We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,500
Open access books available

118,000
International authors and editors

130M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

1

Discovery and Optimization of Inhibitors of DNA Methyltransferase as Novel Drugs for Cancer Therapy

Jakyung Yoo and José L. Medina-Franco
Torrey Pines Institute for Molecular Studies
USA

1. Introduction

The genome contains genetic and epigenetic information. While the genome provides the blueprint for the manufacture of all the proteins required to create a living thing, the epigenetic information provides instruction on how, where, and when the genetic information should be used (Robertson, 2001). The major form of epigenetic information in mammalian cells is DNA methylation that is the covalent addition of a methyl group to the 5-position of cytosine, mostly within the CpG dinucleotide (Robertson, 2001). DNA methylation is involved in the control of gene expression, regulation of parental imprinting and stabilization of X chromosome inactivation as well as maintenance of the genome integrity. It is also implicated in the development of the immune system, cellular reprogramming and brain function and behaviour (Jurkowska et al., 2011). DNA methylation is mediated by a family of DNA methyltransferase enzymes (DNMTs). In mammals, three DNMTs have been identified so far in the human genome, including the two *de novo* methyltransferases (DNMT3A and DNMT3B) and the maintenance methyltransferase (DNMT1), which is generally the most abundant and active of the three (Goll and Bestor, 2005; Robertson, 2001; Yokochi and Robertson, 2002). DNMT3L is a related protein that has high sequence similarity with DNMT3A, 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 (Cheng and Blumenthal, 2008). The protein DNMT2 can also be found in mammalian cells. Despite the fact that the structure of DNMT2 is very similar to other DNMTs, its role is comparably less understood (Schaefer and Lyko, 2010). It has been reported that DNMT2 does not methylate DNA but instead methylates aspartic acid transfer RNA ($tRNA^{Asp}$) (Goll et al., 2006). Recent evidence suggests that DNMT2 activity is not limited to $tRNA^{Asp}$ and that DNMT2 represents a noncanonical enzyme of the DNMT family (Schaefer and Lyko, 2010).

DNMT1 is responsible for duplicating patterns of DNA methylation during replication and is essential for mammalian development and cancer cell growth (Chen et al., 2007). These enzymes are key regulators of gene transcription, and their roles in carcinogenesis have been the subject of considerable interest over the last decade (Jones and Baylin, 2007; Robertson, 2001). Therefore, specific inhibition of DNA methylation is an attractive and novel approach for cancer therapy (Kelly et al., 2010; Lyko and Brown, 2005; Portela and

Esteller, 2010; Robertson, 2001). It is worth noting that DNA methylation inhibitors have also emerged as a promising strategy for the treatment of immunodeficiency and brain disorders (Miller et al., 2010; Zawia et al., 2009).

The structure of mammalian DNMTs can be divided into two major parts, a large N-terminal regulatory domain of variable size, which has regulatory functions, and a C-terminal catalytic domain which is conserved in eukaryotic and prokaryotic carbon-5 DNMTs. The N-terminal domain guides the nuclear localization of the enzymes and mediates their interactions with other proteins, DNA, and chromatine. The smaller C-terminal domain harbors the active center of the enzyme and contains ten amino acids motifs diagnostic for all carbon-5 DNMTs (Jurkowska et al., 2011). Motifs I-III form the cofactor binding pocket, motif IV has the catalytic cysteine, motifs VI, VIII, and X compose the substrate binding site, and motifs V and VII form the target recognition domain (Sippl and Jung, 2009). Human DNMT1 has 1616 amino acids for which limited three-dimensional structural information is available. For example, just recently a crystal structure of human DNMT1 bound to duplex DNA containing unmethylated cytosine-guanine (CG) sites was published (Song et al., 2011). Further details of the structure of DNMTs and other available crystal structures of DNMTs are extensively reviewed elsewhere (Cheng and Blumenthal, 2008; Lan et al., 2010; Sippl and Jung, 2009).

The proposed mechanism of DNA cytosine-C5 methylation is summarized in Fig. 1 (Schermelleh et al., 2005; Sippl and Jung, 2009; Vilkaitis et al., 2001). DNMT forms a complex with DNA, and the cytosine which will be methylated flips out from the DNA. The thiol of the catalytic cysteine from motif IV 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 conducts a nucleophilic attack on the cofactor S-adenosyl-L-methionine (AdoMet) to form the 5-methyl covalent adduct and *S*-adenosyl-L-homocysteine (AdoHcy). The attack on the 6-position is assisted by a transient protonation of the cytosine ring at the endocyclic nitrogen atom N3, which is stabilized by a glutamate residue from motif VI. The same residue also contacts the exocyclic N4 amino group and stabilizes the flipped base. The carbanion may also be stabilized by resonance, where an arginine residue from motif VIII may participate in the stabilization of the cytosine base. 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.

DNA methylation inhibitors have been well characterized and tested in clinical trials (Issa and Kantarjian, 2009). To date, only 5-azacytidine and 5-aza-2'-deoxycytidine (Fig. 2) have been developed clinically. These two drugs are nucleoside analogues, which, after incorporation into DNA, cause covalent trapping and subsequent depletion of DNA methyltransferases (Schermelleh et al., 2005; Stresemann and Lyko, 2008). Aza nucleosides are approved by the Food and Drug Administration of the United States for the treatment of myelodysplastic syndrome, where they demonstrate significant, although usually transient improvement in patient survival and are currently being tested in many solid cancers (Issa et al., 2005; Schrump et al., 2006). Despite the clinical successes achieved with DNA methylation inhibitors, there is still need for improvement since aza nucleosides have relatively low specificity and are characterized by substantial cellular and clinical toxicity (Stresemann and Lyko, 2008). Their exact mechanism of antitumor action – demethylation of aberrantly silenced growth regulatory genes, induction of DNA damage, or other mechanism also remains unclear (Fandy et al., 2009; Issa, 2005; Palii et al., 2008).

Consequently, there is clear need to identify novel and more specific DNMT inhibitors that do not function via incorporation into DNA.

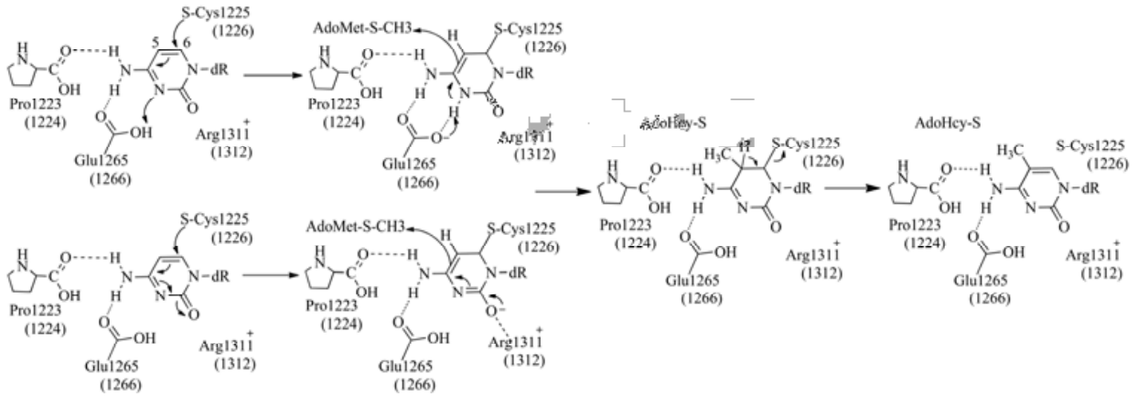


Fig. 1. Mechanism of DNA cytosine-C5 methylation. Amino acid residue numbers are based on the homology model. Equivalent residue numbers in parentheses correspond to the crystal structure.

