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Advances in the computational development of DNA methyltransferase inhibitors

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DNA methylation is an epigenetic change that results in the addition of a methyl group at the carbon-5 position of cytosine residues. The process is mediated by DNA methyltransferases (DNMTs), a family of enzymes for which inhibition is a promising strategy for the treatment of cancer and other diseases. Here, we review the current status of the computational studies directed to rationalize, at the molecular level, the enzymatic activity of DNMT inhibitors. We also review successful virtual screenings to identify inhibitors with novel scaffolds as well as the emerging efforts to characterize the dynamic behavior of DNMTs. Thus, computational approaches form part of multidisciplinary efforts to further advance epigenetic therapies.

DNA methylation is an epigenetic change that results in the addition of a methyl group at the carbon-5 (C5) position of cytosine residues, a process mediated by DNA methyltransferase enzymes (DNMTs). To date, three types of DNMT have been identified in the human genome, including two *de novo* methyltransferases (DNMT3A and DNMT3B) and the maintenance methyltransferase (DNMT1), which is the most abundant of the three [1–3]. The protein DNMT3L has high sequence similarity with the DNMT3A enzyme, but it lacks any catalytic activity owing to the absence of conserved catalytic residues. However, DNMT3L is required for the catalytic activity of DNMT3A and 3B [4]. The protein DNMT2 can be found in mammalian cells. Despite the structure of DNMT2 being similar to that of other DNMTs, its role is comparably less understood [5]. It has been reported that DNMT2 does not methylate DNA but instead methylates aspartic acid transfer RNA ($tRNA^{Asp}$) [6]. More recent experiments suggest that DNMT2 activity is not limited to $tRNA^{Asp}$ and that DNMT2 represents a noncanonical enzyme of the DNMT family [5]. DNMT1 is responsible for duplicating the pattern of DNA methylation during replication; it is essential for proper mammalian development; and it has also been proposed as a more interesting target for experimental cancer therapies [7]. DNA methylation represents a central mechanism for mediating epigenetic gene regulation, and the development of DNMT inhibitors provides novel opportunities for cancer therapy [1,8,9]. In addition, epigenetic alterations are associated not only with cancer, but also with psychiatric and other diseases [7].

The structure of DNMTs has been extensively reviewed [4,10]. Briefly, the structure of DNMT1, 3A and 3B can be divided into an N-terminal regulatory domain and a C-terminal catalytic domain (Fig. 1). DNMT has ten conserved sequence motifs that are described by their function: motifs I–III form the cofactor binding pocket, motif IV has the catalytic cysteine, motifs VI, VIII and X comprise the substrate binding site and motifs V and VII form the target recognition domain [7]. Human DNMT1 is a protein with 1616 amino acids, for which limited three-dimensional (3D) structural information is available. At the time of writing (November, 2010), there was no crystallographic or NMR information available for the catalytic domain of DNMT1. A crystal structure, however, has been recently published for the DNMT1 replication FOCI-targeting sequence [Protein Data Bank (PDB) accession code 3EPZ]. Crystal structures are available for the DNMT3A–DNMT3L C-terminal domain complex (PDB: 2QRV), and different regions of the N-terminal domain of DNMT3A and 3B (PDB: 3FLG, 3LLR, 3A1A and 3A1B). In addition, crystal structures of DNMT3L (PDB: 2PV0) and DNMT2 (PDB: 1G55) have been published.

The mechanism of DNA cytosine-C5 methylation is depicted in Fig. 2 [7,11,12]. DNMT forms a complex with DNA, and the cytosine that will be methylated flips out from the DNA base-pairing position [13]. The thiol of the catalytic cysteine in the

