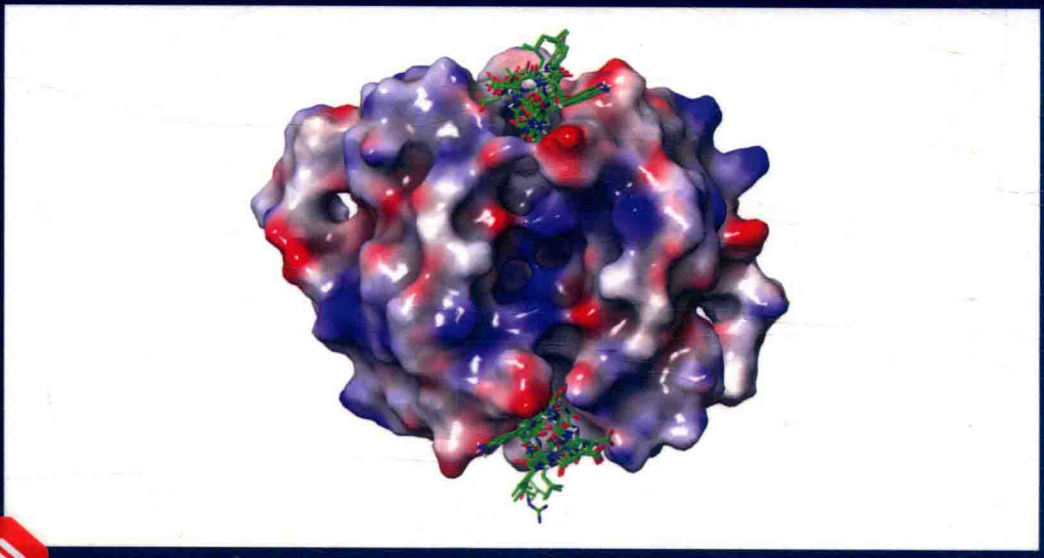
Advances in

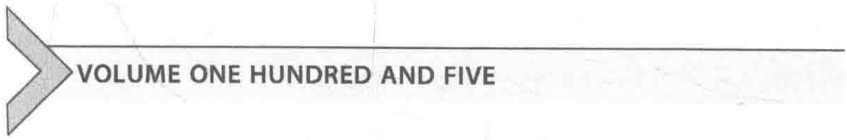
PROTEIN CHEMISTRY and STRUCTURAL BIOLOGY

Insights into Enzyme Mechanisms and Functions from Experimental and Computational Methods



VOLUME 105

Edited by
Christo Z. Christov



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SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

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50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
525 B Street, Suite 1800, San Diego, CA 92101-4495, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom
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First edition 2016

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ISBN: 978-0-12-804825-2
ISSN: 1876-1623

Publisher: Zoe Kruze
Acquisition Editor: Alex White
Editorial Project Manager: Helene Kabes
Production Project Manager: Surya Narayanan Jayachandran
Cover Designer: Mark Rogers

Typeset by SPi Global, India

VOLUME ONE HUNDRED AND FIVE

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PREFACE

Enzymes play a crucial role in molecular processes in living organisms. They are characterized with the ability to increase considerably the rate of chemical reactions in the cells but also with high specificity toward their substrates. The key functions of enzymes in cell biology and unique properties make them important targets for drug design and applications in biotechnology, protein engineering, and synthetic biology.

Recent developments in experimental methods contribute for a rapid growth in our understanding about structure–function relationships in enzymes. Structural methods such as X-ray crystallography and NMR are currently widely used to determine the three-dimensional structures of enzymes, and their complexes with substrates and inhibitors. Homology modeling methods received growing applications to predict 3D structures of enzymes, when experimental structures are not available. In addition, spectroscopic and kinetic methods are broadly applied to complement structural information and understand enzyme structure–function relationships.

The applications of computational methods to enzyme mechanisms of functions received a large increase over the last years. The growth in computational power, the development of parallel supercomputers, and GPUs made possible modeling studies to be performed for very large biomolecular systems, containing hundreds of thousands of atoms. Nowadays, we can routinely perform molecular dynamics in order to understand the conformational flexibility of enzyme–ligand complexes in microsecond timescales, to apply combined quantum mechanical and molecular mechanical methods to explore the reaction mechanisms and the origins of the catalytic power in enzymes and to implement methods for prediction of the binding orientations and free energies of enzyme–ligand complexes.

The present thematic volume of *Advances in Protein Chemistry and Structural Biology* is aimed to address some of the recent developments in enzymes. The selected contributions are focused on state-of-the-art applications of computational and experimental methods to understand different aspects of structure–function relationships and mechanisms of enzymes and their applications in biotechnology, cell biology, drug design, and biomaterials.