There is now an increasing number of substances that are reported to inhibit DNMTs (Lyko and Brown, 2005). Selected DNMT inhibitors and other candidate demethylating agents are depicted in Fig. 2. Some of these compounds are approved drugs for other indications; i.e., the antihypertensive drug hydralazine (Segura-Pacheco et al., 2003), the local anaesthetic procaine (Villar-Garea et al., 2003), and the antiarrhythmic drug procainamide (Lee et al., 2005a). Others like the L-tryptophan derivative RG108, NSC 14778 (Fig. 2) have been identified by docking-based virtual screening (Kuck et al., 2010a; Siedlecki et al., 2006). Several natural products have been implicated in DNA methylation inhibition. Selected examples are the main polyphenol compound from green tea, (-)-epigallocathechin-3-gallate (EGCG) (Fang et al., 2003; Lee et al., 2005b), other tea polyphenols such as catechin and epicatechin, and the bioflavonoids quercetin, fisetin, and myricetin. Curcumin, the major component of the Indian curry spice turmeric, has been reported to inhibit the DNA cytosine C5 methyltransferase M.SssI, an analogue of DNMT1 (Liu et al., 2009). However, more recent studies showed that curcumin did not cause DNA demethylation in three arbitrarily chosen human cancer cell lines (Medina-Franco et al., 2011). Mahanine, a plant-derived carbazole alkaloid, and a fluorescent carbazole analogue, has been reported to induce the Ras-association domain family 1 (RASSF1) gene in human prostate cancer cells, presumably by inhibiting DNMT activity (Jagadeesh et al., 2007; Sheikh et al., 2010). Nanaomycin A, a quinone antibiotic isolated from a culture of *Streptomyces*, has been described as the first non *S*-adenosyl-L-homocysteine (AdoHcy/SAH) analogue 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 (Kuck et al., 2010b). These and several other natural products as putative demethylating agents are extensively reviewed elsewhere (Gilbert and Liu, 2010; Hauser and Jung, 2008; Li and Tollefsbol, 2010; Medina-Franco and Caulfield, 2011). While the substantial number of recent reports may suggest that many natural products inhibit DNA methylation, it should be noted that only a few reports provide compelling evidence for DNMT inhibition in biochemical and in cellular assays. As such, it

remains possible that many of these compounds have an indirect and fortuitous effect on DNA methylation, but do not show a pharmacologically relevant activity that can be developed further for therapeutic purposes (Medina-Franco et al., 2011).

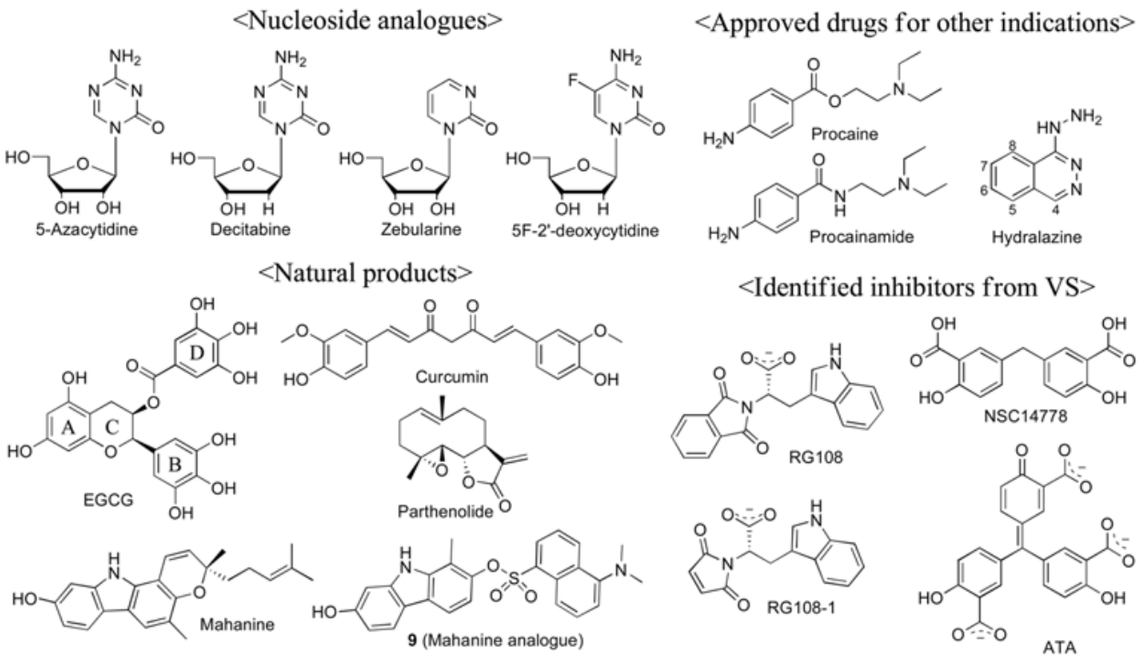


Fig. 2. Chemical structures of selected DNA methyltransferase inhibitors and other compounds with putative demethylating activity.

Until now most compounds associated with DNA methylation inhibition have been identified fortuitously. Remarkable exceptions are RG108 and 5,5′-methylenedisalicylic acid (NSC 14778) that were identified by computational screening followed by experimental evaluation (Kuck et al., 2010a; Siedlecki et al., 2006). In order to accelerate the discovery and optimization of new DNMT inhibitors, rational approaches are increasingly being used. To this end, *in silico* studies have significantly helped to understand the structure and function of DNMTs and the mechanism of DNMT inhibition (Medina-Franco and Caulfield, 2011). This chapter focuses on the different strategies ongoing in our and other research groups for the discovery and optimization of inhibitors of DNMTs with particular emphasis on *in silico* screening (section two) and *in silico* design (section three).

2. *In silico* screening of compound collections to identify novel inhibitors

In silico screening, also called in the literature, computational or virtual screening, consists of the computational evaluation of databases aiming to select a small number of reliable and experimentally testable candidate compounds that have a high probability of being active (Muegge, 2008; Shoichet, 2004). *In silico* screening is one of the most common rational approaches to guide the identification of new hits from large compound libraries. Hit identification using this approach requires several interactive steps that include (1) the compound collection, (2) the computational methods used for screening, and (3) the analysis of the output (López-Vallejo et al., 2011).

2.1 Screening databases

A number of compound databases from different sources can be used in *in silico* screening. These libraries may contain existing or hypothetical; i.e., virtual compounds. Libraries of existing compounds may be proprietary; e.g., in-house libraries, commercial, or public. The sources of screening libraries, with emphasis on libraries in the public domain, have been reviewed (Bender, 2010; Scior et al., 2007). Currently, the ZINC database is one of the most used libraries (Irwin and Shoichet, 2005). The type of screening library utilized should be closely associated with the objective of the particular screening campaign (Shelat and Guy, 2007). Chemically diverse libraries are particular attractive for identifying novel scaffolds for new or relatively unexplored targets such as DNMTs. If the goal is lead optimization, e.g., optimize the activity of known DNMT inhibitors (Fig. 2), focused libraries or collections with high inter-molecular similarity (highly dense libraries) are an attractive source.

2.1.1 Natural product databases

The presence of DNMT inhibitors in dietary products and commonly used herbal remedies (Gilbert and Liu, 2010; Hauser and Jung, 2008; Li and Tollefsbol, 2010) demonstrates the feasibility of identifying additional inhibitors of natural origin. Natural products have unique characteristics attractive for drug discovery. For example, the chemical structures of natural products are, in general, different from the chemical structures of synthetic compounds occupying different areas of chemical space (Ganesan, 2008; Medina-Franco et al., 2008; Singh et al., 2009b). In addition, natural products may be drug candidates themselves or may be the starting point for an optimization program (Ganesan, 2008; Hauser and Jung, 2008). Indeed several natural products are bioavailable, and the rationale of these observations has been recently provided (Ganesan, 2008). Fig. 3 shows a visual representation of the chemical space of natural products, drugs, and DNMT inhibitors. To compare the chemical space, a subset of 1,000 compounds was randomly selected from each database. The visual representation was obtained with principal component analysis (PCA) of the similarity matrix of the databases computed using Molecular ACCess System (MACCS) keys (166 bits) and the Tanimoto coefficient (Maggiora and Shanmugasundaram, 2011). The first three principal components are displayed in Fig. 3 and account for 79% of the variance. This figure clearly shows that most of the DNMT inhibitors, e.g., nucleoside analogues, RG108, RG108-1, procaine, procainamide, SG1027, and hydralazine, share the same chemical space of drugs. This observation is expected from inhibitors such as procaine, procainamide, and hydralazine. In contrast, NSC14778 and DNMT inhibitors from natural origin, EGCG and curcumin, are in a less-dense populated area of the chemical space of drugs. These compounds are characterized by containing one or more hydroxyl groups. Fig. 3b shows that most of the compounds in the natural product database also occupy this second region. Before conducting virtual and experimental screening, it is feasible to filter out natural products with potential toxicity issues using drug- or lead-like filters (Charifson and Walters, 2002).

2.1.2 Combinatorial libraries

Combinatorial libraries, either existing or virtual, are important sources of compound collections that can be used for *in silico* screening (López-Vallejo et al., 2011). Advances in synthetic approaches can generate *libraries from libraries*, *target-oriented* libraries, and *diversity-oriented* libraries which explore the chemical space in different ways (López-Vallejo

et al., 2011) and can be used in lead optimization or hit-identification, depending on the goals of the screening campaign.