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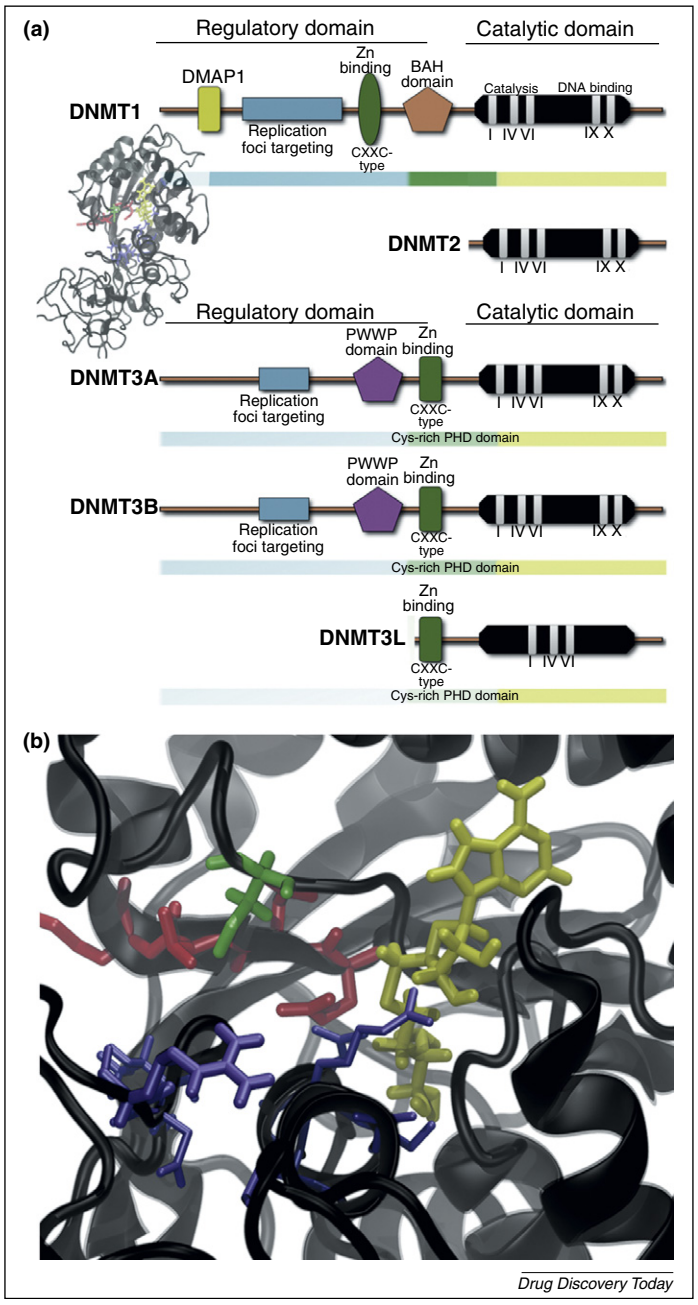

FIGURE 1

Structure of the known DNMTs. **(a)** Schematic representation of the DNMTs, and DNMT-like proteins. DNMT1, 2, 3A, 3B and L can be split into two main domains: regulatory and catalytic. Key conserved regions are shown (roman numerals) and other areas of structural importance are also indicated. The structure inset shows the entire DNMT1 molecule. **(b)** A close up of the catalytic site for DNMT1 with SAH (yellow) bound close to the core conserved residues (red) and catalytic cysteine (green). All other conserved or interacting residues are in blue. Abbreviations: Cys, cysteine; PHD, plant homeodomain.

