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Structural optimization of non-nucleoside DNA methyltransferase inhibitor as anti-cancer agent

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ABSTRACT

Inhibition of DNA methyltransferase 1 (DNMT1) can reverse the malignant behavior of cancer cells by restoring expression of aberrantly silenced genes that are required for differentiation, senescence, and apoptosis. Clinically used DNMT1 inhibitors decitabine and azacitidine inhibit their target by covalent trapping after incorporation into DNA as azacytidine analogs. These nucleoside compounds are prone to rapid enzymatic inactivation in blood, posing challenges to the development of purely epigenetic dosing schedules. Non-nucleoside compounds that suppress expression or function of DNMT1 may overcome this problem. Using a high-throughput PCR-based site specific chromatin condensation assay, we identified a compound that reactivated Cyclin-Dependent Kinase Inhibitor 2A (CDKN2A) in myeloma cells and suppressed expression of DNMT1 from a library of 5120 chemically diverse small molecules. Lead optimization was performed to generate 26 new analogs with lung cancer proliferation and DNMT1 expression as activity readout. Two of the new derivatives showed 2 fold improvement of growth inhibiting potency and also decreased DNMT1 protein levels in lung cancer cells.

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Inhibition of the maintenance DNA methyltransferase 1 (DNMT1) can reverse the malignant behavior of diverse cancer cells by restoring expression of aberrantly silenced genes that are required for differentiation, senescence, and apoptosis. The aza nucleosides decitabine and azacitidine are currently the only clinically used DNMT1 inhibitors and exert their epigenetic effects after incorporation into DNA.[1,2] However, poor stability, rapid metabolism, non-specific incorporation into DNA and cell cycle dependent effect have made design of optimal treatment schedules difficult.[3–5] In addition, the non-specific incorporation of these nucleotide analogs may cause un-wanted side effects. Novel non-nucleoside DNMT1 inhibitors may allow safer, more predictable epigenetic treatment since direct DNA toxicity should be avoidable. To search for alternative epigenetic agents, we have developed a novel high-throughput PCR-based site specific chromatin condensation assay.[6] A library of 5120 chemically diverse small molecules was screened with the assay, and we identified a lead compound {3-(4-chlorophenyl)-5-[(4-hydroxyphenyl) methylidene] imidazolidine-2} from 15 non-nucleoside hits that were epigenetically effective and suppressed DNMT1 protein expression at low micromolar concentrations in myeloma cells (Fig. 1). In the current study we evaluated whether this could be reproduced in lung cancer cells and if the lead could be improved through medicinal chemistry using growth inhibition and DNMT1 suppression as the readout.

The structural modification initially focused on two aromatic rings of the lead compound depicted in Figure 1. We attempted to reveal the relationship between the biological activity of compounds and substituent effects in the A and B position. The chlorine atom at the position A was substituted by different groups such as bromo, iodo, or even replaced by electron-donating groups such as methyl and methoxy. 4-Hydroxyphenyl of the B position can be

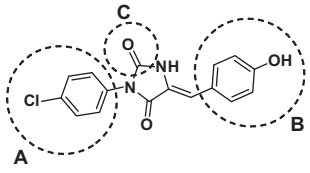

Figure 1. The chemical structure of the lead compound.

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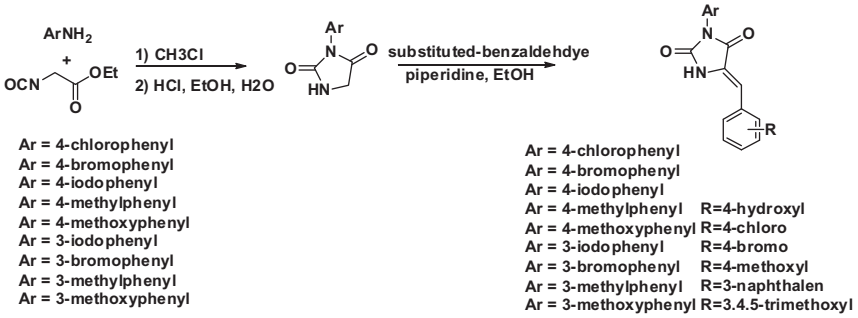
modified to other substituted aryl groups such as 4-bromophenyl, 4-iodophenyl, 4-methylphenyl, 4-methoxyphenyl, 2-naphthyl, and 3,4,5-trimethoxyphenyl. The combinatory strategy was used to generate a series of novel analogs of the lead compound with diversity at the A–B moieties for further biological investigation. The synthesis of the proposed compounds is illustrated in Scheme 1. Substituted aniline reacts with ethyl isocyanatoacetate in chloroform and then cyclizes under acidic condition to form an intermediate 3-substituted hydantoin. 3-Substituted hydantoin condenses with aryl aldehyde to generate the final product in the *Z* configuration. Variations in two aromatic rings can be achieved by using different anilines and aldehydes, respectively.