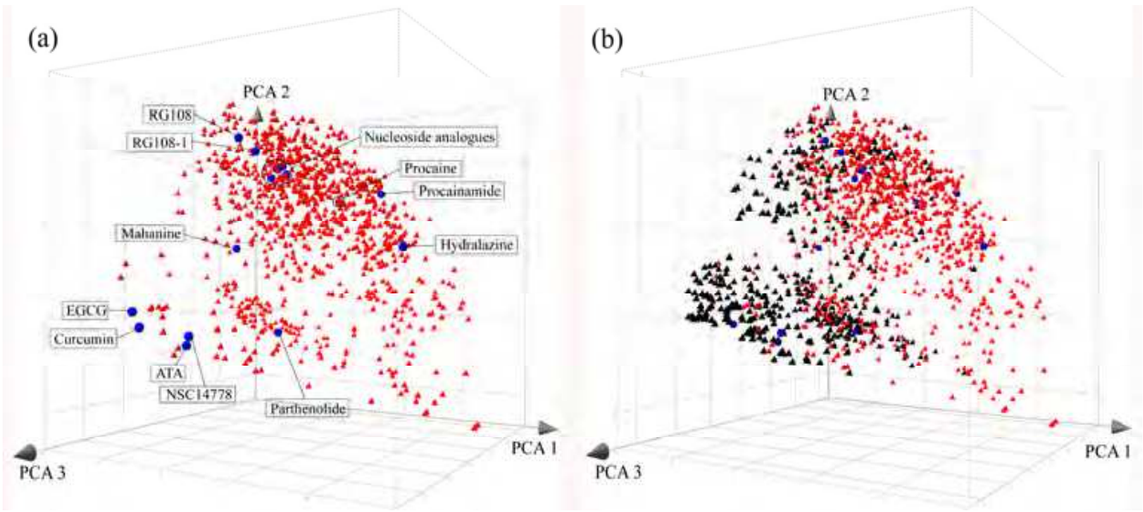


Fig. 3. Comparison of 486 natural products (black triangles), 1,000 drugs (red triangles), and 14 DNMT1 inhibitors (blue spheres). Depiction of a visual representation of the chemical space obtained by PCA of the similarity matrix computed using MACCS keys and Tanimoto similarity. The first three PCs account for 79% of the variance. (a) Comparison of drugs and DNMT1 inhibitors. (b) Comparison of drugs, natural products, and DNMT inhibitors.

2.2 Development and validation of computational approaches

In silico screening can be divided into two general strategies: ligand-based and structure-based (Medina-Franco et al., 2006; Ooms, 2000). Ligand-based approaches use the structural information and biological activity data from a set of known active compounds to select promising candidates for experimental screening. When the three-dimensional structure of the target is known, structure-based methods can be used. Three-dimensional structure information of the target is usually obtained from X-ray crystallography or nuclear magnetic resonance. In the absence of three-dimensional structural information of the receptor, homology models have been successfully used (Grant, 2009; Villoutreix et al., 2009). Perhaps the most common structure-based approach is molecular docking. Docking aims to find the best position and orientation of a molecule within a binding site and gives a score for each docked pose (Hernández-Campos et al., 2010; Kitchen et al., 2004; Villoutreix et al., 2009). Ligand- and structure-based methods can be combined if information for both the experimentally active compounds and the three-dimensional structure of the target are available (Sperandio et al., 2008). The selection of a particular method is generally based on the goal of the project, the information available for the system, and the computational resources available. For structure-based and ligand-based methods, it is highly advisable to validate the virtual screening protocol prior to the selection of compounds for experimental testing. However, the experimental results of the tested candidates will provide full validation of the virtual screening approach.

2.2.1 Structure-based screening

Structure-based screening for novel DNMT inhibitors performed so far has been conducted with homology models of the catalytic domain (Kuck et al., 2010a; Siedlecki et al., 2006). The

construction of useful homology models has been facilitated by the extensive conservation of the catalytic domain of DNMTs (Kumar et al., 1994). Crystal structures of other methyltransferases such as bacterial DNA cytosine C5 methyltransferase from *Haemophilus hemolyticus* (M.HhaI), bacterial cytosine C5 methyltransferase M.HaeIII, and the human DNMT2 (Siedlecki et al., 2003; Yoo and Medina-Franco, 2011) have been used as templates (Medina-Franco and Caulfield, 2011).

We have recently developed two homology models of the catalytic domain of DNMT1. In one model (Yoo and Medina-Franco, 2011), the crystal structures of the DNMTs M.HhaI, M.HaeIII, and DNMT2 were used as templates. The first structure is a ternary complex of M.HhaI, *S*-adenosyl methionine (AdoMet), and DNA containing flipped 4′-thio-2′-deoxcytidine with partial methylation at C5. The crystal structure of M.HaeIII is bound covalently to DNA. In this complex, the substrate cytosine is extruded from the DNA and it is inserted into the active site. The structure of human DNMT2 complexed with *S*-adenosyl-L-homocysteine (AdoHcy/SAH) has high similarities to methyltransferases of both prokaryotes and eukaryotes. A second homology model was developed using only the structure of M.HhaI as template. Both models contain DNA and the conserved residues which are involved in the catalytic mechanism. The target cytosine which is flipped out of the embedded DNA is inserted into the active site. The catalytic loop containing the catalytic cysteine is located above the cytosine as an active site "lid". The target cytosine lies between the nucleophile cysteine residue (Cys1225) and the sulfur atom of AdoHcy. The distance of cytosine C6 to the sulfur atom of Cys1225 is 3.3 Å. The cytosine C5 atom is 3.0 Å away from the sulfur atom of AdoHcy. In the reactive state of Cys1225, the distance between $O^{\varepsilon 1}$ of Glu1265 and N3 of cytidine is 2.8 Å, where the N3 atom is proposed to be protonated making a hydrogen bond with the acidic side chain of Glu1265. In addition, the N3 protonated form of cytosine can make hydrogen bonds with Arg1311 and Pro1223. These key interactions in the catalytic site are commonly observed in both homology models. More specifically, in the first homology model, the α-phosphate backbone and 3′-OH of the sugar moiety of deoxycytidine make a hydrogen bond network with Arg1311, Arg1461, Ser1229, Gly1230, and Gln1396; in the second model, the interactions are observed with the following residues: Gln1226, Ser1229, Gly1230, Arg1268, and Arg1310.

Fig. 4 shows a superimposition of the first homology model of the catalytic site of hDNMT1 (Yoo and Medina-Franco, 2011) with the recently published crystal structure of unmethylated human DNMT1 (Song et al., 2011). The catalytic cores of their methyltransferase domains have similar features, but unmethylated DNA in the crystallographic structure is positioned further away from the active site. In the crystal structure of the human DNMT complex, the key amino acid residues Glu1265 and Arg1311 are positioned in very similar place. In contrast, the catalytic loop adopts a different conformation with respect to the homology model. The catalytic loop has an open conformation, and the catalytic cysteine is far from the binding site, e.g., the distance of superimposed cytosine C6 to the sulfur atom of Cys1225 is 9.5 Å. Taken together, the structural characterization of the catalytic site supports that our homology model is in full agreement with the proposed catalytic mechanism of DNA methylation.

In silico screening has been successfully used to identify novel small molecule inhibitors of DNMT1. In one study, 1990 compounds in the Diversity Set available from the National Cancer Institute were the starting point of a screening using docking with a validated homology model of human DNMT1. Compounds with undesirable size, hydrophobicity, and uncommon atom types were filtered out. Two of the top scoring compounds were

tested experimentally showing activity both *in vitro* and *in vivo*, probably by binding into the DNMT1 catalytic pocket (Siedlecki et al., 2006). In that work, RG108 (Fig. 2) showed an IC_{50} = 0.60 μM with M.SssI (Siedlecki et al., 2006). Additional characterization showed that this L-tryptophan derivative did not cause covalent enzyme trapping and that the carboxylate group plays an essential role in the binding with the enzyme since the analogue without this moiety is inactive (Brueckner et al., 2005).