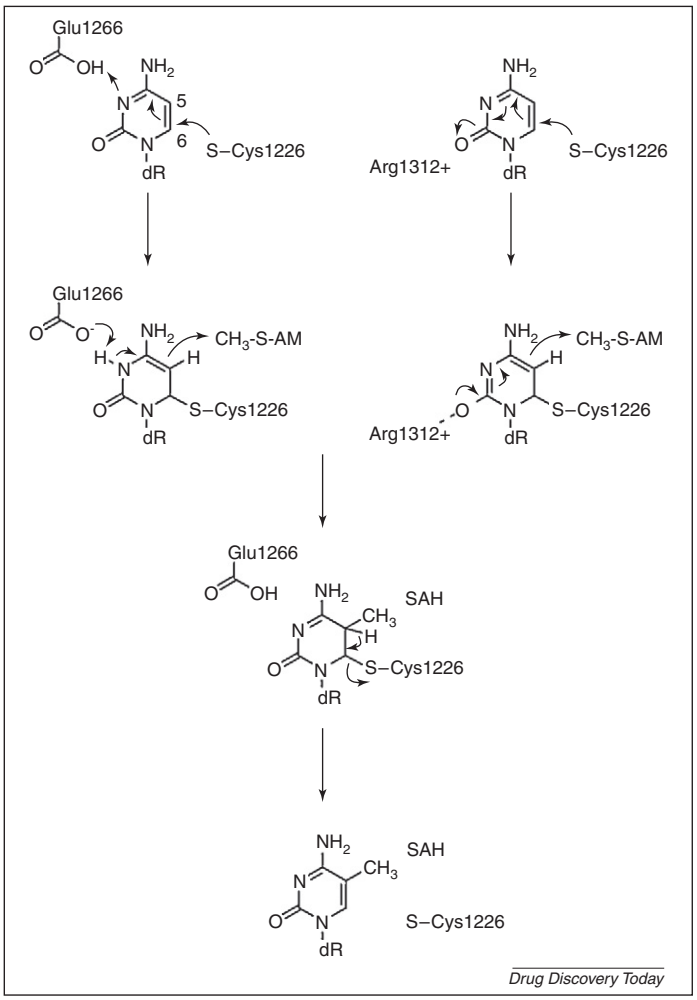

FIGURE 2

Mechanism of DNA methylation. After DNMT forms a complex with DNA and the cytosine that will be methylated flips out of the DNA, the thiol of the catalytic cysteine acts as a nucleophile that attacks the 6-position of the target cytosine to generate a covalent intermediate. The 5-position of the cytosine is activated and performs a nucleophilic attack on the methyl group of SAM to form the 5-methyl covalent adduct and SAH. The attack on the 6-position is assisted by transient protonation of the cytosine ring at the endocyclic nitrogen atom N3, which is stabilized by a glutamate residue. The carbanion might also be stabilized by resonance, where an arginine residue might participate in the stabilization. The covalent complex between the methylated base and the DNA is resolved by deprotonation at the 5-position to generate the methylated cytosine and the free enzyme. The 5-methylated cytosine base then flips back into its original position within the DNA.

active site of DNMTs acts as a nucleophile that attacks the 6-position of the target cytosine to generate a covalent intermediate between the enzyme and DNA. The 5-position of the cytosine is activated and conducts a nucleophilic attack on the methyl group of the methyl-donating cofactor *S*-adenosyl-L-methionine (SAM) to form the 5-methyl covalent adduct and *S*-adenosyl-L-homocysteine (SAH). The attack on the 6-position is assisted by transient protonation of the cytosine ring at the endocyclic nitrogen atom N3, which is stabilized by a glutamate residue. The carbanion might also be stabilized by resonance [14], where an arginine residue might have an important role in the catalytic mechanism. The covalent complex between the methylated base and the DNA is resolved by deprotonation at the 5-position to generate the methylated cytosine and the free enzyme. The 5-methylated cytosine base then flips back into its original position within the DNA. Two DNMT inhibitors approved for human use (discussed below) are based on this mechanism [7].

This review is divided into three main sections. Current DNMT inhibitors are discussed first, followed by molecular modeling studies toward the understanding of the mechanism of action of known DNMT inhibitors at the molecular level. Finally, successful

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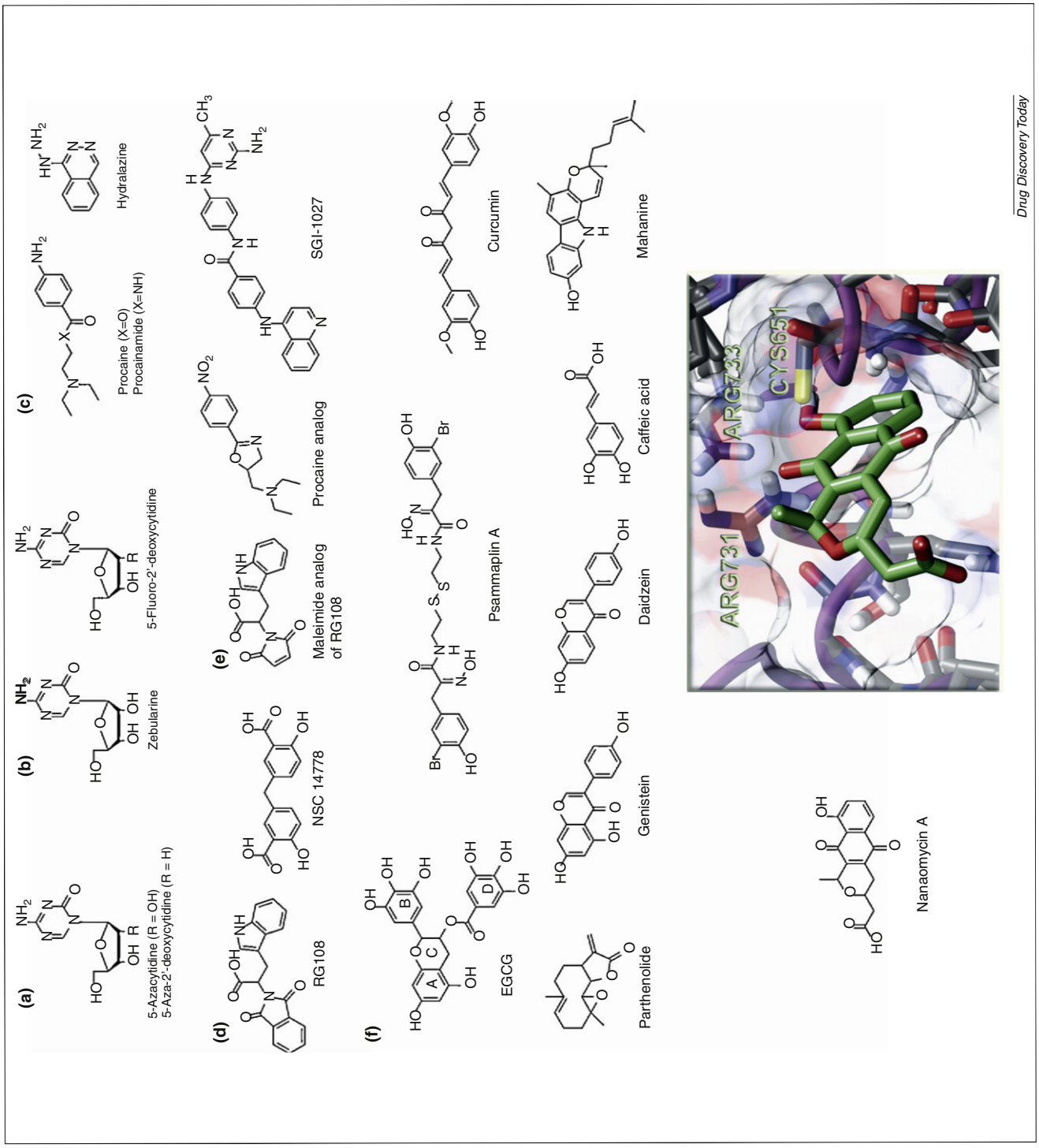