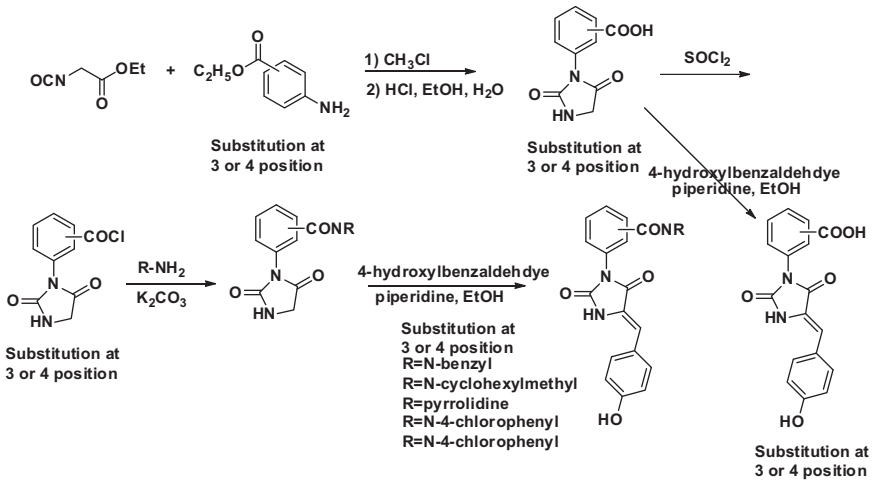
Next, we tried to explore if more hydrophobic and bulky groups at the A moiety could improve the biologic activity. Therefore, we further introduced more hydrophobic and bulky substituent groups on the aromatic ring at the A moiety. The synthesis of this group of compounds is illustrated in Scheme 2.

At last, we explored modifications of the middle imidazole ring at the C moiety (Fig. 1). We were wondering if a sulfur substituted ring could impact the activity of the compound. The synthesis of the sulfur atom substituted analogs is illustrated in Scheme 3. Overall, 26 new derivatives were synthesized, purified and characterized with NMR and MS for the following biological study.

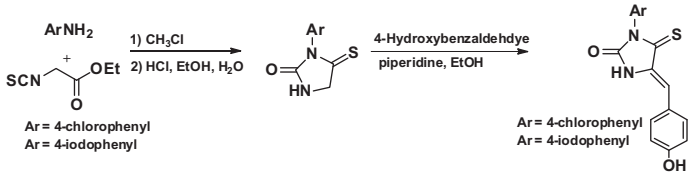
The screening of the compounds was performed using a cell proliferation assay with H292 non-small cell lung cancer cells. We first examined all the compounds at 200 μM, and selected the potent ones for further does-dependent study (Table 1). At the A moiety, replacement of 4-chloro with 3- methoxy, iodo, methyl, carboxylic and bromo all led to the loss of activity (compounds **7**–**9**, **14**, **16**), suggesting that 3 position of the A ring with a small group is not favorable for the biological activity, regardless whether it was electro-donating or electro-withdrawing. However, bulky group substitution at 3 position of the A ring maintains antiproliferative effects (compounds **17**–**19**). Several very bulky groups such as N-benzyl and N-cyclohexylmethyl retained biological



Scheme 1. Synthesis of A and B moiety aromatic ring substituted analogs.



Scheme 2. Synthesis of more bulky A moiety derivatives.



Scheme 3. Synthesis of analogs with sulfur atom substituted imidazole ring.