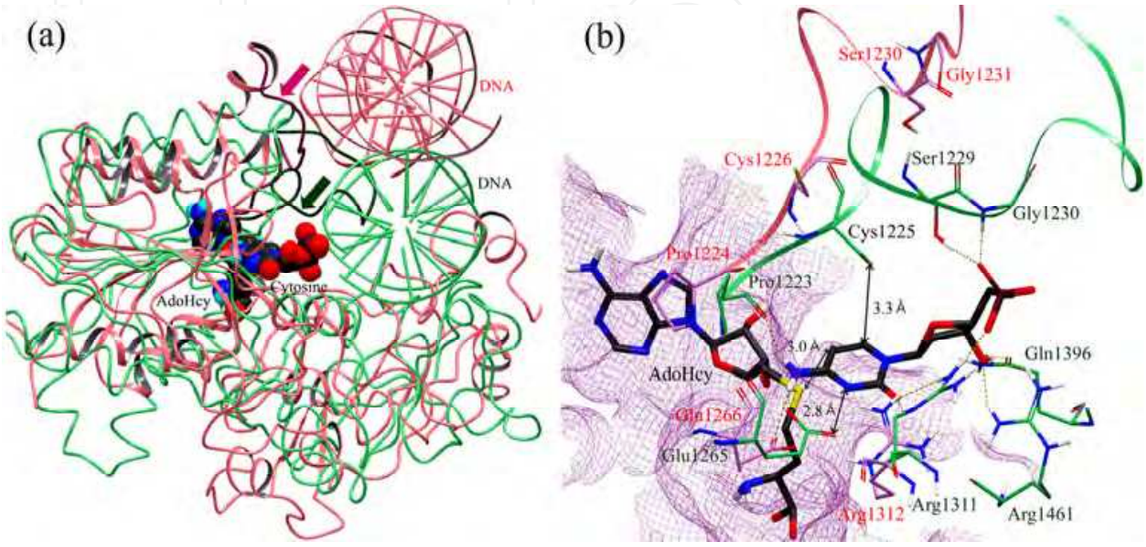


Fig. 4. (a) Superposition of the homology model (green) of the catalytic domain of human DNMT1 with the crystallographic structure (pink) of the unmethylated human DNMT1. The catalytic loops are marked with arrows. AdoHcy and the flipped cytosine in the homology model are shown in space-filling view. (b) Binding model of deoxycytidine (black) with key amino acid residues of homology model (carbon atoms in green) and crystal structure (carbon atoms in pink). Hydrogen bonding interactions are represented by dotted lines.

In a follow-up study, our group screened a larger set of the National Cancer Institute database containing more than 260,000 compounds (Kuck et al., 2010a). In order to focus the screening on compounds that could be promising for further development, we selected a subset of approximately 65,000 lead-like molecules (Charifson and Walters, 2002). The lead-like set was further filtered using a high-throughput *in silico* screening. As part of the screening, three docking programs were used. Favorable docking scores from all three docking approaches were combined to create a total of 24 consensus compounds. Of the 24 molecules that were identified, thirteen were obtained for experimental testing. Seven out of the thirteen consensus hits had detectable DNMT1 inhibitory activity in biochemical assays. Further experimental characterization of active compounds showed that six out of the seven inhibitors appeared selective for DNMT1. The methylenedisalicylic acid derivative, NSC 14778 (Fig. 2), 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. In fact, it is well-known that DNMTs are weak catalysts and are difficult to assay (Hemeon et al., (2011 - ASAP)). Despite the low potency, the *in silico* screening was successful in that it identified diverse scaffolds that were not previously reported for DNMT inhibitors. The new scaffolds represent excellent candidates for optimizing their inhibitory activity and selectivity.

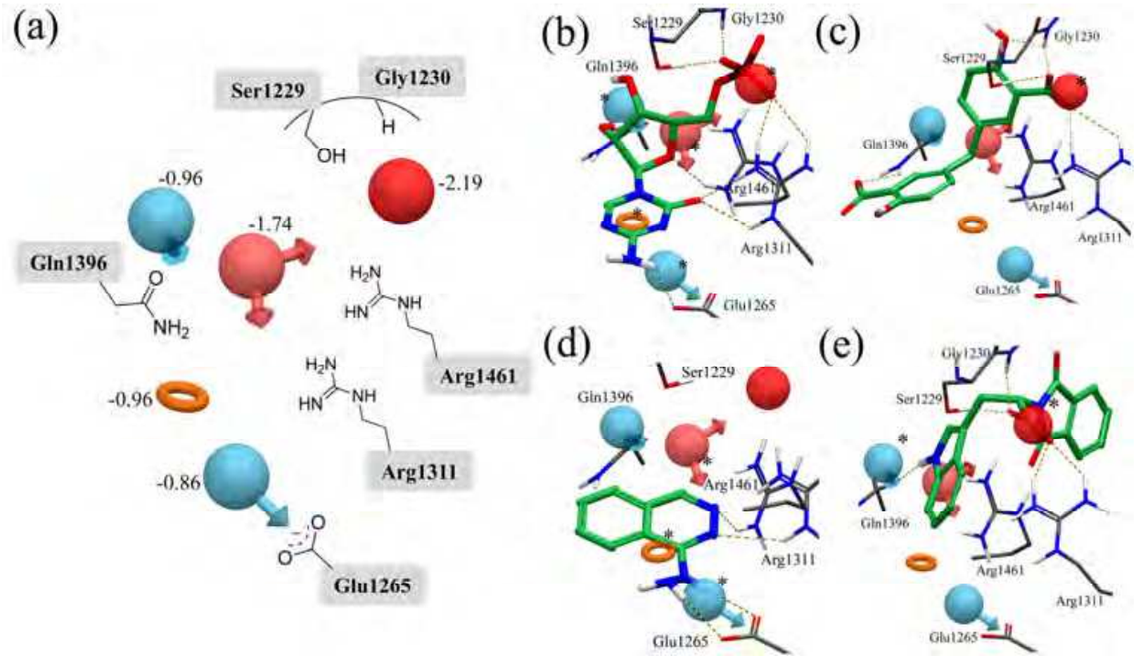


Fig. 5. (a) Structure-based pharmacophore model proposed for human DNMT1 inhibitors. *Red sphere*: negative ionizable, *pink sphere*: hydrogen bond acceptor, *blue sphere*: hydrogen bond donors, and *orange ring*: aromatic ring. Selected amino acid residues in the catalytic site of homology model are schematically depicted for reference. Comparison between the binding mode and pharmacophore hypothesis for representative DNMT inhibitors, (b) 5-azacytidine, (c) NSC14778, (d) hydralazine, and (e) RG108.

Recently, our group developed a structure-based pharmacophore hypothesis for inhibitors of DNMT1 (Yoo and Medina-Franco, 2011). Using the energy optimized hypothesis, 'e-pharmacophore' method (Salam et al., 2009) the pharmacophore model was developed based on the scores and predicted binding modes of 14 known DNMT1 inhibitors docked with a homology model of DNMT1. Fig. 5a shows the pharmacophore model for the 14 DNMT1 inhibitors. The model contains five features which represent the most important interactions of the inhibitors with the catalytic domain. The energetic value assigned to each pharmacophoric feature is displayed in the figure. Nearby amino acids are schematically depicted for reference. The best-scoring feature is a negative charge which is close to the side chains of Ser1229, Gly1230, and Arg1311. The second most favorable feature is an acceptor site that is in close proximity with the side chains of Arg1311 and Arg1461. The third ranked features are an aromatic ring that stabilizes the binding conformation of ligands between AdoHcy and Cys1225, and a donor site that is close to the side chain of Gln1396. The fifth-ranked feature is a donor site that is nearby the side chain of Glu1265 which is a residue implicated in the methylation mechanism. Fig. 5b shows the alignment of representative DNMT inhibitors to the pharmacophore hypothesis. It remains to evaluate the performance of the pharmacophore model in prospective *in silico* screening.

2.2.2 Ligand-based screening

Ligand-based screening can be performed as an alternative approach when the relevant crystal structures are not available on the molecular target. Ligand-based approaches

include similarity searching, substructure, clustering, quantitative structure-activity relationships (QSAR), pharmacophore-, or three-dimensional shape matching techniques (Villoutreix et al., 2007). Several ligand-based methods, including similarity searching and QSAR, can roughly be divided into two- or three-dimensional approaches. Ligand-based virtual screening may be applied even if a single known-ligand has been identified through similarity-based screening. Interestingly, although many more successful structure-based than ligand-based virtual screening applications are reported to date, recent reviews indicate that the potency of hits identified by ligand-based approaches is on average considerably higher than for structure-based methods (Ripphausen et al., 2011; Ripphausen et al., 2010).

If multiple active compounds are known, it is possible to apply QSAR using two- or three-dimensional information of the ligands. One of the main goals of QSAR is to derive statistical models that can be used to predict the activity of molecules not previously tested in the biological assay. Despite the fact that QSAR is a valuable tool, there are potential pitfalls to develop predictive QSAR models (Scior et al., 2009). A major pitfall can occur when the compounds were assayed using different experimental conditions. Other major pitfall is due to the presence of "activity cliffs," i.e., compounds with very high structural similarity but very different biological activity (Maggiora, 2006). Activity cliffs give rise to QSAR with poor predictive ability (Guha and Van Drie, 2008).

3. *In silico* design and optimization of established inhibitors

Concerns about severe toxicity of nucleoside analogues have strongly encouraged not only identifying novel DNMT inhibitors but also developing further established non-nucleoside inhibitors. To this end, medicinal chemistry approaches, either alone or in combination with *in silico* strategies, are being pursued.

