ELSEVIER

feature

DNA methyltransferases: emerging targets for the discovery of inhibitors as potent anticancer drugs

Jie Yu[‡], **Tianli Xie**[‡], **Zhe Wang, Xuwen Wang, Su Zeng, Yu Kang,** yukang@zju.edu.cn **and Tingjun Hou,** tingjunhou@zju.edu.cn

DNA methyltransferases (DNMTs) are a conserved family of cytosine methylases with crucial roles in epigenetic regulation. They have been considered as promising therapeutic targets for the epigenetic treatment of cancer. Therefore, DNMT inhibitors (DNMTis) have attracted considerable interest in recent years for the modulation of the aberrant DNA methylation pattern in a reversible way. In this review, we provide a structure-based overview of the therapeutic importance of DNMTs against different cancer types, and then summarize recently investigated DNMTis as well as their inhibitory mechanisms, focusing on recent advances in the development of DNMTis with specificity and/or selectivity using computational approaches.

Introduction

As one of the most-studied epigenetic phenomena, DNA methylation has been widely proven to be associated with cancer initiation and progression [1], which is typically mediated by DNMTs, which catalyze the transfer of a methyl group to the carbon-5 position of cytosine ring from *S*-adenosyl methionine (SAM), yielding *S*-adenosyl-l-homocysteine (SAH) as an end product [2]. Three active DNMTs in mammals, namely DNMT1, DNMT3A, and DNMT3B [3], have been investigated for over 30 years. Generally, DNMT1 was widely considered responsible for the maintenance of DNA methylation pattern in DNA replication [4], whereas DNMT3A and DNMT3B were then known as two *de novo* methyltransferases that establish new DNA methylation patterns by catalyzing the methylation of unmethylated DNA elements with the help of the catalytically inactive DNMT3 L [5,6]. It was recently recognized that this functional classification of DNMTs might be oversimplified, and that the multiple roles for DNA methylation in mammalian epigenetics depend on the dynamic regulation and targeting of DNMTs modulated by other proteins or nucleic acids [7]. More recently, a new *de novo* DNMT, named DNMT3C, with specialized activity required for mouse fertility, was identified in murine germ cells [8]. Such new results expand and deepen the scope of investigations on DNMTs and their multiple regulating mechanisms.

Many studies have shown that epigenetic disruptions caused by DNMT abnormalities are associate3d with various human diseases, including autoimmune diseases [9], neurological disorders [10], diabetes [11], and, in particular, cancer. Inhibition of DNMTs leads to a reduction in tumor formation in part through the reactivation of tumor suppressor genes (TSGs) [12]. The development of DNMTis has been widely believed to provide opportunities for novel cancer therapy. However, currently only two DNMTis (azacitidine and decitabine) have been approved by the US Food and Drug Administration (FDA); however, such nucleoside analogs function by forming a suicidal covalent complex with DNMTs and demonstrate cytotoxicity [13]. Thus, many efforts have been made to discover non-nucleoside DNMTis using different strategies, although this is a challenge. Most non-nucleoside DNMTis lack adequate activity against DNMTs or sufficient impact on TSG

https://doi.org/10.1016/j.drudis.2019.08.006

re-expression [14]. Recent studies have shown emerging interest in the development of selective DNMTis with better specificity and minimal off-target effects, but their mechanisms of inhibition need to be determined. Computational approaches have an indispensable role in the identification and optimization of DNMTis, as well as in understanding the complicated inhibitory mechanisms of DNMTis at the molecular level [15–18]. Here, we review the molecular structures and related biological functions of active DNMTs, and summarize recently investigated DNMTis as well as their inhibitory mechanisms, focusing on recent advances in selective DNMTi development resulting from molecular simulation techniques.

Structure and binding partners of DNMTs

DNMTs share similar domain architecture with two functional components, an N-terminal regulatory domain and a C-terminal catalytic domain (Fig. 1a) [2,8]. For the three active DNMTs, the C-terminal domain has a common core structure involved in cofactor binding (motifs I and X) and substrate catalysis (motifs IV, VI and VIII), which is suggestive of a common mechanism of catalysis. DNMT3L does not have the essential motifs for DNA and cofactor binding (motifs IX and X) and, therefore, is catalytically inactive [6]. DNMT3C, the new member of DNMT family but with specialized activity, also shows characteristics of conserved methyltransferase motifs [8]. The N-terminal regulatory domain of DNMT1 differs from that of DNMT3A/B; it contains several subdomains that have an important role in the recognition of hemi-methylated DNA (Fig. 1b), whereas those of DNMT3A and DNMT3B only have two defined functional domains, for H3K4me0 and nucleosome recognition (Fig. 1c).

It was recently shown that the catalytic activity of DNMTs could be under allosteric control of the N-terminal domain with autoinhibitory function, more precisely the RFTS and CXXC domains in DNMT1 and the ADD domain in DNMT3A [7]. The detailed allosteric mechanism of DNMTs is reviewed elsewhere [7]. Thus, modulation of the various domains of DNMTs might regulate the activity and specificity of the enzyme via allosteric effects, providing a wider perspective for DNMTi development. Moreover, DNMT activity can be modulated by several interaction partners, including PCNA, UHRF1 protein, HDACs, histone-lysine methyltransferases (e.g., G9a, SUV39H1, EZH2, and SETDB1), transcription factors (e.g., c-Myc), and other proteins [7]. These protein–protein interactions (PPIs) are also relevant to the regulation of the N-terminal domain and to the conformational change of the catalytic domain, which could shed light on the future design of PPI inhibitors.

The catalytic pockets of DNMTs are highly conserved, which poses a challenge for the direct design of potent substrate and/or cofactor competitors toward a specific DNMT isoform. The structural alignment of the catalytic pockets of the crystal structures of hDNMT1 [Protein Data Bank (PDB) code: 4WXX] [19] and hDNMT3A (PDB code: 4U7T) [20] illustrates that several different residues can form different interactions with the ligands and contribute to the selectivity of certain inhibitors, including Val1580/Trp893, Asn1578/Arg891, Trp1170/Cys666, and Met1169/Val665 (Fig. 1d). A recent study discussed in detail the molecular basis of the binding selectivity of inhibitors towards DNMT1 and DNMT3A through computational approaches, which confirmed several residue pairs as the key factors responsible for the binding selectivity of DNMTis [21].

Therapeutic importance of DNMTs

Accumulated studies have shown that DNA methylation changes in many cancer types. The different contribution of each DNMT in various cancers highlights the potential advantage of selective inhibitors for a certain DNMT isoform depending on the tumor type [22]. For example, numerous functional experiments have shown that *DNMT3A* mutations exert an overwhelming influence in hematological malignancies [23]. These mutations, mainly localized at the Arg882 residue of the C-terminal catalytic domain, are most likely associated with a loss of enzymatic function [24]. In breast cancer, *DNMT3B* has shown significantly higher expression changes compared with *DNMT1* and *DNMT3A* [25]. Thus, DNMT3B was then considered to have a predominant role over DNMT3A and DNMT1 in breast cancer, pointing to the design of selective DNMT3B inhibitors as therapy for breast cancer [26–28].