FIGURE 3

Chemical structures of representative DNMT inhibitors and other selected compounds with proposed demethylating activity. **(a)** Nucleoside analogs approved by the FDA. **(b)** Nucleoside analogs in clinical development. **(c)** Drugs used for other indications. **(d)** Compounds identified using virtual screening. **(e)** Synthetic compounds (part of optimization programs). **(f)** Natural products; a binding model reported for nanaomycin A with DNMT3B is also shown [48].

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virtual screening studies to identify novel small molecule inhibitors are reviewed.

DNMT inhibitors

DNMT inhibitors can be classified into two broad categories. One category includes 'mechanistic inhibitors', such as nucleosides and nucleoside derivatives, which are incorporated into DNA and act as suicide inhibitors via a covalent adduct formation. The second category is the non-nucleoside inhibitors [7,9].

To date, only two nucleoside analogs, 5-azacytidine and 5-aza-2′-deoxycytidine, have been developed clinically. These drugs, after incorporation into DNA, cause covalent trapping and subsequent depletion of DNMTs [8,12]. Aza nucleosides are approved by the Food and Drug Administration (FDA) for the treatment of myelodysplastic syndrome, where they demonstrate significant, although usually transient, improvement in patient survival, and are also currently being tested in many solid cancers [15,16]. These drugs have relatively low specificity and are characterized by substantial cellular and clinical toxicity [8]. The exact mechanism of antitumor action for these drugs (by demethylation of aberrantly silenced growth regulatory genes, induction of DNA damage, or other mechanisms) also remains unclear [17,18]. These issues have prompted the urgent need to identify novel and more specific DNMT inhibitors.

Representative DNMT inhibitors and other candidate demethylating agents are depicted in Fig. 3. Promising nucleoside analogs are zebularine and 5-fluoro-2′-deoxycytidine. These are mechanism-based inhibitors that mimic reactive intermediates in the mechanism of methylation. Some of the non-nucleoside DNMT inhibitors are approved drugs for other indications (e.g. the antihypertensive drug hydralazine [19], the local anesthetic procaine [20], and the antiarrhythmic drug procainamide [21]). Constrained analogs of procaine have been reported [22]. A synthetic quinoline-based compound, SGI-1027, has been described as a DNMT1, DNMT3A and DNMT3B inhibitors [23]. SAH analogs presumably binding in the co-factor binding pocket have also been reported as selective inhibitors toward DNMTs [24,25]. The antisense oligodeoxynucleotide MG98 has also been proposed as a DNMT inhibitor [26]. Other DNMT inhibitors have been identified by docking-based virtual screening. Examples of such compounds, reviewed in detail below, include the L-tryptophan derivative RG108 [27], the methylenedisalicylic acid NSC 14778, and NSC 319745 [28]. Synthetic analogs of RG108 have also been recently reported [29] (Fig. 3), and some of these and other DNMT inhibitors are reviewed elsewhere [30,31].