3.1 Optimization of RG108, procaine, and mahanine

As mentioned above, procaine, a local anesthetic drug, and procainamide, a drug for the treatment of cardiac arrhythmias, have been reported as inhibitors of DNA methylation (Fig. 2). In a recent report, constrained analogues of procaine were synthesized and tested for their inhibition against DNMT1 (Castellano et al., 2008). Procaine as a lead structure was modified to partially reduce the high flexibility which can have a detrimental effect for drug-likeness. The most potent inhibitor in an *in vitro* methylation assay also showed demethylation activity in HL60 human myeloid leukemia cells, and it was suggested as a lead compound for further studies (Castellano et al., 2008).

In a separate work, a series of maleimide derivatives of RG108 were reported (Suzuki et al., 2010). In that work, design, chemical synthesis, inhibitory activity assays, and automated docking methods were used. The most active compound of the series was RG108-1 (Fig. 2). A binding model of RG108-1 with the crystal structure of bacterial M.HhaI suggested that this compound could be a covalent blocker of the catalytic cysteine. A more recent molecular modelling study using a model of human DNMT1 (Yoo and Medina-Franco, 2011) supported this hypothesis. Interestingly, in the model obtained with human DNMT, the maleimide moiety of RG108-1 interacts with Arg1311, Arg1461, and lies next to Cys1225, where the conjugate addition of the thiol group of the catalytic cysteine to the maleimide can occur. In addition, the carboxylate anion of RG108-1 overlaps with that of RG108 and

has the same interaction with Arg1311, Ser1229, and Gly1230 (Yoo and Medina-Franco, 2011).

The natural product mahanine (Fig. 2) has the ability to restore RASSF1A expression, and it is a potent inhibitor of androgen dependent (LNCaP) and androgen independent (PC-3) human prostate cancer cell proliferation (Jagadeesh et al., 2007). The antiproliferative activity of mahanine is associated with inhibition of the DNMT activity. Recently, fluorescent carbazole analogues of mahanine were designed and synthesized to find a novel and more potent small molecule with a mechanistic profile similar to that of the parent compound. Compound '9' in Fig. 2 inhibited human prostate cancer cell proliferation at 1.5 μM and also showed DNMT inhibition activity without the cytotoxic effects seen with mahanine treatment. Inhibition of DNMT was proposed as the event leading to the restoration of RASSF1A expression (Sheikh et al., 2010).

3.2 Structure-based optimization of hydralazine

Hydralazine, a potent arterial vasodilator, has been used for the management of hypertensive disorders and heart failure. Using a drug repurposing strategy (Duenas-Gonzalez et al., 2008), clinical trials have demonstrated the antitumor effect of the combination of hydralazine with valporic acid (a histone deacetylase inhibitor). Hydralazine and procainamide were first reported to have DNA methylation inhibition effect in 1988. Despite the fact that numerous studies were conducted with hydralazine, its molecular mechanism has remained unknown. In order to help understand the activity of hydralazine at the molecular level, we developed a binding mode of this compound with a validated homology model of the catalytic domain of DNMT1 using docking and molecular dynamics (Singh et al., 2009a; Yoo and Medina-Franco, 2011).

In molecular modeling studies, hydralazine showed similar interactions within the binding pocket as nucleoside analogues including a complex network of hydrogen bonds with arginine and glutamic acid residues that play a major role in the mechanism of DNA methylation (Yoo and Medina-Franco, 2011). Fig. 6 shows the comparison of the binding modes of hydralazine with 5-azacytidine. The amino group of hydralazine matched well with the amino group of 5-azacytidine, and it is capable of making hydrogen bonds with Glu1265 and Pro1223. The nitrogen of the phthalazine ring overlapped with the carbonyl oxygen of 5-azacytidine and formed hydrogen bonds with Arg1311 and Arg1461. We also identified that the small structure of hydralazine could not occupy the site of the sugar ring and phosphate backbone of nucleoside analogues. This result also suggests that hydralazine can be substituted at the C4 position to yield analogues with enhanced affinity with the enzyme. In contrast, there is a small empty pocket nearby the carbocyclic aromatic ring of hydralazine (C5-C8) that can be occupied by a substituent (Yoo and Medina-Franco, 2011).

The molecule shown in Fig. 6 was designed based on the structure and binding mode of hydralazine. Molecular modeling indicates that the addition of a phenyl group in the C4 position of hydralazine improves the calculated binding affinity with DNMT1. Moreover, adding polar substituents at various positions of the phenyl group can provide additional favorable interactions with the catalytic site. Also based on our molecular modeling analysis, the binding affinity is expected to increase by the addition of polar groups to the carbocyclic aromatic ring of phthalazine. It is *expected* that new compounds with increased calculated affinity with the enzyme will show increased potency in the DNMT1 enzyme inhibition assays.

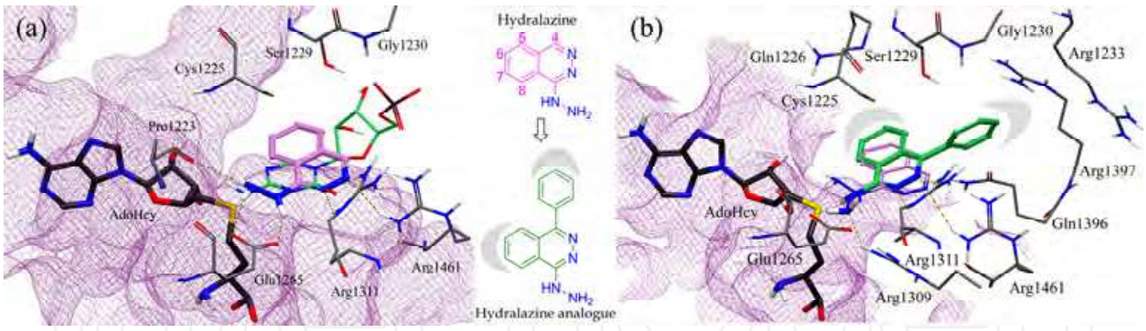


Fig. 6. Design of analogues of hydralazine. (a) comparison of the binding modes of hydralazine (carbon atoms in pink) with 5-azacytidine (carbon atoms in green) (b) structure-guided design of a representative hydralazine analogue (carbon atoms in green).

3.3 Design of focused combinatorial libraries

Computer-assisted combinatorial library design is a powerful tool frequently used in the discovery and optimization of new lead compounds. Molecular diversity has played a critical role in designing combinatorial libraries for screening (Tommasi and Cornella, 2006; Zheng and Johnson, 2008). However, the core chemical scaffolds of some currently used diverse libraries might be inadequate to provide drug-like compounds for new targets. Library design based on bioisosteric replacement or scaffold hopping methods can be used as an alternative to diversity oriented synthesis. Bioisostere searching involves swapping functional groups of a molecule with other functional groups that have similar biological properties. Scaffold hopping is an approach to discover structurally novel compounds starting from known active compounds by modifying the central core structure of the molecule (Brown and Jacoby, 2006). Scaffold hopping is an important drug design strategy to develop novel molecules with potent activity, altered physicochemical attributes, and Absorption, Distribution, Metabolism, Excretion and Toxicity –ADMET- properties. An example of application is phosphodiesterase 5 inhibitors for the treatment of erectile dysfunction. Sildenafil and vardenafil represent a case of heteroaromatic core scaffolds hopping with a small change in the scaffold (Jordan and Roughley, 2009). In contrast, tadalafil has a very different core scaffold, but it has the same biological activity. Computational design of focused libraries or compounds designed using any other strategy has to be in agreement with the experimental synthetic feasibility of the compounds proposed. Ideally, synthetic routes should follow short and easy steps.

Fig. 7 shows additional hydralazine analogues that have been proposed based on the knowledge gained in our previous molecular modeling studies of DNMT inhibitors. Starting from the 1-hydrazinyl-4-phenylphthalazine, polar groups are introduced into the carbocyclic aromatic ring of phthalazine. Based on molecular modeling analysis, substitution at ortho-, meta-, and para- position of the phenyl group with e.g., carboxyl, cyanide, and acetyl, showed a significant improvement in the calculated binding of the new compounds with DNMT1. A carboxyl group introduced into the ortho position plays a key role to make hydrogen bonds with Ser1229 or Gly1230. In contrast, substitution at C8 position of the phthalazine did not fit well into the catalytic site because of the narrow pocket size. Addition of polar groups to other positions slightly increases the predicted

binding affinity with the enzyme. Further chemical modifications to the structures of the lead DNMT inhibitors will be suggested toward the improvement of the *in vitro* and *in vivo* demethylating activity.