To date, there remains controversy over which DNMT isoform would be the best target in different diseases [29]. Therefore, it is necessary to develop selective inhibitors toward a specific DNMT isoform, which could identify the most appropriate DNMT isoform to target in cancer cells. For example, in colon cancer, a SAM-competitive compound, SGI-1027, was reported to inhibit the activity of the three active DNMTs, whereas an unexpected observation suggested that treatment of different cancer cell lines with SGI-1027 resulted in selective degradation of DNMT1 with minimal or no effects on DNMT3A and DNMT3B [30]. In hepatocellular carcinoma (HCC), no evident correlation of a specific DNMT isoform with this type of cancer was observed. Given that DNA methylation is a dynamic process with ongoing methylation and demethylation, in which DNMTs work together to achieve the multiple functions of methylation [7], inhibitors that concomitantly inhibit DNMT1 and DNMT3s might have better efficacy in some cases.

Inhibitors of DNMTs

Several mechanisms can be exploited to inactivate DNMT activity with small molecules, including: (i) DNA incorporation and enzyme trapping; (ii) binding with DNA; (iii) DNA and/or SAM competition; (iv) allosteric inhibition; and (v) targeting the PPIs [31]. The nucleoside inhibitors related to mechanism (i) are mainly derivatives of cytidine, but their clinical development is hindered by this mechanism of action. Therefore, more research is focusing on the design and development of non-nucleoside DNMTis, which would make use of various structure details and mechanisms of action of DNMTs. To date, most non-nucleoside DNMTis have been designed to target the catalytic domain via mechanism (iii), including DNA competitors, SAM competitors, and dual substrate competitors, which combine the structures of two substrate analogs. Other strategies to inhibit DNMT activity also have aroused interest recently, such as targeting the allosteric sites that regulate enzyme activity or the PPIs required for the interactions of DNMTs with their partners; however, more research is still needed.

Over the past 15 years, an increasing number of non-nucleoside inhibitors have been obtained from different sources: (i) drug repurposing (approved for other indications): hydralazine (antihypertensive) [32], procainamide (antiarrhythmic) [33], and procaine (local anesthetic) [34]; (ii) natural products: EGCG [35], nanaomycin A [36], genistein [37], laccaic acid A [38], and other natural products [39]; (iii) molecules discovered by virtual screening (VS): RG108 [15], NSC14778, NSC319745, and NSC137546 [17], and DC_05 [16]; (iv) molecules identified by experimental high-throughput screening (HTS): 3-chloro-3-nitroflavanones [40], diclone [41], and SW155246 [42]; and (v) synthesized molecules: SGI-1027 and its derivatives [30,43,44], RG108 analogs [45–47], NSC compound analogs [48,49], and Δ^2-Isoxazoline derivatives [50]. So far, except for the two FDA-approved nucleoside inhibitors, only another two nucleoside analogs are under assessment in clinical trials (SGI-110 in Phase III and 5-F-CdR in Phase I) [51,52]. In addition, MG98, an oligonucleotide antisense inhibitor of DNMT1, was recently in a Phase I study [51]. For