Natural products as DNMT inhibitors

Several natural products are associated with DNA methylation inhibition, highlighting their potential for cancer chemoprevention [32–34]. The main polyphenol compound from green tea, (−)-epigallocathechin-3-gallate (EGCG), has been proposed to inhibit DNMT1 by blocking the active site of the enzyme, leading to reactivation of methylation-silenced genes in cancer cells [35,36]. Other tea polyphenols, such as catechin and epicathechin, and the bioflavonoids quercetin, fisetin, and myricetin, have also been implicated in DNA methylation inhibition. The catechol containing dietary epicathechin has been suggested to inhibit DNA methylation indirectly owing to the increased formation of SAH, as a consequence of cathechol-*O*-methyltransferase (COMT)-mediated *O*-methylation of epicathechin [36]. Other dietary cathecols that might inhibit DNA methylation by a similar mechanism are the polyphenols from coffee: caffeic acid and chlorogenic acid [37]. Apple polyphenols [38], the major isoflavone from soybean genistein and two other isoflavones (biochanin A and daidzein [39]) have also been reported to have demethylating activity. Mahanine, a plant-derived carbazole alkaloid, has been reported to induce the Ras-association domain family 1 (*RASSF1*) gene in human prostate cancer cells, presumably by inhibiting DNMT activity [40]. Psammaplin A and several other disulfide bromotyrosine derivatives isolated from the marine sponge *Pseudoceratina purpurea* have been described as potent inhibitors of DNMT1 [41]. Other marine natural products with reported DNMT1 inhibitory activity are the peyssonenynes A and B isolated from the red alga *Peyssonnelia caulifera* [42]. Parthenolide, the principal sesquiterpene lactone of feverfew, has been reported to inhibit DNMT1 [43]. Curcumin, the major component of the Indian curry spice turmeric, has been reported to inhibit M.SssI, an analog of DNMT1 [44] (Fig. 3). However, more recent studies showed that curcumin did not cause DNA demethylation in three arbitrarily chosen human cancer cell lines [45].

Mitoxantrone, an antitumor antibiotic and inhibitor of topoisomerase II, has been reported to inhibit DNA methylation in cell-based assays [46]. The antibiotic mithramycin A has also been described to inhibit DNA methylation [47]. More recently, nanaomycin A (Fig. 3), a quinone antibiotic isolated from a culture of *Streptomyces*, has been described as the first non-SAH analog acting as a DNMT3B-selective inhibitor that induces genomic demethylation. Nanaomycin A treatment reduced the global methylation levels in three cell lines and reactivated transcription of the *RASSF1A* tumor suppressor gene [48].

Public repositories with DNMT inhibitors

Chemical databases annotated with bioactivity data are an increasingly important information source available to the scientific community. At the time of writing (November, 2010), ChEMBL (http://www.ebi.ac.uk/chembldb/index.php) contained the chemical structures and activity data of 88 compounds screened with 'DNA (cytosine-5)-methyltransferase 1' (target ID: 321). The Binding Database (http://www.bindingdb.org) contained information for 51 compounds screened for inhibition of 'DNA (cytosine-5)-methyltransferase' and 44 compounds screened for inhibition of 'DNA (cytosine-5)-methyltransferase 3B'. These databases can be searched online. In addition, the chemical structures along with bioactivity data can be downloaded to conduct structure–activity relationships (SAR) and other chemoinformatic studies discussed below. It is anticipated that an increasing number of DNMT inhibitors will be stored in these public databases.

Binding models of active compounds

Several molecular modeling studies published thus far have significantly helped in the understanding of the activity of established DNMT inhibitors at the molecular level (Table 1). Such studies are discussed below.

One of the first contributions of molecular modeling to the research of DNMT inhibitors was the development of a homology model of the catalytic domain of DNMT1 [49]. Given that currently

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TABLE 1

DNMT inhibitors studied using molecular modeling and/or discovered through virtual screening.

Molecule	Target	Computational studies	Major insights of the study	Ref.
Hydralazine	M.HhaI	Docking	Non-covalent inhibitor; hydrogen-bond network with lysine and arginine residues	[55]
	DNMT1	Docking and MD	Non-covalent stabilization with residues involved in the mechanism of methylation; similar interactions as nucleoside analogs	[53]
Procaine	DNMT1	Docking	Non-covalent inhibitor with binding mode similar to 2′-deoxycytidine and procainamide	[53]
Procainamide	DNMT1	Docking	Non-covalent inhibitor with binding mode similar to 2′-deoxycytidine and procaine	[53]
Maleimide analog of RG108	M.HhaI	Docking	Alkylates the catalytic cysteine with the maleimide moiety	[29]
EGCG	DNMT1	Homology modeling and docking	Non-covalent inhibitor; gallate moiety is crucial for binding	[35,36]
Parthenolide	DNMT1	Homology modeling and docking	Alkylates the catalytic cysteine with the γ-methylene lactone	[43]
Curcumin	DNMT1	Homology modeling and docking	Alkylates the catalytic cysteine with the α-carbon atom of the *bis*-α,β-unsaturated ketone or competes with the cofactor	[44]
Mithramycin A	DNMT1	Docking	The trisaccharide moiety can fit into the cytosine pocket	[47]
Nanaomycin A	DNMT3B	Homology modeling and docking	Alkylates the catalytic cysteine with the α,β-unsaturated carbonyl system; non-covalent stabilization with residues involved in methylation	[48]
RG108	DNMT1	Docking-based virtual screening	One of the top-ranked hits; non-covalent inhibitor with a new scaffold	[27]
NSC 14778	DNMT1	Multistep docking-based virtual screening	One of the top-ranked consensus hits; non-covalent inhibitor with a new scaffold	[28]

known DNMTs show extensive conservation of their catalytic domain [50], the available crystal structures of bacterial MTase M.HhaI (PDB: 6MHT) [13], M.HaeIII (PDB: 1DCT) [51] and the human DNMT2 (PDB: 1G55) [52] were used to construct a homology model of the catalytic domain of DNMT1. The model showed not only a significant conformational preservation of the catalytic site, but also unique features. Differences included a replacement of a histidine amino acid (His1459) by an alanine at the corresponding position in both bacterial MTase, different side-chain conformations of two arginines (Arg1310 and Arg1312) in human DNMT1 and bacterial MTase, and the charged amino acids that are close to the active site. In the same work, the homology model was used to design N4-fluoroacetyl-5-azacytidine, which showed efficient inhibition of DNA methylation in human tumor cell lines [49].

The homology model described above was also used to develop a binding mode of hydralazine (Fig. 3) with the catalytic domain of DNMT1 using docking and molecular dynamics (MD) [53]. The docking protocol used was validated by predicting and comparing the binding mode of substrate 2′-deoxycytidine and its analogs 5-azacytidine and 5-aza-2′-deoxycytidine. The docking-based binding modes of the nucleoside analogs were in agreement with the proposed mechanism of methylation discussed in Fig. 2. Docking and MD simulations of hydralazine showed similar interactions within the binding pocket as nucleoside analogs, namely, a complex network of hydrogen bonds with arginine and glutamic acid residues that also have a major role in the mechanism of DNA methylation. In the same work, the putative binding models of procaine and procainamide (Fig. 3) were explored, with the conclusion that the predicted binding modes of these two drugs are similar to those of 2′-deoxycytidine and other deoxycytidine analogs; for example, the carbonyl oxygen of procainamide and procaine occupy approximately the same position as that of the oxygen of the ribose ring of 2′-deoxycytidine [53]. However, it has been proposed that procaine and procanamide bind to GC-rich DNA sequences [20]. The binding mode of hydralazine with bacterial MTase M.HhaI (PDB: 5MHT) [54] has also been studied using docking [55], concluding that the four nitrogen atoms of hydralazine might form a hydrogen-bond network with lysine and arginine amino acids [55].

The maleimide analog of RG108 (Fig. 3) showed improved enzymatic inhibitory activity with DNMT1 over RG108 [29]. The binding mode of the maleimide was docked into the catalytic site of bacterial MTase M.HhaI (PDB: 1M0E) [56]. In the binding model, the carboxylate and carbonyl groups of the maleimide formed hydrogen bonds with arginine residues. In addition, the maleimide moiety is positioned close to the catalytic cysteine, suggesting a putative conjugate addition of a thiol to the maleimide [29]. The binding mode of this compound with human DNMT1 remains to be explored.