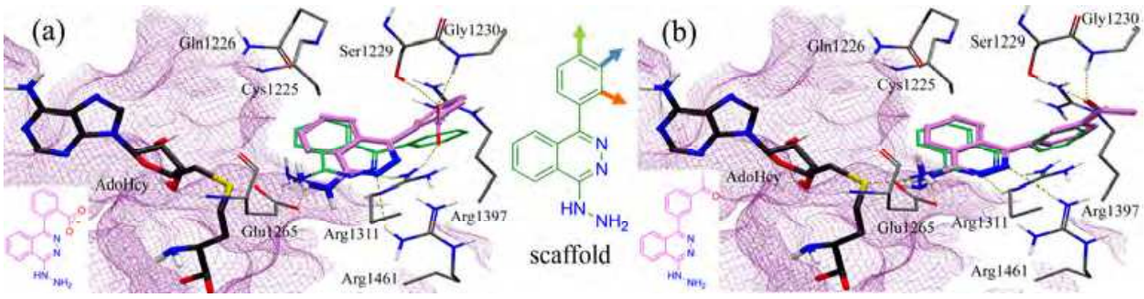


Fig. 7. Binding mode of hydralazine analogues (carbon atoms in pink) designed by scaffold hopping. The carbon atoms of new core scaffold are in green. Analogues with (a) ortho-carboxylate substitution and (b) meta-acetyl substitution on the phenyl moiety.

3.4 Characterization of structure-activity relationships

Currently, DNMT inhibitors have been screened in different assays using different conditions, and QSAR studies may not be reliable. However, once quality activity data has been gathered for several compounds assayed under comparable experimental conditions, it is feasible to conduct structure-activity relationships (SAR) of the compounds tested. When there is a significant amount of data, for example, activity data for more than 100 or 200 compounds, systematic analysis of the SAR can be performed via chemoinformatic approaches using the concept of "activity landscape modelling" (Wawer et al., 2010). The goal of activity landscape modeling of molecular data sets is to help rationalize the underlying SAR identifying key compounds and structural features for further exploration. The concept of activity landscape is strongly associated with the basic relationships between molecular structure and biological activity. While predictive SAR methods, such as pharmacophore modelling and traditional QSAR, focus on specific molecular descriptors or arrangements of substructures or functional groups associated with activity, descriptive activity landscape models rely on the "similarity property principle," i.e., similar structures should have similar biological properties (Bender and Glen, 2004; Maggiora and Shanmugasundaram, 2011) and employ whole-molecular similarity measures (Wawer et al., 2010). Systematic approaches to model activity landscapes and to detect "activity cliffs" using multiple representations are published elsewhere (Medina-Franco et al., 2009; Pérez-Villanueva et al., 2010; Pérez-Villanueva et al., 2011).

4. Conclusion

DNA methyltransferases are promising epigenetic targets for the treatment of cancer and other diseases. Clinical data demonstrates the potential of DNMT inhibitors for the therapeutic treatment of cancer. This is evidenced by the two DNMT inhibitors approved by the Food and Drug Administration of the United States for the treatment of patients with high-risk myelodysplastic syndrome. However, current approved drugs are nucleoside analogues that are not specific and still present issues such as cellular and clinical toxicity. A

wide range of computational approaches are being used to assist in the discovery and development of novel DNMT inhibitors. Molecular docking, pharmacophore modelling and molecular dynamics have been used to better understand the mechanism of action of established DNMT inhibitors; *in silico* screening of large compound libraries followed by experimental testing has been successful in identifying non-nucleoside inhibitors with novel chemical scaffolds; structure-based design is being used to guide the optimization of inhibitors such as hydralazine. Homology models of the catalytic domain of DNMT1 has played an important role to conduct the computational approaches that rely on the three dimensional structure of the target. It is expected that the recently published crystal structure of human DNMT1 bound to duplex DNA containing unmethylated CG sites will be the starting point of future structure-based studies with inhibitors of DNA methylation. It is also anticipated that the synergistic combination of computational approaches with combinatorial chemistry, and the systematic *in silico* and experimental screening of natural products will boost the discovery and optimization of inhibitors of DNMT for cancer therapy.

5. Acknowledgment

Discussions with Dr. Fabian López-Vallejo and other members of the group are highly appreciated. Authors are also very grateful to Karen Gottwald for proofreading the chapter. This work was supported by the State of Florida, Executive Office of the Governor's Office of Tourism, Trade, and Economic Development. J.L.M-F. also thanks the Menopause & Women's Health Research Center for funding.

6. References

Bender, A., 2010. Databases compound bioactivities go public. *Nat. Chem. Biol.* 6, 309-309.

Bender, A., Glen, R.C., 2004. Molecular similarity: A key technique in molecular informatics. *Org. Biomol. Chem.* 2, 3204-3218.

Brown, N., Jacoby, E., 2006. On scaffolds and hopping in medicinal chemistry. *Mini-Rev. Med. Chem.* 6, 1217-1229.

Brueckner, B., Boy, R.G., Siedlecki, P., Musch, T., Kliem, H.C., Zielenkiewicz, P., Suhai, S., Wiessler, M., Lyko, F., 2005. Epigenetic reactivation of tumor suppressor genes by a novel small-molecule inhibitor of human DNA methyltransferases. *Cancer Res.* 65, 6305-6311.

Castellano, S., Kuck, D., Sala, M., Novellino, E., Lyko, F., Sbardella, G., 2008. Constrained analogues of procaine as novel small molecule inhibitors of DNA methyltransferase-1. *J. Med. Chem.* 51, 2321-2325.

Charifson, P.S., Walters, W.P., 2002. Filtering databases and chemical libraries. *J. Comput.-Aided Mol. Des.* 16, 311-323.

Chen, T.P., Hevi, S., Gay, F., Tsujimoto, N., He, T., Zhang, B.L., Ueda, Y., Li, E., 2007. Complete inactivation of DNMT1 leads to mitotic catastrophe in human cancer cells. *Nat. Genet.* 39, 391-396.

Cheng, X.D., Blumenthal, R.M., 2008. Mammalian DNA methyltransferases: A structural perspective. *Structure* 16, 341-350.

Dueñas-González, A., García-López, P., Herrera, L.A., Medina-Franco, J.L., González-Fierro, A., Candelaria, M., 2008. The prince and the pauper. A tale of anticancer targeted agents. *Mol. Cancer* 7, 33.

Fandy, T.E., Herman, J.G., Kerns, P., Jiemjit, A., Sugar, E.A., Choi, S.H., Yang, A.S., Aucott, T., Dauses, T., Odchimar-Reissig, R., Licht, J., Mcconnell, M.J., Nasrallah, C., Kim, M.K.H., Zhang, W.J., Sun, Y.Z., Murgo, A., Espinoza-Delgado, I., Oteiza, K., Owoeye, I., Silverman, L.R., Gore, S.D., Carraway, H.E., 2009. Early epigenetic changes and DNA damage do not predict clinical response in an overlapping schedule of 5-azacytidine and entinostat in patients with myeloid malignancies. *Blood* 114, 2764-2773.

Fang, M.Z., Wang, Y.M., Ai, N., Hou, Z., Sun, Y., Lu, H., Welsh, W., Yang, C.S., 2003. Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res.* 63, 7563-7570.

Ganesan, A., 2008. The impact of natural products upon modern drug discovery. *Curr. Opin. Chem. Biol.* 12, 306-317.

Gilbert, E.R., Liu, D., 2010. Flavonoids influence epigenetic-modifying enzyme activity: Structure-function relationships and the therapeutic potential for cancer. *Curr. Med. Chem.* 17, 1756-1768.

Goll, M.G., Bestor, T.H., 2005. Eukaryotic cytosine methyltransferases. *Annu. Rev. Biochem.* 74, 481-514.

Goll, M.G., Kirpekar, F., Maggert, K.A., Yoder, J.A., Hsieh, C.-L., Zhang, X., Golic, K.G., Jacobsen, S.E., Bestor, T.H., 2006. Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. *Science* 311, 395-398.

Grant, M.A., 2009. Protein structure prediction in structure-based ligand design and virtual screening. *Comb. Chem. High Throughput Screening* 12, 940-960.

Guha, R., Van Drie, J.H., 2008. Assessing how well a modeling protocol captures a structure-activity landscape. *J. Chem. Inf. Model.* 48, 1716-1728.

Hauser, A.T., Jung, M., 2008. Targeting epigenetic mechanisms: Potential of natural products in cancer chemoprevention. *Planta Med.* 74, 1593-1601.

Hemeon, I., Gutierrez, J.A., Ho, M.-C., Schramm, V.L., (2011). Characterizing DNA methyltransferases with an ultrasensitive luciferase-linked continuous assay. *Anal. Chem.*, 83, 4996-5004.

Hernández-Campos, A., Velázquez-Martínez, I., Castillo, R., López-Vallejo, F., Jia, P., Yu, Y., Giulianotti, M.A., Medina-Franco, J.L., 2010. Docking of protein kinase B inhibitors: Implications in the structure-based optimization of a novel scaffold. *Chem. Biol. Drug Des.* 76, 269-276.