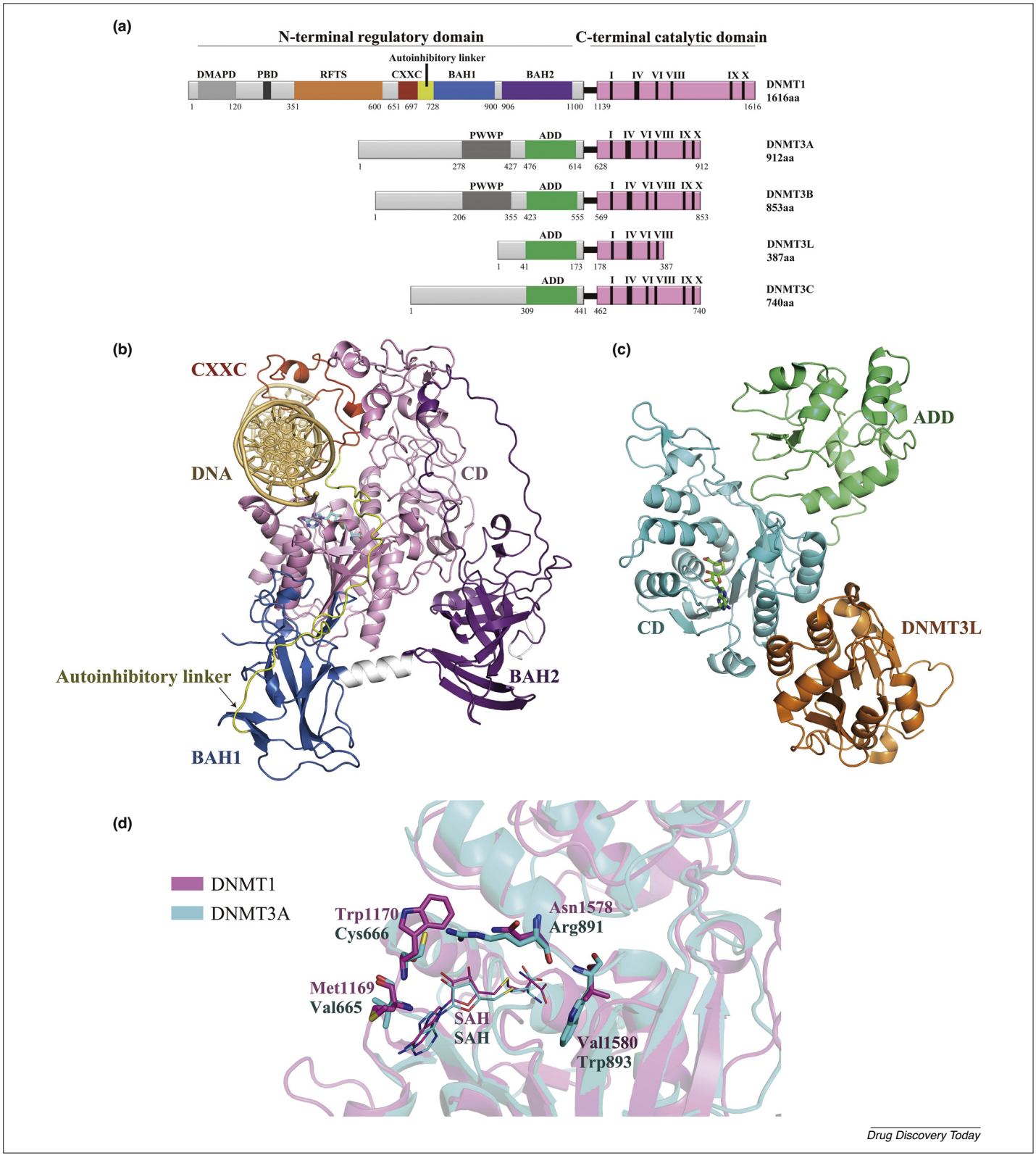

FIGURE 1

Title. **(a)** Domain architecture of the mammalian DNMTs. **(b)** Structural overview of the mDNMT1-DNA complex bound to *S*-adenosyl-l-homocysteine (SAH) [Protein Data Bank (PDB) code: 3PT6], the SAH is shown as sticks. **(c)** Structural overview of the hDNMT3A-DNMT3L complex bound to SAH (PDB code: 4U7P), the SAH is shown as sticks. **(d)** Structural superposition of the catalytic pockets in the crystal structure of hDNMT1 (PDB code: 4WXX) and hDNMT3A (PDB code: 4U7T). Comparison of the comparatively important pairs of different residues in the binding sites are shown as sticks.

non-nucleoside DNMTis, other than hydralazine, which is currently in a Phase III trial, and EGCG, which is in a Phase II clinical trial as anticancer drugs, other non-nucleoside DNMTis remain in preclinical studies [51,52]. The chemical structures of the most studied DNMTis are summarized in Fig. 2, and the recently reported potent and/or selective non-nucleoside DNMTis are shown in Fig. 3, along with their inhibitory activities (IC_{50}/EC_{50}), which are further summarized in Table 1. It appears that the potency and selectivity of novel non-nucleoside DNMTis have partly improved, but most still have relatively low bioactivity at micromolar levels and/or weak DNA demethylation capability in cells. Thus, the development of novel DNMTis is receiving increasing attention and >100 compounds have been tested as DNMTis [53]. Several earlier discovered DNMTis have been reviewed elsewhere [29,54], encouraging further efforts to design potent and selective DNMTis. Here, we focus

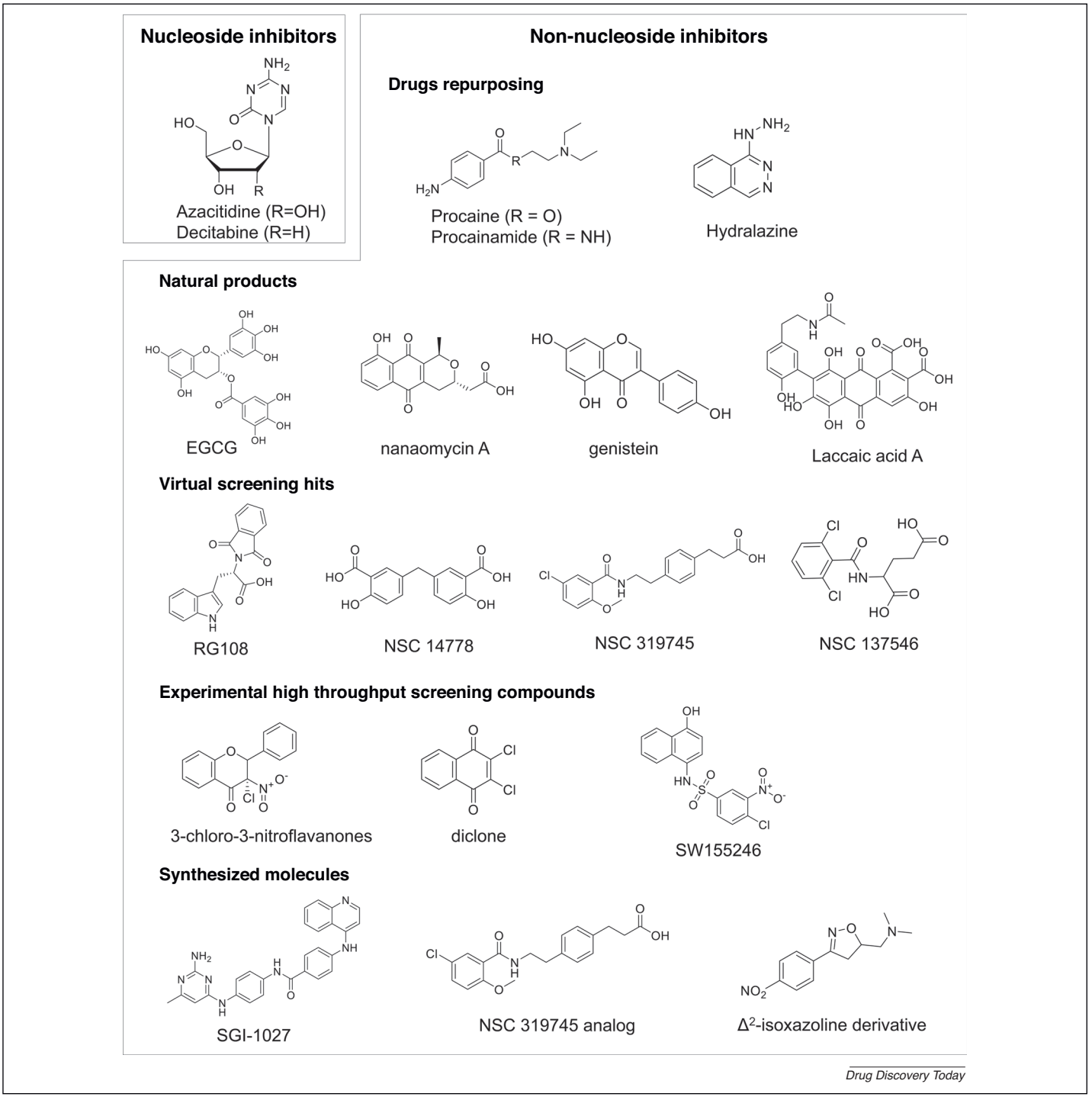

FIGURE 2

Chemical structures of the most studied DNA methyltransferase inhibitors (DNMTis) classified by the method used to reveal them.

