Bioorganic & Medicinal Chemistry Letters 26 (2016) 1272–1275

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Bo Zhong [a], Sergei Vatolin [b], Nethrie D. Idippily [a], Rati Lama [a], Laila A. Alhadad [a], Frederic J. Reu [b,*], Bin Su [a,c,*]

[a] Department of Chemistry, College of Sciences and Health Professions, Cleveland State University, 2121 Euclid Ave., Cleveland, OH 44115, USA
[b] Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, 9500 Euclid Ave., Cleveland, OH 44195, USA
[c] Center for Gene Regulation in Health and Disease, College of Sciences & Health Professions, Cleveland State University, 2121 Euclid Ave., Cleveland, OH 44115, USA

ARTICLE INFO

Article history:
Received 11 November 2015
Revised 6 January 2016
Accepted 8 January 2016
Available online 9 January 2016

Keywords:
DNMT1
Cancer
Non-nucleosides
Drug development

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.

Published by Elsevier Ltd.

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

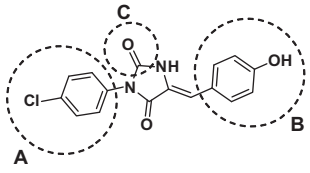


Figure 1. The chemical structure of the lead compound.

* Corresponding authors. Tel.: +1 216 368 3378; fax: +1 216 368 1300 (F.J.R.); tel.: +1 216 687 9219; fax: +1 216 687 9298 (B.S.).
E-mail addresses: reuf@ccf.org (F.J. Reu), B.su@csuohio.edu (B. Su).

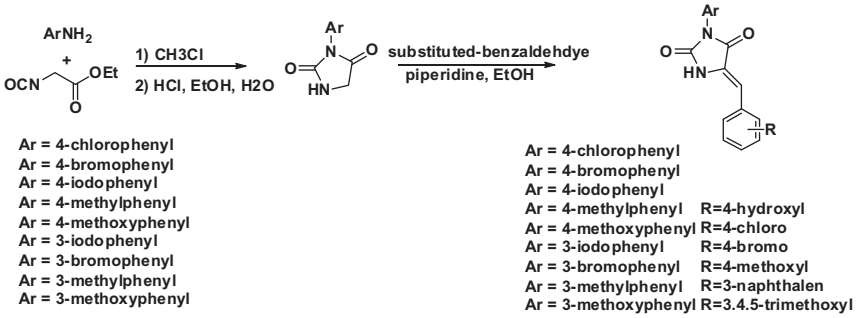
http://dx.doi.org/10.1016/j.bmcl.2016.01.020
0960-894X/Published by Elsevier Ltd.

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.

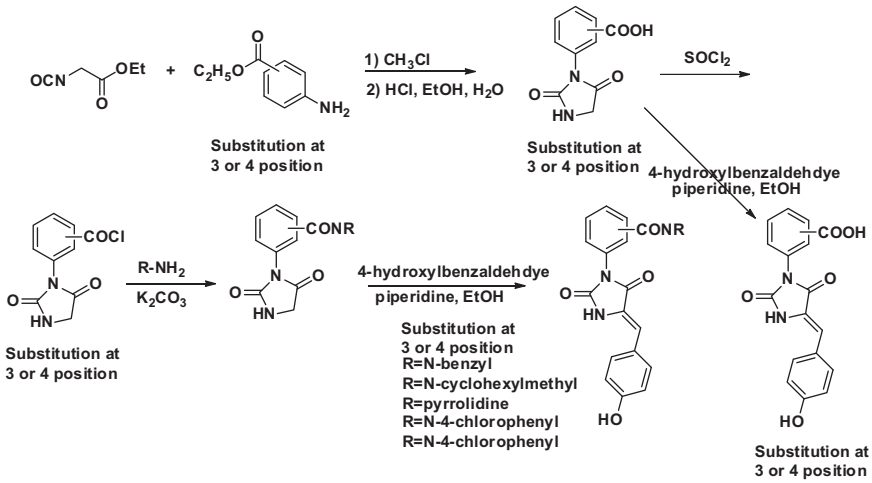
Next, we tried to explore if more hydrophobic and bulky groups at the A moiety could improve the biologic activity. Therefore, we further introduced more hydrophobic and bulky substituent groups on the aromatic ring at the A moiety. The synthesis of this group of compounds is illustrated in Scheme 2.

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.

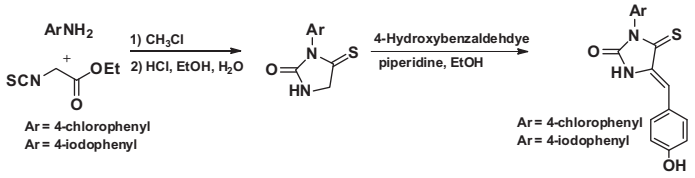
The screening of the compounds was performed using a cell proliferation assay with H292 non-small cell lung cancer cells. We first examined all the compounds at 200 μM, and selected the potent ones for further does-dependent study (Table 1). At the A moiety, replacement of 4-chloro with 3- methoxy, iodo, 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.

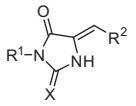


Scheme 2. Synthesis of more bulky A moiety derivatives.



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

Table 1
Growth inhibitory effects of the DNMT1 inhibitors on H292 cells

Entry		IC_{50} (μM)
Lead	R^1 = 4-Chlorobenzene, R^2 = 4-hydroxylbenzene, X = O	41.3 ± 12.5
1	R^1 = 4-iodobenzene, R^2 = 4-hydroxylbenzene, X = O	25.5 ± 13.2
2	R^1 = 4-Chlorobenzene, R^2 = 4-Chlorobenzene, X = O	35.6 ± 13.8
3	R^1 = 4-Chlorobenzene, R^2 = 4-bromobenzene, X = O	46.6 ± 12.6
4	R^1 = 4-Chlorobenzene, R^2 = 4-methoxylbenzene, X = O	32.1 ± 11.4
5	R^1 = 4-methylbenzene, R^2 = 4-hydroxylbenzene, X = O	27.9 ± 11.5
6	R^1 = 4-methoxylbenzene, R^2 = 4-hydroxylbenzene, X = O	>200
7	R^1 = 3-methoxylbenzene, R^2 = 4-hydroxylbenzene, X = O	>200
8	R^1 = 3-iodobenzene, R^2 = 4-hydroxylbenzene, X = O	>200
9	R^1 = 3-methylbenzene, R^2 = 4-hydroxylbenzene, X = O	>200
10	R^1 = ethyl 4-benzoate, R^2 = 4-hydroxylbenzene, X = O	>200
11	R^1 = 4-carboxylbenzene, R^2 = 4-hydroxylbenzene, X = O	>200
12	R^1 = 4-Chlorobenzene, R^2 = 3-naphthalen, X = O	>200
13	R^1 = 4-Chlorobenzene, R^2 = 3,4,5-trimethoxylbenzene, X = O	>200
14	R^1 = 3-carboxylbenzene, R^2 = 4-hydroxylbenzene, X = O	>200
15	R^1 = 4-bromobenzene, R^2 = 4-hydroxylbenzene, X = O	69.9 ± 24.4
16	R^1 = 3-bromobenzene, R^2 = 4-hydroxylbenzene, X = O	>200
17	R^1 = 3-*N*-(4-chlorophenyl)benzamide, R^2 = 4-hydroxylbenzene, X = O	124.6 ± 44.2
18	R^1 = 3-*N*-benzyl-benzamide, R^2 = 4-hydroxylbenzene, X = O	23.5 ± 10.9
19	R^1 = 3-*N*-cyclohexylmethyl-benzamide, R^2 = 4-hydroxylbenzene, X = O	17.9 ± 8.3
20	R^1 = 3-(pyrrolidine-1-carbonyl)phenyl, R^2 = 4-hydroxylbenzene, X = O	>200
21	R^1 = 4-*N*-(4-chlorophenyl)benzamide, R^2 = 4-hydroxylbenzene, X = O	>200
22	R^1 = 4-*N*-benzyl-benzamide, R^2 = 4-hydroxylbenzene, X = O	>200
23	R^1 = 4-*N*-cyclohexylmethyl-benzamide, R^2 = 4-hydroxylbenzene, X = O	>200
24	R^1 = 4-(pyrrolidine-1-carbonyl)phenyl, R^2 = 4-hydroxylbenzene, X = O	>200
25	R^1 = 4-chlorobenzene, R^2 = 4-hydroxylbenzene, X = S	28.5 ± 14.3
26	R^1 = 4-iodobenzene, R^2 = 4-hydroxylbenzene, X = S	19.7 ± 6.8

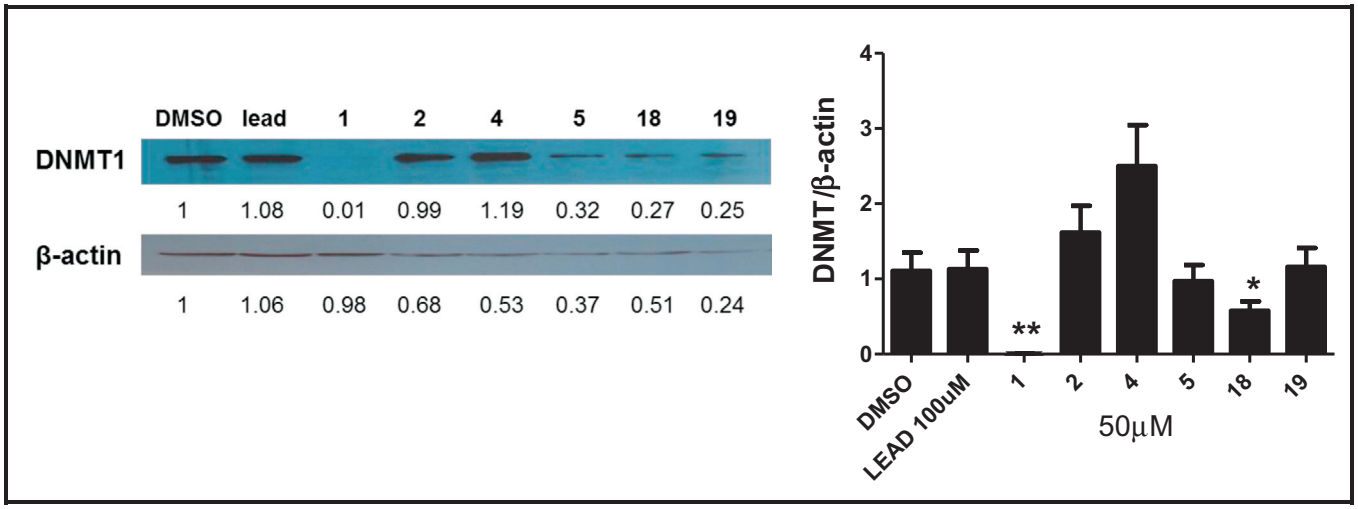


Figure 2. Compounds affect DNMT1 protein levels. H292 cells were treated with DMSO and the compounds for 48 h. Levels of DNMT1 and β-actin were analyzed by Western blot of cell extracts with specific antibodies. Bands of DNMT1 (upper panel) and β-actin (lower panel) were quantified using ImageJ (NIH) and normalized to β-actin. The experiments were repeated three times and the representative one is listed in the figure. $^{**}P$ <0.001 versus control, * <0.01 versus control.

activity. On the other hand, these bulky groups at 4-positon of the A ring all led to the loss of activity (compounds **21**–**23**). Pyrrolidine group erased all the activity no matter if it was connected to 3 or 4 position at A ring (compounds **20** and **24**). Bulky groups to replace the B ring decreased activity (compounds **12** and **13**). The naphthalene and 2,3,4-trimethoxylbenzene all impaired the activity.

We identified 11 new derivatives from the 26 compounds pool to perform dose-dependent cell proliferation investigation, and summarized a brief structure activity relationship (SAR) based on the results of the IC_{50} values. The 4-hydroxyl group of the B ring is critical for activity. Replacement of the hydroxyl group with other halogens such as chloro or bromo groups impaired activity compared to the lead compound (compounds **2** and **3**). A 4-methoxyl group at the B ring also decreased the activity (compound **4**). Sulfur atoms did not affect the activity of the compound (compounds **25** and **26**). The IC_{50} values for the corresponding oxygen substituted imidazole or sulfur substituted imidazole are in the similar range, as demonstrated by lead compound versus compound **25** and compound **1** versus compound **26**.

The lead compound decreased DNMT1 protein levels in myeloma cells. We were wondering if the new analogs had this activity in lung cancer cells. We first examined the compound with IC_{50}

values below 100 μM via western blot in H292 lung cancer cells, and identified compounds **1**, **2**, **4**, **5**, **18** and **19** that decreased the DNMT1 protein level. Interestingly, the lead compound did not affect DNMT1 expression in lung cancer cells but substitution of the A ring with 4-iodobenzene or 3-*N*-benzyl-benzamide led to significant DNMT1 suppression at doses 50% lower than the ones used for parent compound (Fig. 2). This suggests that cell specific differences in networks controlling DNMT1 expression may exist and that analogs of this class would have to be evaluated in multiple cell lines before development into DNMT1 suppressing drugs begins. Furthermore, results suggest that this class of compounds can have anti-neoplastic effects that are independent of DNMT1 suppression which warrant further study.

In summary, our findings indicate that non-nucleoside inhibitors of DNMT1 expression can be developed. Further research into their mechanism of action and optimization of DNMT1 suppressing potency may yield novel epigenetic therapies with improved therapeutic index.

Acknowledgments

This work was supported by Center for Gene Regulation in Health and Disease (GRHD) of Cleveland State University and Ohio Department of Development (ODOD) to B. Su and Scott Hamilton CARES grant to F.J. Reu.

References and notes

1. Chen, Z.; Liu, S.; Tian, L.; Wu, M.; Ai, F.; Tang, W.; Zhao, L.; Ding, J.; Zhang, L.; Tang, A. *Oncotarget* **2015**, *6*, 38139.
2. Zhou, D.; Wan, Y.; Xie, D.; Wang, Y.; Wei, J.; Yan, Q.; Lu, P.; Mo, L.; Xie, J.; Yang, S.; Qi, X. *Exp. Mol. Med.* **2015**, *47*, e182.
3. Wijermans, P. W.; Lubbert, M.; Verhoef, G.; Klimek, V.; Bosly, A. *Ann. Hematol.* **2005**, *84*, 9.
4. Momparler, R. L. *Semin. Oncol.* **2005**, *32*, 443.
5. Marcucci, G.; Silverman, L.; Eller, M.; Lintz, L.; Beach, C. L. *J. Clin. Pharmacol.* **2005**, *45*, 597.
6. Vatolin, S.; Khan, S. N.; Reu, F. J. *PLoS One* **2012**, *7*, e44690.