Irwin, J.J., Shoichet, B.K., 2005. ZINC - A free database of commercially available compounds for virtual screening. *J. Chem. Inf. Model.* 45, 177-182.

Issa, J.-P., 2005. Optimizing therapy with methylation inhibitors in myelodysplastic syndromes: Dose, duration, and patient selection. *Nat. Clin. Pract. Oncol.* 2 Suppl 1, S24-9.

Issa, J.-P.J., Kantarjian, H.M., 2009. Targeting DNA methylation. *Clin. Cancer Res.* 15, 3938-3946.

Issa, J.P.J., Kantarjian, H.M., Kirkpatrick, P., 2005. Azacitidine. *Nat. Rev. Drug Discovery* 4, 275-276.

Jagadeesh, S., Sinha, S., Pal, B.C., Bhattacharya, S., Banerjee, P.P., 2007. Mahanine reverses an epigenetically silenced tumor suppressor gene RASSF1A in human prostate cancer cells. *Biochem. Biophys. Res. Commun.* 362, 212-217.

Jones, P.A., Baylin, S.B., 2007. The epigenomics of cancer. *Cell* 128, 683-692.

Jordan, A.M., Roughley, S.D., 2009. Drug discovery chemistry: A primer for the non-specialist. *Drug Discovery Today* 14, 731-744.

Jurkowska, R.Z., Jurkowski, T.P., Jeltsch, A., 2011. Structure and function of mammalian DNA methyltransferases. *ChemBioChem* 12, 206-222.

Kelly, T.K., De Carvalho, D.D., Jones, P.A., 2010. Epigenetic modifications as therapeutic targets. *Nat. Biotechnol.* 28, 1069-1078.

Kitchen, D.B., Decornez, H., Furr, J.R., Bajorath, J., 2004. Docking and scoring in virtual screening for drug discovery: Methods and applications. *Nat. Rev. Drug Discov.* 3, 935-949.

Kuck, D., Singh, N., Lyko, F., Medina-Franco, J.L., 2010a. Novel and selective DNA methyltransferase inhibitors: Docking-based virtual screening and experimental evaluation. *Bioorg. Med. Chem.* 18, 822-829.

Kuck, D., Caulfield, T., Lyko, F., Medina-Franco, J.L., 2010b. Nanaomycin A selectively inhibits DNMT3B and reactivates silenced tumor suppressor genes in human cancer cells. *Mol. Cancer Ther.* 9, 3015-23.

Kumar, S., Cheng, X.D., Klimasauskas, S., Mi, S., Posfai, J., Roberts, R.J., Wilson, G.G., 1994. The DNA (cytosine-5) methyltransferases. *Nucleic Acids Res.* 22, 1-10.

Lan, J., Hua, S., He, X.N., Zhang, Y., 2010. DNA methyltransferases and methyl-binding proteins of mammals. *Acta Biochim. Biophys. Sin.* 42, 243-252.

Lee, B.H., Yegnasubramanian, S., Lin, X.H., Nelson, W.G., 2005a. Procainamide is a specific inhibitor of DNA methyltransferase 1. *J. Biol. Chem.* 280, 40749-40756.

Lee, W.J., Shim, J.Y., Zhu, B.T., 2005b. Mechanisms for the inhibition of DNA methyltransferases by tea catechins and bioflavonoids. *Mol. Pharmacol.* 68, 1018-1030.

Li, Y., Tollefsbol, T.O., 2010. Impact on DNA methylation in cancer prevention and therapy by bioactive dietary components. *Curr. Med. Chem.* 17, 2141-2151.

Liu, Z.F., Xie, Z.L., Jones, W., Pavlovicz, R.E., Liu, S.J., Yu, J.H., Li, P.K., Lin, J.Y., Fuchs, J.R., Marcucci, G., Li, C.L., Chan, K.K., 2009. Curcumin is a potent DNA hypomethylation agent. *Bioorg. Med. Chem. Lett.* 19, 706-709.

López-Vallejo, F., Caulfield, T., Martínez-Mayorga, K., Giulianotti, M.A., Nefzi, A., Houghten, R.A., Medina-Franco, J.L., 2011. Integrating virtual screening and combinatorial chemistry for accelerated drug discovery. *Comb. Chem. High Throughput Screening* 14, 475-487.

López-Vallejo, F., Nefzi, A., Bender, A., Owen, J.R., Nabney, I.T., Houghten, R.A., Medina-Franco, J.L., 2011. Increased diversity of libraries from libraries: Chemoinformatic analysis of bis-diazacyclic libraries. *Chem. Biol. Drug Des.* 77, 328-342.

Lyko, F., Brown, R., 2005. DNA methyltransferase inhibitors and the development of epigenetic cancer therapies. *J. Natl. Cancer Inst.* 97, 1498-1506.

Maggiora, G.M., 2006. On outliers and activity cliffs-why QSAR often disappoints. *J. Chem. Inf. Model.* 46, 1535-1535.

Maggiora, G.M., Shanmugasundaram, V., 2011. Molecular similarity measures, In: *Chemoinformatics and Computational Chemical Biology, Methods in Molecular Biology*, J. Bajorath, (Ed.), pp. 39-100, Springer, New York.

Medina-Franco, J., López-Vallejo, F., Kuck, D., Lyko, F., 2011. Natural products as DNA methyltransferase inhibitors: A computer-aided discovery approach. *Mol. Diversity* 15, 293-304.

Medina-Franco, J.L., Caulfield, T., 2011. Advances in the computational development of DNA methyltransferase inhibitors. *Drug Discovery Today* 16, 418-425.

Medina-Franco, J.L., López-Vallejo, F., Castillo, R., 2006. Diseño de fármacos asistido por computadora. *Educ. Quim.* 17, 452-457.

Medina-Franco, J.L., Martínez-Mayorga, K., Giulianotti, M.A., Houghten, R.A., Pinilla, C., 2008. Visualization of the chemical space in drug discovery. *Curr. Comput.-Aided Drug Des.* 4, 322-333.

Medina-Franco, J.L., Martínez-Mayorga, K., Bender, A., Marín, R.M., Giulianotti, M.A., Pinilla, C., Houghten, R.A., 2009. Characterization of activity landscapes using 2D and 3D similarity methods: Consensus activity cliffs. *J. Chem. Inf. Model.* 49, 477-491.

Miller, C.A., Gavin, C.F., White, J.A., Parrish, R.R., Honasoge, A., Yancey, C.R., Rivera, I.M., Rubio, M.D., Rumbaugh, G., Sweatt, J.D., 2010. Cortical DNA methylation maintains remote memory. *Nat. Neurosci.* 13, 664-666.

Muegge, I., 2008. Synergies of virtual screening approaches. *Mini-Rev. Med. Chem.* 8, 927-933.

Ooms, F., 2000. Molecular modeling and computer aided drug design. Examples of their applications in medicinal chemistry. *Curr. Med. Chem.* 7, 141-158.

Palii, S.S., Van Emburgh, B.O., Sankpal, U.T., Brown, K.D., Robertson, K.D., 2008. DNA methylation inhibitor 5-aza-2'-deoxycytidine induces reversible genome-wide DNA damage that is distinctly influenced by DNA methyltransferases 1 and 3B. *Mol. Cell. Biol.* 28, 752-771.

Pérez-Villanueva, J., Santos, R., Hernández-Campos, A., Giulianotti, M.A., Castillo, R., Medina-Franco, J.L., 2010. Towards a systematic characterization of the antiprotozoal activity landscape of benzimidazole derivatives. *Bioorg. Med. Chem.* 18, 7380-7391.

Pérez-Villanueva, J., Santos, R., Hernandez-Campos, A., Giulianotti, M.A., Castillo, R., Medina-Franco, J.L., 2011. Structure-activity relationships of benzimidazole derivatives as antiparasitic agents: Dual activity-difference (DAD) maps. *Med. Chem. Comm.* 2, 44-49.

Portela, A., Esteller, M., 2010. Epigenetic modifications and human disease. *Nat. Biotechnol.* 28, 1057-1068.

Ripphausen, P., Nisius, B., Bajorath, J., 2011. State-of-the-art in ligand-based virtual screening. *Drug Discovery Today* 16, 372-376.

Ripphausen, P., Nisius, B., Peltason, L., Bajorath, J.R., 2010. *Quo vadis*, virtual screening? A comprehensive survey of prospective applications. *J. Med. Chem.* 53, 8461-8467.

Robertson, K.D., 2001. DNA methylation, methyltransferases, and cancer. *Oncogene* 20, 3139-3155.

Salam, N.K., Nuti, R., Sherman, W., 2009. Novel method for generating structure-based pharmacophores using energetic analysis. *J. Chem. Inf. Model.* 49, 2356-2368.