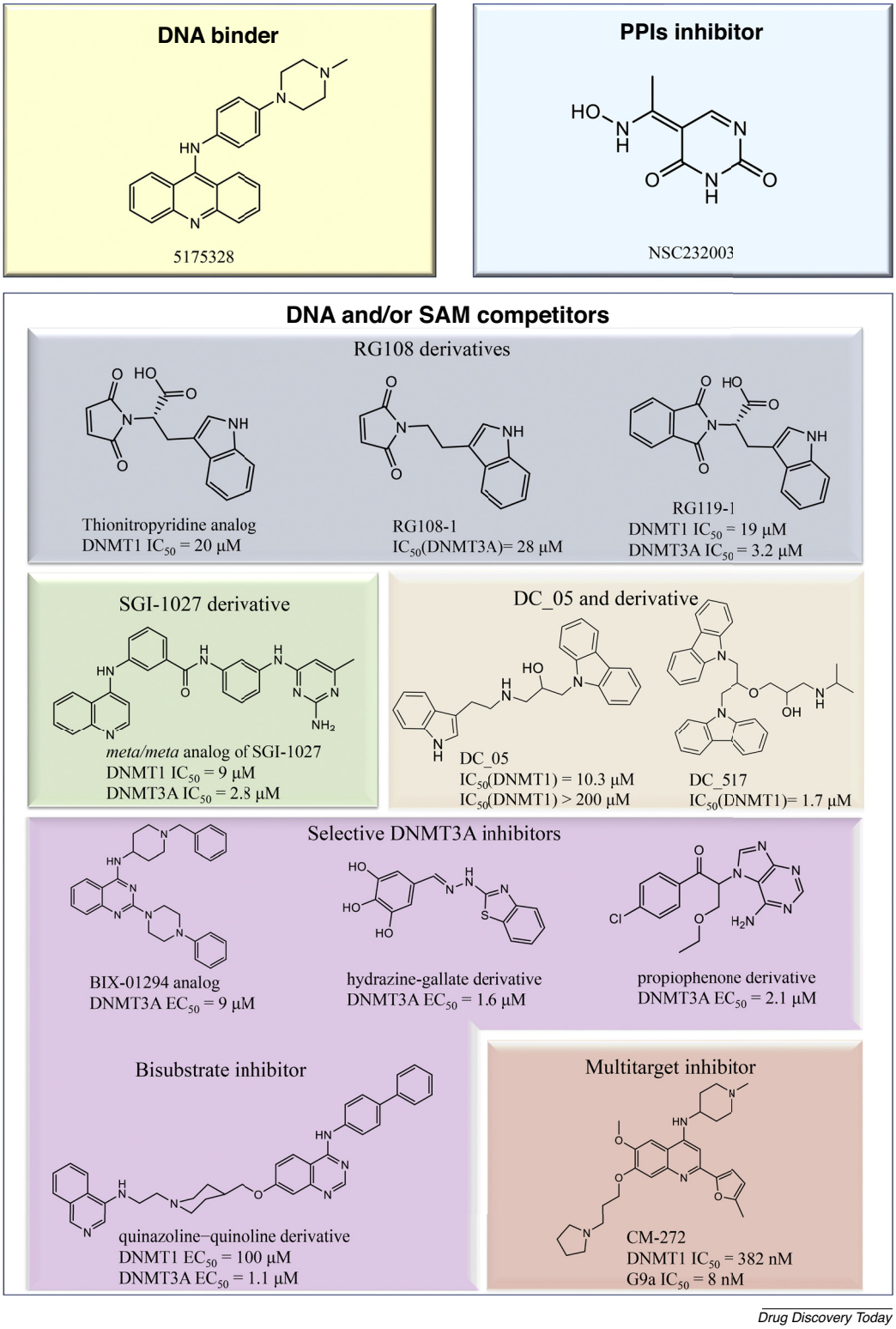

FIGURE 3

Chemical structures of the recently reported more potent and/or selective non-nucleoside DNA methyltransferase inhibitors (DNMTis) with their IC_{50}/EC_{50} values classified based on their action modes: DNA binders, protein–protein interacting (PPI) inhibitors, or DNA and/or *S*-adenosyl methionine (SAM) competitors. The group of DNA and/or SAM competitors are further listed with different colors for clarity.

TABLE 1

Summary of recently reported potent and/or selective non-nucleoside DNMTis and their biological properties

Compound	Source	Enzymatic inhibition, (IC_{50}/EC_{50}, μM)[a]	Mechanism	Effect	Refs
Acridine compound (5175328)	High-throughput cell-based screening	Not described	DNA binder	Reactive TSGs in MiaPaCa2 and RKO cells	[55]
NSC232003	VS	Not described	PPIs inhibitor	Disrupts DNMT1/UHRF1 interaction in U251 glioma cells	[56]
RG108	VS	IC_{50}(DNMT1) = 390; IC_{50}(DNMT3A/3L) = 315	SAM competitor	Reactive TSGs in HCT116 cells	[15]
RG108 thionitropyridine analog	Lead optimization	IC_{50}(DNMT1) = 20	DNA and SAM competitor	Cytotoxicity on DU145 cell line	[46]
RG108-1	Lead optimization	IC_{50}(DNMT3A) = 28	SAM competitor	Not described	[45]
RG119-1	Lead optimization	IC_{50}(DNMT1) = 19; IC_{50}(DNMT3A) = 3.2	SAM competitor	Cytotoxicity on mesothelioma cells	[47]
SGI-1027	Synthetic compounds	IC_{50}(DNMT1) = 6; IC_{50}(DNMT3A) = 8; IC_{50}(DNMT3B) = 7.5	DNA competitor	Reactive *TIMP3, MLH1,* and *P16* in HCT116 cells	[30]
SGI-1027 meta/meta analog	Lead optimization	IC_{50}(DNMT1) = 9; IC_{50}(DNMT3A) = 2.8	DNA competitor	Comparable activity as SGI-1027 but with less toxicity	[43]
DC_05	VS	IC_{50}(DNMT1) = 10.3; IC_{50}(DNMT3A)>200	DNA and/or SAM competitor	Cytotoxicity on HCT116 and Capan-1 cells	[16]
DC_517	Lead optimization	IC_{50}(DNMT1) = 1.7	DNA and/or SAM competitor	High antiproliferative effects in HCT116 and Capan-1 cells	[16]
BIX-01924 analog	Lead optimization	EC_{50}(DNMT3A) = 9	DNA and/or SAM competitor	High antiproliferative effects in human lymphoma U-937 and RAJI cells	[57]
Hydralazine-gallate derivative	Enzymatic screening and optimization	EC_{50}(DNMT3A) = 1.6	DNA and/or SAM competitor	Reactivates reporter gene in leukemia KG-1 cells	[58]
Propiophenone derivatives	HTS	EC_{50}(DNMT3A) = 2.1	Covalent bond with catalytic cysteine	Cytotoxicity on leukemia KG-1 and colon cancer HCT116 cell lines	[59]
Bisubstrate-type inhibitor	Synthetic compounds	EC_{50}(DNMT1) = 100; EC_{50}(DNMT3A) = 1.1	DNA and SAM competitor	Reactive CDKN2A in HCT116	[60]
CM-272	Lead optimization	IC_{50}(DNMT1) = 0.382	DNA competitor (dual inhibitor of G9a/DNMTs)	Prolongs survival in *in vivo* models of hematological malignancies	[61]

[a] The IC_{50}/EC_{50} values of inhibitors tested under different experimental conditions are not considered to be directly comparable, because these could be influenced by the choice of the C5 DNA methyltransferase, the concentration of the cofactor, the concentration and nature of the DNA duplex, and the method of detection [54].

mainly on the most recent advances in the discovery of novel inhibitors targeting DNMTs through lead optimization, VS, HTS, and computer-aided drug design (CADD). We also emphasize the increasingly important role of computational approaches in discovering DNMTis, guiding lead optimization, and understanding the biological functions of DNMTis.

