, Ar), 7.84–7.82 (m, 2H, Ar), 7.76–7.74 (m, 2H, Ar), 2.33, 2.26 (Aziridine, 4H, CH_2CH_2, J_{AB} = 6.2 Hz); ^{13}C NMR: (100 MHz, d_6-DMSO) 181.4, 181.2, 180.5, 177.1, 154.9, 144.9, 141.5, 135.4, 135.2, 135.1, 134.2, 131.9, 131.7, 131.6 (2), 127.6, 127.4, 126.8, 126.2, 121.5, 28.5 (2); MS (ESI) *m/z* calcd for $C_{22}H_{12}ClNO_4$ (M^+): 389.1, found: 390.0 (M+H$^+$). (**3**) Aziridine (63 μL, 1.2 mmol, 6 equiv), **1** (76 mg, 0.2 mmol, 1 equiv), Et_3N (84 mL, 0.6 mmol, 3 equiv), THF, RT, 16 h, 44%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16–8.11 (m, 4 H, Ar), 7.75–7.73 (m, 4H, Ar), 2.31–2.26 (m, 8H, 2 × CH_2CH_2); ^{13}C NMR (100 MHz, d_6-DMSO): 182.5, 180.9, 154.3, 134.8, 133.8, 132.5, 131.7, 126.5, 126.2, 122.9, 20.5; MS (ESI) *m/z* calcd for $C_{24}H_{16}N_2O_4$ (M^+): 396.1, found: 397.0 (M+H$^+$). (**4**) Dimethylamine hydrochloride (0.329 g, 4.03 mmol), **1** (0.218 g, 0.569 mmol), *N,N-diisopropylethylamine* (0.7 mL), CH_2Cl_2 (10 mL), pressure tube, RT, 48 h, 48%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01–7.99 (d, 2H, Ar), 7.91 (d, 2H, Ar), 7.81–7.78 (m, 4H, Ar), 2.89 (s, 12H); MS (ESI) *m/z* calcd for $C_{24}H_{20}N_2O_4$ (M^+): 400.1, found: 401.1 (M+H$^+$).