Several molecular modeling and docking studies have been reported with EGCG (Fig. 3) and have been reviewed recently [33]. In one study [35], a homology model of the catalytic domain of DNMT1 was constructed using the crystal structure of bacterial MTase M.HhaI (PDB: 5MHT) as a template. EGCG and five analogs were docked into the putative cytosine pocket of the homology model. In the binding model, the gallate moiety of EGCG forms hydrogen bonds with glutamate and proline residues that appear

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to stabilize the flipped cytosine through hydrogen bonding and participate in the mechanism of methylation. The D ring of EGCG occupies a similar binding pocket as the pyrimidyl ring of cytosine in the structural model of DNMT1, and the catalytic cysteine makes additional hydrogen bonds with hydroxyl groups in the A and B rings of EGCG [35]. In a later study, EGCG was also docked into the catalytic site of a homology model of DNMT1, which was constructed from M.HhaI (PDB: 5MHT). Results also suggested the formation of hydrogen bonds between the gallate moiety and a glutamate residue that participate in the mechanism of methylation [36].

To rationalize the activity of parthenolide (Fig. 3), this natural product was docked into the catalytic site of a homology model of DNMT1, therein constructed based on M.HhaI (PDB: 5MHT) [43]. A notable feature of the binding model proposed is that the γ-methylene lactone of parthenolide overlaps with the C-5 atom of the modeled cytosine ring in the catalytic site; the γ-carbon atom of parthenolide is close to the *S*-atom of the catalytic cysteine. These results suggest that parthenolide inhibits DNMT1 through alkylation of the catalytic cysteine [43]. A similar mechanism of inhibition was proposed for curcumin based on a docking study with a homology model of the catalytic site of DNMT1 [44]. In the docking model, the α-carbon atom of the *bis*-α,β-unsaturated ketone is close to the catalytic cysteine [44]. Suicide inhibition without toxic damage in genomic DNA has been proposed as an attractive strategy to develop DNMT inhibitors [57]. A docking study with mithramycin A has been published suggesting that this natural product binds into the catalytic domain of DNMT1 [47].

As discussed earlier, nanaomycin A (Fig. 3) is the first reported non-SAH analog acting as a DNMT3B-selective inhibitor [48]. To rationalize at the molecular level the activity of the natural product, in that study nanaomycin A was docked with a homology model of the catalytic site of DNMT3B [48]. The homology model of DNMT3B was constructed using the crystal structure of DNMT3A (PDB: 2QRV) as a template [58]. In the binding model, the carboxylic acid, hydroxyl group and adjacent carbonyl oxygen atoms were predicted to form an extensive hydrogen-bond network with the side chains of arginine amino acids. Additionally, the hydroxyl group of nanaomycin A formed a hydrogen bond with the side chain of a glutamic acid residue. Interestingly, similar hydrogen bond interactions with the equivalent glutamic acid and arginine residues that participate in the mechanism of methylation were not observed in the docking studies with DNMT1. These results provide a possible structural explanation for the enzyme selectivity of the natural product [48]. Furthermore, based on the binding model of nanaomycin A with DNMT3B, along with the experimental and theoretical evidence of the reaction between quinones and cysteine-rich proteins, it was hypothesized that the catalytic cysteine performs a nucleophilic Michael 1,4 addition to the α,β-unsaturated carbonyl system of nanaomycin A. Interestingly, a similar plausible reaction was not observed in the binding model with DNMT1, which further provided a theoretical basis to the experimental selectivity toward DNMT3B [48].

A protein dynamics study of the catalytic domains of four DNMTs upon binding of SAM has been recently published [59]. In this study, the apo protein, SAM-bound and SAH-bound complexes were studied for crystal structures of DNMT3A (PDB: 2QRV), DNMT2 (PDB: 1G55) and homology models of DNMT1 and DNMT3B. The homology models of DNMT1 and DNMT3B were constructed based on the crystal structures of the catalytic domains of bacterial HhaI (PDB: 2HMY) and DNMT3A, respectively. No dramatic conformational changes were observed upon the SAM or SAH binding in any of the four DNMTs on a nanosecond scale. It was also concluded that cytosine DNMTs appear to use internal dynamic events to facilitate the cofactor binding for the catalysis [59].

Virtual screening for small molecule inhibitors

Until now, most compounds associated with DNA methylation inhibition have been identified fortuitously, and even fewer have been validated as directly targeting DNMTs *in vitro* or *in vivo*. Limited studies have been reported thus far to explore systematically the DNMT inhibition properties of large compound databases. Experimental characterization of hundreds or thousands of small molecules as demethylating agents would be a daunting task in terms of cost and time. To accelerate systematic screening, computational techniques, including virtual screening, are increasingly being used to identify active compounds.

Screening of the National Cancer Institute database

Application of a docking-based virtual screening approach, with a validated homology model of DNMT1 [49], has been published [27]. In a first study, 1990 compounds available from the Diversity Set of the National Cancer Institute (NCI; http://dtp.nci.nih.gov/) were the starting point for a virtual screening. Compounds with undesirable size, hydrophobicity and uncommon atom types were filtered out. Two top-scoring compounds were experimentally tested that showed activity both *in vitro* and *in vivo*. RG108 (Fig. 3) showed an $IC_{50} = 0.60$ μM with M.SssI [27]. Additional characterization showed that this L-tryptophan derivative did not cause covalent enzyme trapping and that the carboxylate group has an essential role in the binding with the enzyme because the analog without this moiety is inactive [60].

In a follow-up study, a larger set of the NCI database totaling over 260,000 compounds was the starting point to identify additional DNMT1 inhibitors [28]. To focus the computational screening on compounds that could be promising for further development, the authors selected a subset of approximately 65,000 lead-like molecules. The NCI lead-like set was further filtered using a high-throughput virtual screening protocol. As part of the screening, three docking programs were used. Favorable docking scores from all three docking approaches were pooled to create a total of 24 consensus compounds. Of the 24 molecules that were identified, 13 were obtained for experimental testing. Seven out of the 13 consensus hits had detectable DNMT1 inhibitory activity in biochemical assays. Further experimental characterization of active compounds showed that six out of these seven inhibitors appeared selective for DNMT1 and did not inhibit the DNMT3B methyltransferase. The methylenedisalicylic acid derivative, NSC 14778 (Fig. 3), showed an $IC_{50} = 92$ μM with DNMT1 and an $IC_{50} = 17$ μM with DNMT3B. The observed potency was comparably low for most test compounds, which was partially attributed to the high amount of protein used in the biochemical assay. Despite the low potency, the virtual screening was successful in that it identified diverse scaffolds that were not previously reported for DNMT inhibitors and that represent

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excellent candidates for optimizing their inhibitory activity and selectivity.

Screening of natural products

As discussed above, natural products are attractive sources of DNMT inhibitors. Active compounds from natural sources can be drug candidates themselves or can be the starting point of optimization programs [32]. In an initial effort to screen systematically the large chemical space covered by compounds of natural origin and identify potential DNMT1 inhibitors, a public collection of natural products was screened computationally [45]. The starting point was a collection with more than 89,000 natural products obtained from a public database. A selected subset of 14,053 lead-like compounds was subjected to a multistep docking-based virtual screening approach with a homology model of the catalytic site of DNMT1. A set of 58 consensus hits was identified. Interestingly, one of the hits was a methylenedisalicylic acid derivative that also was identified as a hit in the virtual screening of the NCI database previously reported [28]. The experimental evaluation of the natural products that were consensus hits is ongoing [45].

Liu *et al.* [44] have reported the docking-based screening of a set of 44 natural germacrolides with a homology model of DNMT1. It was concluded that γ-methylactone compounds could be effective inhibitors, and these studies resulted in the discovery of pathenolide discussed above.

Concluding remarks

DNA methylation represents a central mechanism for mediating epigenetic gene regulation, and the development of DNMT inhibitors provides novel opportunities for cancer therapy and other diseases. Limited but informative crystallographic structures of DNMT3A, DNMT3L and DNMT2 are currently available. Recent structural studies of the catalytic site of DNMTs using molecular dynamics simulations are starting to shed light on the dynamic behavior of these enzymes. Despite the current lack of a crystal structure of the catalytic domain of DNMT1, one of the most attractive isoforms for experimental cancer therapies, several homology models have been constructed. Docking and molecular dynamics simulations with homology models of DNMT1, DNMT3B and crystallographic structures of other isoforms have helped to rationalize, at the molecular level, the enzymatic activity of several natural products and other small-molecule inhibitors, including hydralazine and RG108.

Several of these studies have generated hypotheses that can guide future experimental and theoretical studies directed to increase current knowledge of the mechanism of inhibition and optimization of DNMT inhibitors. Successful docking-based virtual screenings have been conducted with DNMT1 yielding novel scaffolds as starting points for optimization programs. The binding models developed for active compounds with DNMT1 or DNMT3B might serve as a basis for structure-guided optimization cycles. These efforts can be complemented with ligand-based approaches, such as similarity searching and pharmacophore modeling. The last two approaches are currently unexploited for DNMT inhibitors. Ligand-based methods, including SAR studies, can benefit from the information stored in public molecular databases, where activity information for DNMT inhibitors is starting to be deposited. Taken together, computational studies conducted with DNMTs and DNMT inhibitors form part of the multidisciplinary and synergistic efforts to further develop DNMTs as promising therapeutic agents for the treatment of cancer and other diseases.

Conflict of interest

The authors declare that they do not have any conflict of interest related to this manuscript.

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