Chemical synthesis and optimization

Chemistry optimization has contributed to optimize existing inhibitors obtained from VS, HTS, or other approaches, leading to the identification of more potent and/or selective inhibitors. Computational approaches and resolved crystal structures were used to explain the selectivity or potency improvement observed by biological assays, and also help to guide lead optimization [46,47,55,56]. Several typical examples of structure-guided optimization of DNMTis are provided elsewhere [57], and also discussed here. In 2006, Siedlecki *et al.* discovered the first DNMT1 inhibitor RG108 (*N*-phthaloyl-L-tryptophan) by VS based on a homology model of hDNMT1 [15], which is considered to be the starting point for lead optimization of DNMTis. Based on this most investigated inhibitor, several derivatives with improved potency were subsequently designed and synthesized, such as the maleimide derivative (RG108-1) [45] and the constraint analog (thionitropyridine analog) [46]. The putative binding model of the constraint analog was predicted by induced-fit docking, and the simulation results indicated that it is positioned in both the SAM and DNA pockets, thereby explaining its higher inhibition activity compared with RG108 [46]. More recently, Rondelet *et al.* studied the inhibition mechanism of the maleimide analogs of RG108 against DNMTs, which suggested a competitive mode of action with the SAM cofactor. They synthesized RG119-1, a tryptamine derivative that presents better enzymatic and cellular activities (low micromolar) compared with RG108-1 [47], as shown in Fig. 3 and Table 1. Noncovalent and covalent docking studies provided detailed insights into their binding modes and revealed essential residues for the stabilization of such compounds inside DNMTs [47]. Other VS hits, such as NSC14778, NSC319745, and NSC137546, were also further optimized for more promising derivatives [48,49]. More recently, the resynthesized VS hits, NSC14778 and NSC106084, were found to be inactive against DNMTs [58], which can be explained by the difference of assay conditions used, such as the method of detection, concentration of the cofactor, and the concentration and nature of the DNA duplex [54]. Another representative example is SGI-1027, a quinoline-based compound that was described by Datta *et al.* in 2009. It inhibits the activity of DNMT1, DNMT3A, and DNMT3B and results in the re-expression of

some silenced TSGs in colon cancer cell lines [30]. It was quickly considered as the starting point to design more promising derivatives [43,44]. Among these derivatives, the meta/meta analogs showed substantial improvement in potency (Table 1) [43]. Biophysical studies revealed that SGI-1027 and its derivatives interact with DNA and result in SAM-noncompetitive but DNA-competitive inhibition [59].

Besides optimizing the existing DNMTis, Rotili *et al*. disclosed a series of quinazoline-based DNMTis based on the chemical modification of the G9a/GLP inhibitor BIX-01294 [56]. Interestingly, these compounds inhibited DNMT3A at a relatively low micromolar level (EC_{50} = 935 μM) but could not inhibit DNMT1 and G9a effectively [56]. The binding modes of the two most potent compounds in the binding site of DNMT3A were explored by molecular docking studies [56].

Multitarget approaches are also promising strategies to develop inhibitors with enhanced anticancer activity because of the crosstalk between DNA and histone methylation [31]. In 2017, San José-Enériz *et al.* postulated that inhibitors simultaneously targeting G9a and DNMTs might be an improved strategy because of the crosstalk between DNA and H3K9 methylation. They reported the discovery of a quinoline-based compound CM-272 as the inhibitor of G9a (IC_{50} = 8 nM) and DNMT1 (IC_{50} = 382 nM) with *in vivo* efficacy in acute myeloid leukemia (AML), acute lymphoblastic leukaemia (ALL), and diffuse large B cell lymphoma xenogeneic models [55]. Molecular docking studies were used to guide the rational design of inhibitors. In addition, enzymatic competition assays confirmed that the compound is a DNA-competitive inhibitor but not a SAM competitor of DNMT1. This study of a multitarget inhibitor might represent a novel strategy in cancer therapeutics.

In 2017, Halby *et al*. designed a group of quinazoline-quinoline derivatives as potent inhibitors of DNMT3A and DNMT1 [60]. These novel bisubstrate-type inhibitors targeted both the cytidine and AdoMet binding pockets by mimicking SAH and deoxycytidine, and linking them together, with some showing certain isoform selectivity. In addition, a new scaffold (a quinazoline-quinoline conjugate scaffold) could be used as a promising starting point for the development of selective inhibitors of DNMTs.

Another recent example also showed the development of DNMT is with isoform selectivity. In 2016, Erdmann *et al*. found a novel hydrazine-gallate derivative through chemical optimization based on enzymatic screening and structure–activity relationship (SAR) studies (Table 1) [61]. This compound was able to inhibit DNMT3A with micromolar potency (EC_{50} = 1.6 μM), but did not inhibit DNMT1; moreover was able to reactivate the expression of the luciferase gene in leukemia KG-1 cells but without substantial cytotoxicity. DNMT3A selectivity was supported by a docking study revealing that the compound is stabilized in the catalytic pocket by interacting with Trp893 and Arg891, which is consistent with the observation of the structural alignment we proposed earlier (Fig. 1d).

High-throughput screening

Advances in HTS and development of DNMTis have been reviewed previously [62]. Here, we focus on the most recent examples of the application of HTS to the discovery of novel DNMTis. In 2013, Hossain *et al*. reported four acridine compounds previously identified by high-throughput cell-based screening. These were able to inhibit DNMT1 *in vitro* and rapidly reactivated methylated TSGs through binding the DNA [63]. Given that acridine compounds would not require covalent incorporation into DNA for their activity, demethylation could be observed after only one round of cell division, whereas nucleoside analogs inhibit DNMTs after at least two rounds of cell division. However, as DNA binders, these compounds would be at risk of interfering with other DNA enzymes. In 2015, Erdmann *et al*. identified a family of propiophenone derivatives by HTS based on an enzymatic assay against the catalytic DNMT3A [64], in which a new inhibitor with an EC_{50} of 2.1 μM was obtained after chemical optimization. The new propiophenone derivative containing a highly reactive Michael acceptor was able to inhibit DNMT3A by forming a covalent bond with the catalytic cysteine of the enzyme. Covalent non-nucleoside inhibitors can be considered as the most powerful type of compounds because they could form a covalent bond with the azanucleoside inhibitors, without the drawbacks of incorporation into DNA.