Schaefer, M., Lyko, F., 2010. Solving the dnmt2 enigma. *Chromosoma* 119, 35-40.

Schermelleh, L., Spada, F., Easwaran, H.P., Zolghadr, K., Margot, J.B., Cardoso, M.C., Leonhardt, H., 2005. Trapped in action: Direct visualization of DNA methyltransferase activity in living cells. *Nat. Methods* 2, 751-756.

Schrump, D.S., Fischette, M.R., Nguyen, D.M., Zhao, M., Li, X.M., Kunst, T.F., Hancox, A., Hong, J.A., Chen, G.A., Pishchik, V., Figg, W.D., Murgo, A.J., Steinberg, S.M., 2006. Phase I study of decitabine-mediated gene expression in patients with cancers involving the lungs, esophagus, or pleura. *Clin. Cancer Res.* 12, 5777-5785.

Scior, T., Bernard, P., Medina-Franco, J.L., Maggiora, G.M., 2007. Large compound databases for structure-activity relationships studies in drug discovery. *Mini-Rev. Med. Chem.* 7, 851-860.

Scior, T., Medina-Franco, J.L., Do, Q.T., Martinez-Mayorga, K., Yunes Rojas, J.A., Bernard, P., 2009. How to recognize and workaround pitfalls in QSAR studies: A critical review. *Curr. Med. Chem.* 16, 4297-4313.

Segura-Pacheco, B., Trejo-Becerril, C., Perez-Cardenas, E., Taja-Chayeb, L., Mariscal, I., Chavez, A., Acuña, C., Salazar, A.M., Lizano, M., Dueñas-Gonzalez, A., 2003. Reactivation of tumor suppressor genes by the cardiovascular drugs hydralazine and procainamide and their potential use in cancer therapy. *Clin. Cancer Res.* 9, 1596-1603.

Sheikh, K.D., Banerjee, P.P., Jagadeesh, S., Grindrod, S.C., Zhang, L., Paige, M., Brown, M.L., 2010. Fluorescent epigenetic small molecule induces expression of the tumor suppressor Ras-association domain family 1a and inhibits human prostate xenograft. *J. Med. Chem.* 53, 2376-2382.

Shelat, A.A., Guy, R.K., 2007. The interdependence between screening methods and screening libraries. *Curr. Opin. Chem. Biol.* 11, 244-251.

Shoichet, B.K., 2004. Virtual screening of chemical libraries. *Nature* 432, 862-865.

Siedlecki, P., Boy, R.G., Musch, T., Brueckner, B., Suhai, S., Lyko, F., Zielenkiewicz, P., 2006. Discovery of two novel, small-molecule inhibitors of DNA methylation. *J. Med. Chem.* 49, 678-683.

Siedlecki, P., Boy, R.G., Comagic, S., Schirrmacher, R., Wiessler, M., Zielenkiewicz, P., Suhai, S., Lyko, F., 2003. Establishment and functional validation of a structural homology model for human DNA methyltransferase 1. *Biochem. Biophys. Res. Commun.* 306, 558-563.

Singh, N., Dueñas-González, A., Lyko, F., Medina-Franco, J.L., 2009a. Molecular modeling and dynamics studies of hydralazine with human DNA methyltransferase 1. *ChemMedChem* 4, 792-799.

Singh, N., Guha, R., Giulianotti, M.A., Pinilla, C., Houghten, R.A., Medina-Franco, J.L., 2009b. Chemoinformatic analysis of combinatorial libraries, drugs, natural products, and molecular libraries small molecule repository. *J. Chem. Inf. Model.* 49, 1010-1024.

Sippl, W., Jung, M., 2009. DNA methyltransferase inhibitors, In: *Epigenetic Targets in DrugDiscovery*, W. Sippl, and M. Jung (Eds.), pp. 163-183, Wiley-VCH, Weinheim.

Song, J., Rechkoblit, O., Bestor, T.H., Patel, D.J., 2011. Structure of DNMT1-DNA complex reveals a role for autoinhibition in maintenance DNA methylation. *Science* 331, 1036-1040.

Sperandio, O., Miteva, M.A., Villoutreix, B.O., 2008. Combining ligand- and structure-based methods in drug design projects. *Curr. Comput.-Aided Drug Des.* 4, 250-258.

Stresemann, C., Lyko, F., 2008. Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine. *Int. J. Cancer* 123, 8-13.

Suzuki, T., Tanaka, R., Hamada, S., Nakagawa, H., Miyata, N., 2010. Design, synthesis, inhibitory activity, and binding mode study of novel DNA methyltransferase 1 inhibitors. *Bioorg. Med. Chem. Lett.* 20, 1124-1127.

Tommasi, R., Cornella, I., 2006. Focused libraries: The evolution in strategy from large-diversity libraries to the focused library approach, In: *Exploiting Chemical Diversity for Drug Discovery,* A. Bartlett and M. Entzeroth, (Eds.), pp. 163-183, The Royal Society of Chemistry, Cambridge.

Vilkaitis, G., Merkiene, E., Serva, S., Weinhold, E., Klimasauskas, S., 2001. The mechanism of DNA cytosine-5 methylation. Kinetic and mutational dissection of HhaI methyltransferase. *J. Biol. Chem.* 276, 20924-20934.

Villar-Garea, A., Fraga, M.F., Espada, J., Esteller, M., 2003. Procaine is a DNA-demethylating agent with growth-inhibitory effects in human cancer cells. *Cancer Res.* 63, 4984-4989.

Villoutreix, B.O., Eudes, R., Miteva, M.A., 2009. Structure-based virtual ligand screening: Recent success stories. *Comb. Chem. & High Throughput Screening* 12, 1000-1016.

Villoutreix, B.O., Renault, N., Lagorce, D., Sperandio, O., Montes, M., Miteva, M.A., 2007. Free resources to assist structure-based virtual ligand screening experiments. *Curr. Protein Pept. Sci.* 8, 381-411.

Wawer, M., Lounkine, E., Wassermann, A.M., Bajorath, J., 2010. Data structures and computational tools for the extraction of SAR information from large compound sets. *Drug Discovery Today* 15, 630-639.

Yokochi, T., Robertson, K.D., 2002. Preferential methylation of unmethylated DNA by mammalian de novo DNA methyltransferase dnmt3a. *J. Biol. Chem.* 277, 11735-11745.

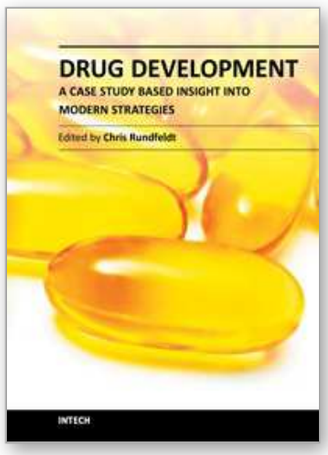
Yoo, J., Medina-Franco, J.L., 2011. Homology modeling, docking, and structure-based pharmacophore of inhibitors of DNA methyltransferase. *J. Comp.-Aided Mol. Des.* 25, 555-567.

Zawia, N.H., Lahiri, D.K., Cardozo-Pelaez, F., 2009. Epigenetics, oxidative stress, and Alzheimer disease. *Free Radical Biol. Med.* 46, 1241-1249.

Zheng, W., Johnson, S.R., 2008. Compound library design - principles and applications, In: *Chemoinformatics Approaches to Virtual Screening,* A. Varnek and A. Tropsha, (Eds.), pp. 268-294, Royal Society of Chemistry, Cambridge.

IntechOpen

IntechOpen



Drug Development - A Case Study Based Insight into Modern Strategies

Edited by Dr. Chris Rundfeldt

ISBN 978-953-307-257-9
Hard cover, 654 pages
Publisher InTech
Published online 07, December, 2011
Published in print edition December, 2011

This book represents a case study based overview of many different aspects of drug development, ranging from target identification and characterization to chemical optimization for efficacy and safety, as well as bioproduction of natural products utilizing for example lichen. In the last section, special aspects of the formal drug development process are discussed. Since drug development is a highly complex multidisciplinary process, case studies are an excellent tool to obtain insight in this field. While each chapter gives specific insight and may be read as an independent source of information, the whole book represents a unique collection of different facets giving insight in the complexity of drug development.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Jakyung Yoo and José L. Medina-Franco (2011). Discovery and Optimization of Inhibitors of DNA Methyltransferase as Novel Drugs for Cancer Therapy, Drug Development - A Case Study Based Insight into Modern Strategies, Dr. Chris Rundfeldt (Ed.), ISBN: 978-953-307-257-9, InTech, Available from: http://www.intechopen.com/books/drug-development-a-case-study-based-insight-into-modern-strategies/discovery-and-optimization-of-inhibitors-of-dna-methyltransferase-as-novel-drugs-for-cancer-therapy

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen