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Curcumin is a potent DNA hypomethylation agent

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ABSTRACT

Molecular docking of the interaction of curcumin and DNMT1 suggested that curcumin covalently blocks the catalytic thiolate of C1226 of DNMT1 to exert its inhibitory effect. This was validated by showing that curcumin inhibits the activity of M. SssI with an IC_{50} of 30 nM, but no inhibitory activity of hexahydrocurcumin up to 100 μM. In addition, curcumin can induce global DNA hypomethylation in a leukemia cell line.
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DNA methylation of cytosine residues in the context of the sequence 5′-cytosine–guanosine (CpG) in gene promoter regions is an epigenetic mechanism that controls gene transcription, genome stability and genetic imprinting.[1] This process is regulated by DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b) in the presence of S-adenosyl-methionine (SAM) that serves as a methyl donor for methylation of cytosine residues at the C-5 position to yield 5-methylcytosine.[1] Aberrant hypermethylation of promoter CpG islands (>55% CG content) of tumor suppressor genes (TSGs) results in their transcriptional silence in a variety of solid and blood cancers.[1] In vitro and in vivo treatment with hypomethylating agents has proven to be effective in restoring gene expression and normal patterns of differentiation and apoptosis in malignant cells.[2]

Two nucleoside analogs (azanucleosides) with hypomethylating activity, that is, decitabine or 5-azacytidine, have recently been approved by the Food and Drug Administration for the treatment of myelodysplastic syndrome.[3,4] Clinical trials with these agents are ongoing in other type of cancers, for example, acute myeloid leukemia (AML) with encouraging results.[5] However, toxicities (i.e., myelosuppression) inherent to the cell cycle phase specificity of nucleoside analogs pose significant limitations to the use of these drugs, especially in patients with solid tumors.[5] Thus, discovery and development of novel hypomethylating agents that are more effective and have a more favorable toxicity profile are essential to broaden the spectrum of epigenetic targeting therapeutic strategies.[6]

Several categories of DNMT1 inhibitors have been identified and their structures were depicted in Scheme 1. These compounds work mainly through the following mechanisms: [1] covalent-trapping of DNMT1 through incorporation into DNA (i.e., nucleoside analogs decitabine, 5-azacytidine and zebularine),[3,4,6] [2] non-covalent blocking of DNMT1 catalytic active site (i.e., EGCG and RG108),[7,8] [3] interruption of binding site of DNMT1 to DNA (i.e., procaine and procainamide),[9,10] [4] degradation of DNMT1 (i.e., decitabine),[11] and [5] suppression of DNMT1 expression (i.e., antisense MG-98).[12] Among these, DNMT1 covalent-trapping compounds represented by azanucleosides appear to be the most effective in inducing global DNA hypomethylation and reactivation of epigenetically silenced tumor suppressor genes (TSG) in malignant cells.[13] All non-nucleoside hypomethylating agents showed relatively low efficacy compared to 5-azacitidine and decitabine.[13]

We hypothesized that small molecules that covalently block the catalytic C1226 of DNMT1 inhibit the enzymatic activity of this protein without requiring incorporation into DNA, which results in significant hypomethylating activity with reduced aforementioned toxicity nucleoside hypomethylating agents. Hence, we initially sought to identify lead compounds from plant-derived natural agents (phytochemicals), which have been shown to exert inhibition activity through covalently binding to thiol groups of enzymes/transcriptional factors. These phytochemicals in general are

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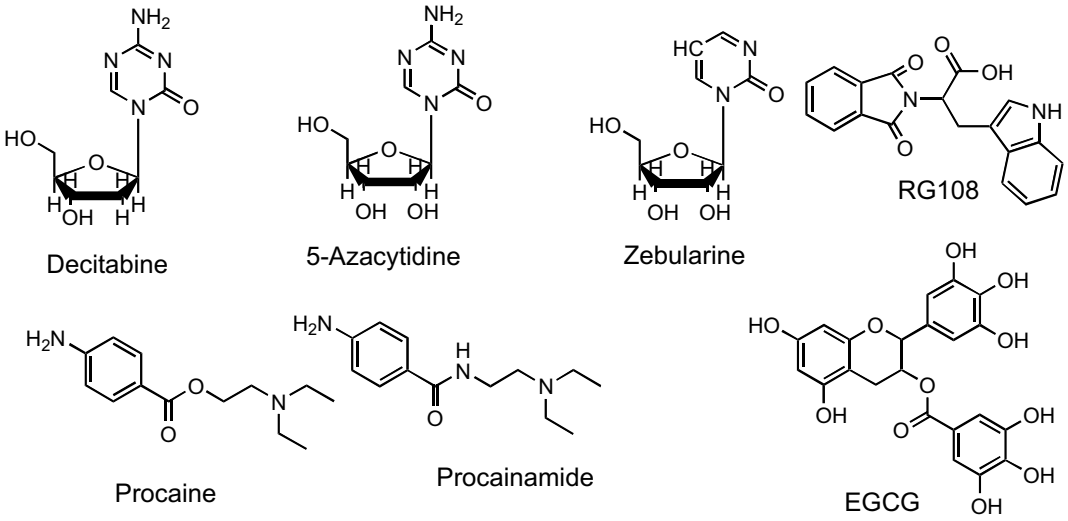

Scheme 1. Chemical structures of selected DNMT1 inhibitors.

expected to have low toxicity and therefore could be potentially used for chemoprevention alone or in combination with other anti-cancer agents.

Recently, DNMT1 homology modeling[14] has been built to provide an opportunity for virtual screening of compounds that inhibit human DNA methyltransferases in a mechanism-independent manner. Recently, as proof of principle, we adapted and improved the hDNMT1 homology modeling and docked 44 natural germacrolides, one subtype of sesquiterpene lactones, in our DNMT1 homology modeling and found that that γ-methylene lactone-like molecules might be particularly effective in inhibiting DNMT activity through covalent binding of the thiol group of DNMT1 and partially through competitive non-covalent binding to the catalytic pocket of DNMT1 with the substrate cytosine and cofactor SAM. Parthenolide has been identified and characterized as an effective hypomethylating agent (#1466, AACR, 2007).

Based on their structural activity, we are assuming that phytochemicals with α,β-unsaturated carbonyl moieties might covalently block DNMTs depending on their conformation. Curcumin, the major component of tumeric, has shown strong anti-inflammatory, anti-angiogenic, and antioxidant, wound healing and anticancer effects and used for various diseases.[15]

Therefore, we tested the potential binding activity of curcumin to the catalytic site of DNMT1 using the DNMT1 homology model. As shown in Figure 1, the simulated bindings of curcumin (compound **1**), demethoxycurcumin (compound **2**), bisdemethoxycurcumin (compound **3**) (Fig. 1A) and its two saturated analogs tetrahydrocurcumin (compound **4**) (Fig. 1B) and hexahydrocurcumin (compound **5**) (not shown) onto the catalytic site of DNMT1 homology model, the DNMT1 catalytic site is a deep pocket buttressed by a typical pseudo-Rossmann fold in the bottom and walled by helices and loops. The pocket is largely hydrophobic with polar residues in the binding sub-pockets on the methionine end of cofactor and at the side of pyrimidine aromatic ring of substrate. There are two potential binding modes of compounds **1**–**3** in the catalytic site of DNMT1 in a similar way. First, compounds **1**–**3** can compete with the methionine of the cofactor SAM with the hydrophobic pocket in the middle, and the pyrimidine ring of substrate with aromatic ring. The binding affinity of compounds **1**–**5**: curcumin, demethoxycurcumin, bisdemethoxycurcumin tetrahydrocurcumin, and hexahydrocurcumin are −14.26, −12.89, −11.68, 14.15 and 14.05 kcal/mol, respectively. The α-carbon atom of bis-α,β-unsaturated ketone of curcumin, demethoxycurcumin and bisdemethoxycurcum overlaps with the C6 atom of the cytosine ring in the catalytic space. The distance between the α-carbon atom of bis-α,β-unsaturated ketone of compounds **1**–**3** are 4.5, 4.4, 4.3 Å away from the S atom of catalytic cysteine of DNMT1, respectively. The model clearly demonstrates that curcumin has the potential to inhibit the DNMT1 catalytic function through either blocking the catalytic cysteine (C1226) of DNMT1 by its bis-α,β-unsaturated ketones as a suicide inhibitor for compounds **1**–**3** or compete with cofactor SAM as a reversible competitive inhibitor for compounds **4**–**5**.

Recently, Payton et al.[16] demonstrated that curcumin exist predominantly as the enol form. Therefore, we postulated that tetrahydrocurcumin, like curcumin, can tautomerize between diketone and keto-enol, which may serve as another Michael acceptor and potential covalent blocking the catalytic thiol group in the DNMT1 through the C3 of the keto-enol moiety of compounds **1**–**3** and **5**. Therefore, this mode was evaluated in the hDNMT1 homology mode. The distance of the C3 of the keto-enol tautomer of compounds **1**–**3** to the S atom of C1226 of DNMT1 is 7.3 Å, suggesting the lack of potential covalency. However, in the case of tetrahydrocurcumin, it can adapt another favorable conformation(s) with a distance of 4.1 or 3.7 Å from C3 or C5 keto-enol of compound **4** to the S atom of C1226, respectively, as shown in Figure 1B. This result suggests that there a different type of covalency in **4** because of its flexibility arisen from the single bond (α,β-saturated ketone) instead of double bond (α,β-unsaturated ketone) in compounds **1**–**3**. Notably, R1312 in the catalytic domain of DNMT1 can form two H-bonds with the keto-enol center in compound **4** with distances of 3.0 and 3.1 Å in addition to a common 2.7 Å H-bond of E1168 with one side of the aromatics of in compounds **1**–**4** (Fig. 1B).

To validate the results of the DNMT1 homology modeling study, the inhibition of the enzymatic activity of M. SssI, an analog of DNMT1, by commercial curcumin, a mixture of compounds **1**–**3** was tested in vitro. M. SssI has been reported to have a robust methylation activity and its catalytic domain is structurally similar to DNMT1. Thus, we employed a 38 bp double strand (ds)-oligonucleotide labeled with 3′-biotin in one strand and 3′-digoxigenin-NHS ester in its complementary strand as a substrate for M. SssI in a solution containing SAM, and the endonuclease HapII. The ds-oligonucleotide contains a CCGG sequence, which, when cleaved by a HapII, resulted in a loss of the 3′-digoxigenin-NHS ester. When anti-digoxigenin-AP antibody and the substrate Attophos were added to the

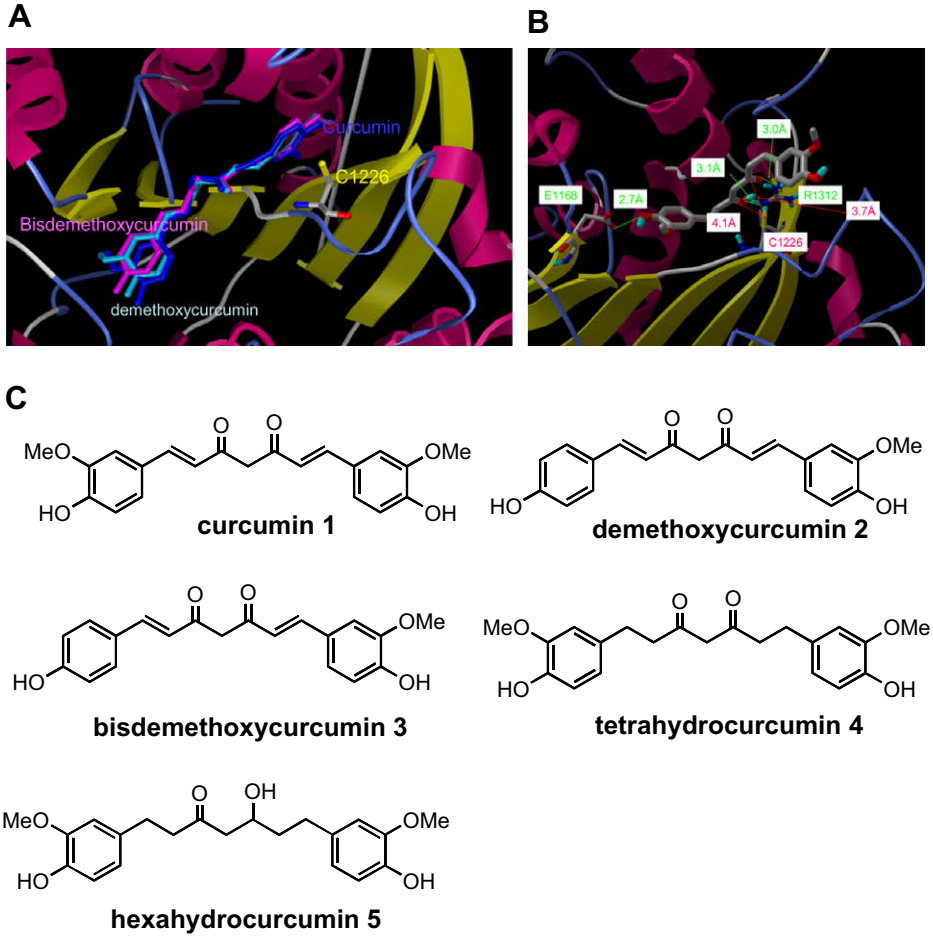

Figure 1. The modeling of the interaction of curcumin (**1**) and its two analogs (**2**–**3**) (A) and tetrahydrocurcumin (**4**, B) with DNMT1, the DNMT1 catalytic domain is represented by ribbon model. Docked curcumin (blue), demethoxycurcumin (light blue), bisdemethoxycurcumin (pink), tetrahydrocurcumin (gray in B) catalytic Cys1126, and anchoring E1668, R1312 are shown in ball and stick models (A and B) and the chemical structures (C) of curcumin (**1**) and its analogs (**2**–**5**).

solution, no detectable fluorescence signal was detected. In contrast, when the CCGG sequence of the ds-oligonucleotide is methylated to $CC^{m}GG$, it resulted in resistance to the cleavage activity of HapII, maintenance of the 3′-digoxigenin-NHS ester of the ds-oligonucleotide and in turn generation of fluorescence signal under the same condition. The methylation level of the ds-oligonucleotide was found to directly correlate with the intensity of the assay fluorescence signal and in turn with the enzymatic activity of M. SssI (data not shown). Exposure to various concentrations (1, 10, 30, and 300 nM, 1, 3, 10, 30, and 100 μM) of curcumin resulted in a dose-dependent decrease in fluorescent intensity, reflecting inhibition of the M. SssI methylation activity (Fig. 2A). The apparent IC_{50} of curcumin with respect to M. SssI inhibition was found to be 30 nM. Then, curcumin and its analogs 2–3 were purified from commercial curcumin mixture by HPLC (See Support Information). Similarly, the inhibitory activity of curcumin and its two analogs **2** and **3** on M. SssI is almost same to that of commercial curcumin with an IC_{50} of 30 nM.

Two saturated curcumin analogs: tetrahydrocurcumin **4** and hexahydrocurcumin **5** were prepared by reduction of curcumin according to the reported procedure[17] and their inhibitory activity on M. SssI was also examined. No inhibitory activity of hexahydrocurcumin **5** up to 100 μM was observed although its binding affinity (−14.05 kcal/mol) estimated from the DNMT1 homology modeling is comparable to that of curcumin (−14.26 kcal/mol). However, tetrahydrocurcumin showed a similar activity to that of curcumin (data not shown), which is consistent with our modeling prediction as shown in Figure 1B. Apparently, these results demonstrated that the inhibitive activity of curcumin **1** and its analogs **2** and **3** may be dependent on either one of bis-α,β-unsaturated ketones and the inhibitory activity of tetrahydrocurcumin **4** is dependent on its keto-enol tautomer. The similar inhibitory activity of compounds **1**–**4** with significant different binding affinity (−14.26, −12.89, −11.68, 14.15 kcal/mol, respectively) implicates a potential covalency of these compounds with M. SssI to level off the different inhibitory activity arisen from their different binding affinity. However, the exact binding model of curcumin and its analogs remains unknown. Further exploration of their binding mode to DNMT1 catalytic mode using mass spectrometry, structural analog probe and enzymatic kinetics are warranted.

Given similar DNMT inhibitory effects of curcumin and its analogs **2**–**3**, we next investigated whether the commercial curcumin mixture can induce global DNA hypomethylation in cell lines. Approximately 500 ng of genomic DNA from a leukemia cell line, MV4-11 cells exposed to various concentration of curcumin (0, 1, 3, 30 μM) for 72 h was hydrolyzed. The global DNA methylation level of these cells was measured using the LC–MS/MS method recently developed by our group.[14] During the 72 h incubation, the global DNA methylation of MV4-11 cells remains unchanged at 1.0 μM curcumin, but decreased about 15–20% at 3.0 and 30.0 μM curcumin with respect to untreated basal methylation level of the cell line (Fig. 2B), which is comparable to that of decitabine induced global DNA methylation in the cells.

Taken together, we demonstrated for the first time that curcumin and one of its major metabolites, tetrahydrocurcumin can inhibit M. SssI, an DNMT1 analog, activity and its inhibitory activity may arisen from a potential covalent blocking the catalytic thiol

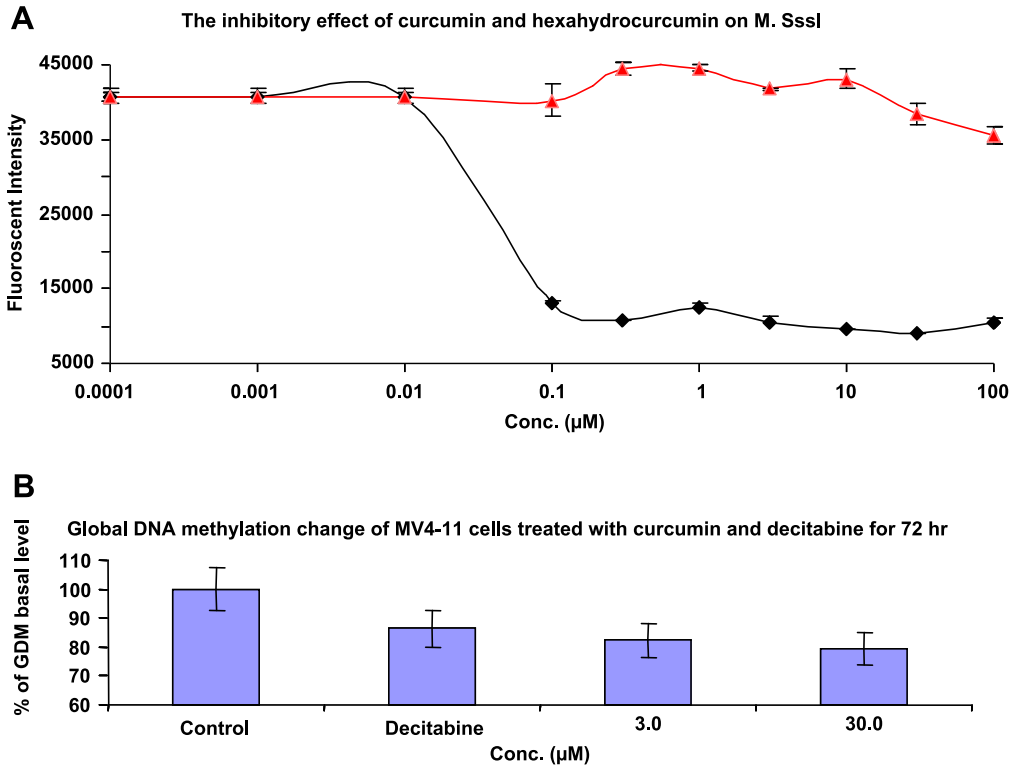

Figure 2. The DNA methylation inhibitory activity of commercial curcumin (black line with square) and hexahydrocurcumin (red line with triangle) on M. SssI (A) and the global DNA hypomethylation effect of curcumin (1.0, 3.0 and 30 μM) on MV4-11 cells for 72 h ($n = 3$, B).

group of C1226 in DNMT1. In consistent with the in vitro cell free inhibitive activity on M. SssI, curcumin can induce about 15–20% decrease of global DNA methylation level at 3.0 μM, a in vivo achievable concentration, which may provide a novel scaffold for discovery and development of novel hypomethylation agents and may steer its future direction for its clinical development as a DNA methylation inhibitor.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.12.041.

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