Computer-aided drug design

Over the past 15 years, CADD techniques have been widely used to discover novel non-nucleoside DNMTis. Many DNMTis have been found by docking-based or pharmacophore-based VS. Furthermore, molecular modeling techniques have become efficient tools to use to study the functions of DNMTs and the interactions between DNMTs and DNMTis. Early applications of computational approaches to develop DNMTis have been reviewed by Medina-Franco *et al*. [62], where the authors surveyed the applications of several *in silico* approaches such as molecular docking, VS, pharmacophore modeling, molecular dynamics (MD) simulations, and similarity searching to advance the development of DNMTis. Most recently, efforts through CADD techniques have been devoted to systematically screen natural products as DNMTis [39]. For example, DC_05 was identified by VS as a selective DNMT1 inhibitor, and was further optimized to a more potent inhibitor (DC_517, Table 1). Another interesting example is a natural product, nanaomycin A, which was initially identified by VS against DNMT1 and then revealed as a selective inhibitor toward DNMT3B by bioassays [36]. Here, we provide a detailed summary of the latest development of the *in silico* design of targeting DNMTs.

In 2015, Maldonado-Rojas *et al*. developed an innovative computational strategy to identify DNMTis [65]. First, they built a quantitative (Q) SAR model with linear discriminant analysis (LDA) based on 47 compounds with known activities against DNMTs, and then identified six natural products as potential DNMTis through a multistep computational screening strategy by combining the ligand-binding domain (LBD)-QSAR model and molecular docking using AutoDock Vina and Surflex-Dock. Unfortunately, the *in vitro* DNMT activities of these six compounds were not tested in the study.

In 2016, Joshi *et al*. developed inhibitors targeting the 5-methylcytosine (5-mC) binding site of hDNMT1 because of its crucial role in the recognition of hemi-methylated DNA [66]. They generated a structural model of hDNMT1 in active form, and performed multiple nanosecond-timescale MD simulations followed by ensemble-based VS. Two compounds (ASINEX ID: BAS 12771472 and BAS 00872020) were discovered to inhibit hDNMT1 *in vitro*, one of which also showed *in vivo* activity [66]. This study was an innovative attempt to validate a unique site of hDNMT1 that could be harnessed for rational design of highly selective and potent hypomethylating agents.

In 2017, Krishna *et al*. designed a workflow by integrating various ligand-based and structure-based approaches to discover new DNMT1 inhibitors. First, they established a pharmacophore model based on 85 DNMT1 inhibitors by using the Hypogen module of Discovery Studio 4.1, and then virtually screened the Maybridge chemical library based on the pharmacophore model, a Naïve Bayesian classification model, and ensemble docking. Three out of the ten tested compounds showed DNMT1 inhibitory activity at a compound concentration of 20 μM [67]. Hassanzadeh *et al*. developed a

ligand-based pharmacophore model based on known nucleoside-derived DNMTis and virtually screened a database containing 500 small non-nucleoside molecules [68]. The interactions between the selected molecules and the substrate binding site of DNMT1 were then investigated by molecular docking [68]. After *in vitro* DNA inhibition assays, one novel compound was identified to selectively inhibit DNMT1 with IC_{50} of 4.1 μM, whereas its inhibition towards DNMT3 was at least 50-fold weaker. Miletić *et al.* designed an adenosyl-1-methyl-pyrimidin-2-one derivative as the lead compound [18], and a quantum mechanics/molecular mechanics (QM/MM) analysis showed that the lead compound was able to form a covalent adduct with the active site of DNMT1 upon binding. The authors then analyzed 69 modifications in the lead compound structure to create a family of mechanism-based inhibitors toward DNMT1. Finally, 18 out of the presented 69 modifications were used to prepare a family of highly specific DNMT1 inhibitors over DNMT3A [18].

With the better understanding of the molecular basis of PPIs in DNMT-involving complexes, disruption of such PPIs represents an attractive strategy for cancer treatment. Recently, an uracil derivative, NSC232003, was found through VS to inhibit *in vitro* DNA methylation by disrupting the DNMT1/UHRF1 interaction at a cellular level (Table 1) [69]. More such inhibitors could be expected in the near future.

Other related molecular modeling studies

Molecular modeling techniques are also used to study conformational changes of DNMTs. In 2018, Ye *et al.* conducted a mechanistic investigation of the dynamic transition of DNMT1 [70]. They performed long-term MD simulations and found that two mutations allosterically reduced enzymatic activities *in vitro* by decreasing kcat/Km for SAM by disturbing the proper structural transition of DNMT1 between the two conformation states.

Another conformational dynamics study of DNMT3A was performed with the application of elastic network models coupled with information theory, the Protein Structure Network, and sequence evolution analysis [71]. The authors suggested that the DNMT3A homo-dimer acts as the basic functional unit and the dimer interface of DNMT3A provides essential features for the functionality of the enzyme, which mediates important allosteric regulation in both the autoinhibitory and active states of the DNMT3A/3L complex. These studies shed new light on the design of allosteric DNMTis, and more allosteric sites are expected to be investigated in the near future.

Concluding remarks and future perspectives

DNMTs have been identified as promising therapeutic targets for the treatment of epigenetic-related cancer and other diseases. Several crystal structures of human and murine DNMTs with different domains in complex with DNA, cofactors, and its analogs have been solved over the past decade, in which only one with small-molecule reversible inhibitor is currently available (sinefungin, PDB code: 3SWR). On the one hand, it reflects the challenge of identifying characteristics of more SAM-binding site inhibitors, and the corresponding structure-based mechanism of the (non)selective DNMTis-mediated DNA methylation. On the other hand, many crystallographic studies have provided important information for understanding the autoinhibitory mechanism regulated by certain key residues on the N-terminal parts, which offers new thoughts on ways to regulate DNMT activity via allosteric controls.

Currently, although the shortcomings of the nucleoside DNMTis have been widely recognized, they remain the main DNMTis used in the clinic for the treatment of epigenetics-related cancer. In recent years, the design of novel non-nucleoside small molecule DNMTis has aroused continuous interest, but none have been pushed into clinical trials. The major focus of non-nucleoside DNMTis discovery remains the highly conserved SAM-binding pocket, an approach that does not appear particularly successful, given the low level of activity obtained in cells and the failure of compounds in clinical trials. It is necessary to design and develop novel DNMTis based on our deeper understanding of the structure-based function of active DNMTs. Furthermore, given that the various DNMT isoforms have different roles in different cancers, designing selective inhibitors towards DNMT1 or DNMT3s would become more crucial for the identification of DNMT isoforms as the 'best' therapeutic targets for certain diseases. Although controversy remains about whether targeting certain DNMT isoforms or working against all DNMTs would have better efficacy, especially for different cancers or other diseases, attention has been paid to the investigation of DNMTi selectivity and specificity, perhaps the most important issues that need to be addressed currently. A combination of multiple molecular modeling and CADD techniques is believed to be an indispensable way in improve the success rate of structure-based mechanism investigations and rational DNMTi design.

Acknowledgments

This study is supported by National Science & Technology Major Project of China 'Key New Drug Creation and Manufacturing Program' (2018ZX09711002-007), the National Natural Science Foundation of China (21603189, 21575128, and 81773632), and Zhejiang Provincial Natural Science Foundation of China (LZ19H300001).

References

1 Esteller, M. (2008) Epigenetics in cancer. *N. Engl. J. Med.* 358, 1148–1159
2 Jurkowska, R.Z. *et al.* (2011) Structure and function of mammalian DNA methyltransferases. *Chembiochem* 12, 206–222
3 Okano, M. *et al.* (1998) Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nat. Genet.* 19, 219–220
4 Goll, M.G. and Bestor, T.H. (2005) Eukaryotic cytosine methyltransferases. *Annu. Rev. Biochem.* 74, 481–514
5 Okano, M. *et al.* (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 99, 247–257
6 Jia, D. *et al.* (2007) Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation. *Nature* 449, 248–251
7 Jeltsch, A. and Jurkowska, R.Z. (2016) Allosteric control of mammalian DNA methyltransferases - a new regulatory paradigm. *Nucleic Acids Res.* 44, 8556–8575
8 Barau, J. *et al.* (2016) The DNA methyltransferase DNMT3C protects male germ cells from transposon activity. *Science* 354, 909–912
9 Teitell, M. and Richardson, B. (2003) DNA methylation in the immune system. *Clin. Immunol.* 109, 2–5
10 Weng, Y.L. *et al.* (2013) DNA modifications and neurological disorders. *Neurotherapeutics* 10, 556–567
11 Arguelles, A.O. *et al.* (2016) Are epigenetic drugs for diabetes and obesity at our door step? *Drug Discov. Today* 21, 499–509
12 Subramaniam, D. *et al.* (2014) DNA methyltransferases: a novel target for prevention and therapy. *Front. Oncol.* 4, 80
13 Stresemann, C. and Lyko, F. (2008) Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine. *Int. J. Cancer* 123, 8–13
14 Lopez, M. *et al.* (2016) DNA methyltransferase inhibitors: development and applications. *Adv. Exp. Med. Biol.* 945, 431–473
15 Siedlecki, P. *et al.* (2006) Discovery of two novel, small-molecule inhibitors of DNA methylation. *J. Med. Chem.* 49, 678–683
16 Chen, S. *et al.* (2014) Identifying novel selective non-nucleoside DNA methyltransferase 1 inhibitors through docking-based virtual screening. *J. Med. Chem.* 57, 9028–9041
17 Kuck, D. *et al.* (2010) Novel and selective DNA methyltransferase inhibitors: Docking-based virtual screening and experimental evaluation. *Bioorg. Med. Chem.* 18, 822–829
18 Miletic, V. *et al.* (2017) In silico design of the first DNA-independent mechanism-based inhibitor of

mammalian DNA methyltransferase Dnmt1. *PLoS ONE* 12, e0174410
19 Zhang, Z.M. *et al.* (2015) Crystal structure of human DNA methyltransferase 1. *J. Mol. Biol* 427, 2520–2531
20 Guo, X. *et al.* (2015) Structural insight into autoinhibition and histone H3-induced activation of DNMT3A. *Nature* 517 640–U281
21 Xie, T. *et al.* (2019) Insight into the selective binding mechanism of DNMT1 and DNMT3A inhibitors: a molecular simulation study. *Phys. Chem. Chem. Phys.* 21, 12931–12947
22 Zhang, W. and Xu, J. (2017) DNA methyltransferases and their roles in tumorigenesis. *Biomark. Res.* 5, 1
23 Yang, L. *et al.* (2015) DNMT3A in haematological malignancies. *Nat. Rev. Cancer* 15, 152–165
24 Russler-Germain, D.A. *et al.* (2014) The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. *Cancer Cell* 25, 442–454
25 Girault, I. *et al.* (2003) Expression analysis of DNA Methyltransferases 1, 3A, and 3B in sporadic breast carcinomas. *Clin. Cancer Res.* 9, 4415–4422
26 Butcher, D.T. and Rodenhiser, D.I. (2007) Epigenetic inactivation of BRCA1 is associated with aberrant expression of CTCF and DNA methyltransferase (DNMT3B) in some sporadic breast tumours. *Eur. J. Cancer* 43, 210–219
27 Roll, J.D. *et al.* (2008) DNMT3b overexpression contributes to a hypermethylator phenotype in human breast cancer cell lines. *Mol. Cancer* 7, 15
28 Zhang, L. and Long, X.H. (2015) Association of BRCA1 promoter methylation with sporadic breast cancers: evidence from 40 studies. *Sci. Rep.* 5, 17869
29 Xu, P. *et al.* (2016) DNA methyltransferase inhibitors: an updated patent review (2012-2015). *Expert Opin. Ther. Pat.* 26, 1017–1030
30 Datta, J. *et al.* (2009) A new class of quinoline-based DNA hypomethylating agents reactivates tumor suppressor genes by blocking DNA methyltransferase 1 activity and inducing its degradation. *Cancer Res.* 69, 4277–4285
31 Castillo–Aguilera, O. *et al.* (2017) DNA methylation targeting: the DNMT/HMT crosstalk challenge. *Biomolecules* 7, 3
32 Cornacchia, E. *et al.* (1988) Hydralazine and procainamide inhibit T-Cell DNA methylation and induce autoreactivity. *J. Immunol.* 140, 2197–2200
33 Lee, B.H. *et al.* (2005) Procainamide is a specific inhibitor of DNA methyltransferase 1. *J. Biol. Chem.* 280, 40749–40756
34 Villar-Garea, A. *et al.* (2003) Procaine is a DNA-demethylating agent with growth-inhibitory effects in human cancer cells. *Cancer Res.* 63, 4984–4989
35 Fang, M.Z. *et al.* (2003) Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res.* 63, 7563–7570
36 Kuck, D. *et al.* (2010) Nanaomycin A selectively inhibits DNMT3B and reactivates silenced tumor suppressor genes in human cancer cells. *Mol. Cancer Ther.* 9, 3015–3023
37 Fang, M.Z. *et al.* (2005) Reversal of hypermethylation and reactivation of p16(INK4a) RAR beta, and MGMT genes by genistein and other isoflavones from soy. *Clin. Cancer Res* 11, 7033–7041
38 Fagan, R.L. *et al.* (2013) Laccaic acid A is a direct, DNA-competitive inhibitor of DNA methyltransferase 1. *J. Biol. Chem.* 288, 23858–23867
39 Saldivar–Gonzalez, F.I. *et al.* (2018) Inhibitors of DNA methyltransferases from natural sources: a computational perspective. *Front. Pharmacol.* 9, 1144
40 Ceccaldi, A. *et al.* (2011) C5-DNA methyltransferase inhibitors: from screening to effects on zebrafish embryo development. *ChemBiochem* 12, 1337–1345
41 Ceccaldi, A. *et al.* (2013) Identification of novel inhibitors of DNA methylation by screening of a chemical library. *ACS Chem. Biol.* 8, 543–548
42 Kilgore, J.A. *et al.* (2013) Identification of DNMT1 selective antagonists using a novel scintillation proximity assay. *J. Biol. Chem.* 288, 19673–19684
43 Valente, S. *et al.* (2014) Selective non-nucleoside inhibitors of human DNA methyltransferases active in cancer including in cancer stem cells. *J. Med. Chem.* 57, 701–713
44 Rilova, E. *et al.* (2014) Design, synthesis and biological evaluation of 4-amino-N(4-aminophenyl) benzamide analogues of quinoline-based SGI-1027 as inhibitors of DNA methylation. *ChemMedChem* 9, 590–601
45 Suzuki, T. *et al.* (2010) Design, synthesis, inhibitory activity, and binding mode study of novel DNA methyltransferase 1 inhibitors. *Bioorg. Med. Chem. Lett.* 20, 1124–1127
46 Asgatay, S. *et al.* (2014) Synthesis and evaluation of analogues of N-phthaloyl-L-tryptophan (RG108) as inhibitors of DNA methyltransferase 1. *J. Med. Chem.* 57, 421–434
47 Rondelet, G. *et al.* (2017) Inhibition studies of DNA methyltransferases by maleimide derivatives of RG108 as non-nucleoside inhibitors. *Future Med. Chem.* 9, 1465–1481
48 Kabro, A. *et al.* (2013) Preparation of phenylethylbenzamide derivatives as modulators of DNMT3 activity. *MedChemComm* 4, 1562–1570
49 Garella, D. *et al.* (2016) Design and synthesis of N-benzoyl amino acid derivatives as DNA methylation inhibitors. *Chem. Biol. Drug Des.* 88, 664–676
50 Castellano, S. *et al.* (2011) Synthesis and biochemical evaluation of delta(2)-Isoxazoline derivatives as DNA methyltransferase 1 inhibitors. *J. Med. Chem* 54, 7663–7677
51 Pechalrieu, D. *et al.* (2017) DNA methyltransferase inhibitors in cancer: From pharmacology to translational studies. *Biochem. Pharmacol.* 129, 1–13
52 Pan, Y. *et al.* (2018) DNA methylation profiles in cancer diagnosis and therapeutics. *Clin. Exp. Med.* 18, 1–14
53 Naveja, J.J. and Medina-Franco, J.L. (2018) Insights from pharmacological similarity of epigenetic targets in epipolypharmacology. *Drug Discov. Today* 23, 141–150
54 Erdmann, A. *et al.* (2015) Targeting DNA methylation with small molecules: what's next? Miniperspective. *J. Med. Chem.* 58, 2569–2583
55 Jose-Eneriz, E.S. *et al.* (2017) Discovery of first-in-class reversible dual small molecule inhibitors against G9a and DNMTs in hematological malignancies. *Nat. Commun.* 8, 15424
56 Rotili, D. *et al.* (2014) Properly substituted analogues of BIX-01294 lose inhibition of G9a histone methyltransferase and gain selective anti–DNA methyltransferase 3A Activity. *PLoS ONE* 9, e96941
57 Erdmann, A. *et al.* (2016) Structure-guided optimization of DNA methyltransferase inhibitors. In *Epi-Informatics* (Medina-Franco, J.L., ed.), pp. 53–73, Academic Press
58 Leroy, M. *et al.* (2019) Synthesis of NSC 106084 and NSC 14778 and evaluation of their DNMT inhibitory activity. *Bioorg. Med. Chem. Lett.* 29, 826–831
59 Gros, C. *et al.* (2015) New insights on the mechanism of quinoline-based DNA Methyltransferase inhibitors. *J. Biol. Chem.* 290, 6293–6302
60 Halby, L. *et al.* (2017) Rational design of bisubstrate-type analogues as inhibitors of DNA methyltransferases in cancer cells. *J. Med. Chem.* 60, 4665–4679
61 Erdmann, A. *et al.* (2016) Identification and optimization of hydrazone- gallate derivatives as specific inhibitors of DNA methyltransferase 3A. *Future Med. Chem.* 8, 373–380
62 Medina-Franco, J.L. *et al.* (2015) Discovery and development of DNA methyltransferase inhibitors using in silico approaches. *Drug Discov. Today* 20, 569–577
63 Hossain, M.Z. *et al.* (2013) DNA-intercalators causing rapid re-expression of methylated and silenced genes in cancer cells. *Oncotarget* 4, 298–309
64 Erdmann, A. *et al.* (2015) Design and synthesis of new non nucleoside inhibitors of DNMT3A. *Bioorg. Med. Chem.* 23, 5946–5953
65 Maldonado-Rojas, W. *et al.* (2015) Computational fishing of new DNA methyltransferase inhibitors from natural products. *J. Mol. Graph. Model.* 60, 43–54
66 Joshi, M. *et al.* (2016) Ensemble-based virtual screening and experimental validation of inhibitors targeting a novel site of human DNMT1. *Chem. Biol. Drug Des.* 88, 5–16
67 Krishna, S. *et al.* (2017) Identification of potent inhibitors of DNA methyltransferase 1 (DNMT1) through a pharmacophore-based virtual screening approach. *J. Mol. Graph. Model.* 75, 174–188
68 Hassanzadeh, M. *et al.* (2017) Discovery of novel and selective DNA methyltransferase 1 inhibitors by pharmacophore and docking-based virtual screening. *Chemistryselect* 2, 8383–8392
69 Myrianthopoulos, V. *et al.* (2016) Tandem virtual screening targeting the SRA domain of UHRF1 identifies a novel chemical tool modulating DNA methylation. *Eur. J. Med. Chem.* 114, 390–396
70 Ye, F. *et al.* (2018) Biochemical studies and molecular dynamic simulations reveal the molecular basis of conformational changes in DNA methyltransferase-1. *ACS Chem. Biol.* 13, 772–781
71 Liang, Z. *et al.* (2018) Deciphering the role of dimer interface in intrinsic dynamics and allosteric pathways underlying the functional transformation of DNMT3A. *Biochim. Biophys. Acta Gen. Subj.* 1862, 1667–1679

Jie Yu[‡]
Tianli Xie[‡]
Zhe Wang
Xuwen Wang
Su Zeng
Yu Kang*
Tingjun Hou*

College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, China

**Corresponding authors.*

[‡]These authors contributed equally.