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CONTENTS

CHAPTER ONE

Molecular Modeling and Chemoinformatics to Advance the Development of Modulators of Epigenetic Targets: A Focus on DNA Methyltransferases

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Contents

Abstract

In light of the emerging field of *Epi-informatics*, ie, computational methods applied to epigenetic research, molecular docking, and dynamics, pharmacophore and activity landscape modeling and QSAR play a key role in the development of modulators of DNA methyltransferases (DNMTs), one of the major epigenetic target families. The increased chemical information available for modulators of DNMTs has opened up the avenue to explore the epigenetic relevant chemical space (ERCS). Herein, we discuss

Advances in Protein Chemistry and Structural Biology, Volume 105
ISSN 1876-1623
http://dx.doi.org/10.1016/bs.apcsb.2016.05.001

recent progress on the identification and development of inhibitors of DNMTs as potential epi-drugs and epi-probes that have been driven by molecular modeling and chemoinformatics methods. We also survey advances on the elucidation of their structure–activity relationships and exploration of ERCS. Finally, it is illustrated how computational approaches can be applied to identify modulators of DNMTs in food chemicals.

1. INTRODUCTION

DNA methylation is one of the most important epigenetic regulation mechanisms and it is mediated primarily by the family of enzymes DNA methyltransferases (DNMTs). This process involves the addition of a methyl group at the C-5 position of a DNA cytosine residue by the cofactor *S*-adenosyl methionine (SAM). This family of enzymes is formed by DNMT1, DNMT3A, DNMT3B, and DNMT3L. DNMT1 is associated with the maintenance of methylation patterns in DNA. DNMT3A and DNMT3B are de novo DNMTs and are able to transfer a methyl group in nonmethylated CpG's islands. DNMT3L is related to DNMT3A and DNMT3B enhancing their activity (Robertson, 2001).

DNMTs are promising epigenetic targets for the treatment of a number of diseases. For instance, DNA hypermethylation is related to cancer metastasis by silencing the expression genes linked to cell division. DNMTs are implicated in autoimmune diseases and inherited disorders (Gros et al., 2012) and are also promising molecular targets for the treatment of other chronic and degenerative diseases such as Alzheimer's and psychiatric conditions (Gros et al., 2012), and diabetes (Arguelles, Meruvu, Bowman, & Choudhury, 2016). As such, inhibitors of DNMTs (DNMTis) are attractive compounds to be developed as clinical candidates for diverse diseases either alone or as part of combination therapies. Moreover, the development of DNMTis as *epi-probes* is also attractive for the further biochemical understanding of the role of DNMTs in epigenetics. In fact, there are not known good molecular probes for DNMTs although selective probe molecules have been developed for other epigenetic targets (Arrowsmith et al., 2015).

Thus far, 5-azacytidine and decitabine (Fig. 1) are DNMTis that have been approved by the USA Food and Drug Administration—FDA—for the treatment of myelodysplastic syndrome (Derissen, Beijnen, & Schellens, 2013). However, these two compounds are prodrugs that need to be incorporated into DNA. Their high toxicity and low specificity have prompted the search for specific inhibitors preferably of the nonnucleosidic type. To date, several nonnucleosidic inhibitors have been reported which

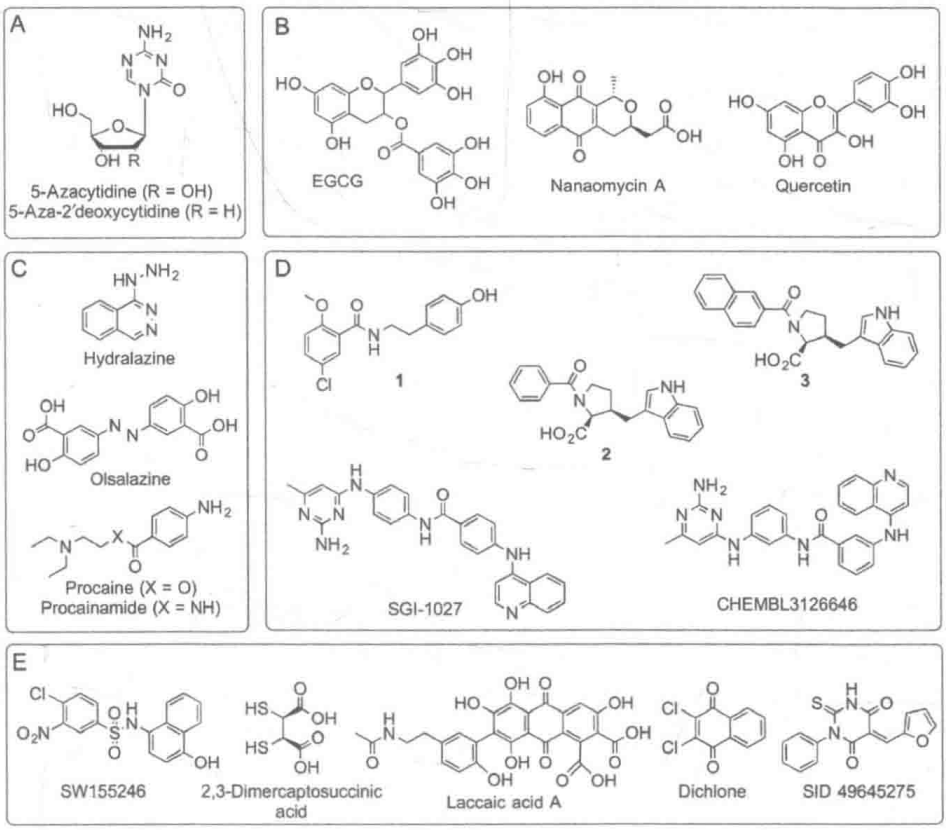

Fig. 1 Representative DNMT inhibitors and compounds with proposed demethylating properties. Compounds are classified by their source: (A) approved for clinical use, (B) natural products, (C) drugs approved for other indications, (D) synthetic compounds coming from optimization programs, and (E) molecules obtained from high-throughput screening.

have been identified from diverse sources such as drugs approved for other indications, natural products, virtual (in silico) screening, high-throughput screening, and synthetic compounds including molecules initially identified from computational screening and later optimized using medicinal chemistry approaches. The chemical structures of representative compounds are shown in Fig. 1. In the figure, compounds are classified by their source in five major groups, namely; DNMTis approved for clinical use (group A), natural products (B), drugs approved for other indications (C), synthetic compounds coming from optimization programs (D), and molecules obtained from high-throughput screening (E). The reader is also referred to reviews of DNMTis that have been published (Erdmann, Arimondo, & Guianvarc'h, 2016; Erdmann, Halby, Fahy, & Arimondo, 2014; Guianvarc'h & Arimondo, 2014).

Computational approaches have played a major role in the identification, optimization, and understanding of the biological activity of DNMTis at the molecular level. Application of in silico techniques continues to increase not only for DNMTs but also for several other epigenetic targets as reflected in the emerging research field called *Epi-informatics* (Medina-Franco, 2016). Reviews on the progress of computational approaches applied to DNMT have been published (Medina-Franco, Méndez-Lucio, Yoo, & Dueñas, 2015; Yoo & Medina-Franco, 2012). However, since these last reviews, major contributions from computational applications have been published in subsequent studies. In particular, a significant and relevant amount of structure–activity information (SAI) has been released and stored in public databases. Herein, we review recent advances on the chemical information resources that have been made available recently to chart the chemical space of DNMTs and elucidate the SAR of DNMTis. We also review major progress on the identification and optimization of hit compounds as well as on the computer-aided discovery of novel hits with putative novel binding sites. All together, these studies emphasize the continued synergy between molecular modeling and chemoinformatic to further advance epigenetic drug and probe development. Representative computational studies, techniques, and major outcomes discussed throughout this chapter are summarized in Table 1.

2. PROGRESS ON CHEMICAL INFORMATION

The SAI of compounds tested as DNMTis has been growing in the last few years. In a recent study, Fernández-de Gortari et al. surveyed public resources and collected chemical databases that contain SAI of DNMTis. Major compound collections included human epigenetic enzyme and modulator database (HEMD) (Huang et al., 2012), ChEMBL (Gaulton et al., 2012), and Binding Database (Liu, Lin, Wen, Jorissen, & Gilson, 2007). An additional database is EpiDBase (Loharch et al., 2015), a curated epigenetic database that includes 11,422 small molecules with activity against different epigenetic targets.

In addition to the availability of molecular databases with experimental data coming from research publications, advances in assay developments and rapid screening technologies for DNMTs (Medina-Franco, Yoo, & Dueñas-Gonzalez, 2015) are facilitating the access to medium- and high-throughput screening data. Table 2 summarizes the information of representative

Table 1 Representative Computational Studies on DNMT Is Recently Reported

Study	Approach	Major Outcomes	References
ERCS focused on DNMTs: comparison with other general compound collections	Chemoinformatic analysis based on physicochemical properties, molecular fingerprints, and scaffolds	The structure of DNMTis is diverse; occupy the traditional chemical space of drugs but also cover other regions. Four tentative privileged epigenetic scaffolds were identified	Fernández-de Gortari and Medina-Franco (2015)
ERCS focused on DNMTs: comparison with epigenetic reference databases	Chemoinformatic analysis including metrics of structural complexity	The structures of DNMTis are different from inhibitors of histone deacetylases and bromodomains. There is a large potential to develop DNMTis with increased complexity	Prieto-Martínez, Fernández-de Gortari, Méndez-Lucio, Medina-Franco (2016)
Chemoinformatic-based pharmacophore model	Pharmacophore models based on the predicted protein–ligand interaction profiles of selected chemical scaffolds enriched with active compounds	A model with three main pharmacophoric points was identified. The model had high sensitivity and specificity in a validation study	This chapter
Activity landscape modeling	Systematic comparison of structure similarity and potency difference of 280 compounds using SAS maps. The landscape of two major groups of inhibitors was assessed	Inhibitors related to the cofactor have a rough landscape with significant activity cliffs. Structure-based interpretation of an activity cliffs suggested distinct molecular interactions in the binding site. Also, a novel "activity landscape sweeping" approach was proposed	Naveja and Medina-Franco (2015)

Continued

Table 1 Representative Computational Studies on DNMT Is Recently Reported—cont'd

Study	Approach	Major Outcomes	References
Linear discriminant analysis-based QSAR and virtual screening	QSAR model for 47 molecules was used to classify natural products as active/inactive; predicted active were docked with DNMT1 and 3A	Six natural products as consensus hits from two docking programs were suggested as potential inhibitors of DNMTs	Maldonado-Rojas, Olivero-Verbel, and Marrero-Ponce (2015)
Virtual screening with experimental validation	Docking-based screening of commercial compounds followed by analog searching	Identification of compound DC_05, DC_501, and DC_517 as low micromolar and selective inhibitors of DNMT1. DC_517 had activity in cell-based assays	Chen et al. (2014)
Molecular dynamics and virtual screening with experimental validation	Ensemble docking using conformations of the protein obtained from molecular dynamics. Multistep docking-based screening of a commercial compound collection	Two experimentally validated hits were identified: ASINEX ID BAS 12771472 and BAS 00872020. The former compound showed in vivo activity. A newly putative binding site in hDNMT1 is proposed	Joshi, Rajpathak, Narwade, and Deobagkar (2016)
Docking of newly designed selective compounds inspired in a virtual screening hit	Flexible docking	The selectivity of the most potent compounds toward DNMT3 over DNMT1 is rationalized at the molecular level	Aldawsari et al. (2016)
Toward computer-assisted drug repurposing	Similarity searching and data fusion	Potential DNMT inhibitors are identified	Naveja, Dueñas-González, and Medina-Franco (2016)

Table 2 Representative Confirmatory Assays in PubChem Related to DNMTs

AID	Name	Title	Assay Data	Source
1066238	Inhibition of human recombinant DNMT1 expressed in H19 cells assessed as inhibition of tritiated methyl incorporation from [3H]-labeled AdoMet into hemimethylated DNA duplex after 2 h by liquid scintillation counting analysis	Synthesis and evaluation of analogs of *N*-phthaloyl-L-tryptophan (RG108) as inhibitors of DNA methyltransferase 1	2 active, 9 tested	ChEMBL
461004	Inhibition of human recombinant DNMT3B expressed in baculovirus–insect cell system by scintillation counting	Novel and selective DNA methyltransferase inhibitors: docking-based virtual screening and experimental evaluation	2 active, 1 activity $\leq$1 μM, 4 tested	ChEMBL
424255	Inhibition of human recombinant DNMT1	Constrained (L-)-*S*-adenosyl-L-homocysteine (SAH) analogs as DNA methyltransferase inhibitors	18 active, 2 activity $\leq$1 μM, 25 tested	ChEMBL
736572	Inhibition of human DNMT1 using AdoMet and poly dI-dC after 2 h by radioactive assay	Synthetic approaches to DNMT inhibitor SGI-1027 and effects on the U937 leukemia cell line	1 active, 1 tested	ChEMBL
424222	Inhibition of human recombinant DNMT3b2 expressed in baculovirus infected high five insect cells	SAR around (L)-*S*-adenosyl-L-homocysteine, an inhibitor of human DNA methyltransferase (DNMT) enzymes	16 active, 7 activity $\leq$1 μM, 20 tested	ChEMBL
675178	Inhibition of DNMT1 in human HeLa cell nuclear extract assessed as methylated substrate level at 10 μM by ELISA	New cytosine derivatives as inhibitors of DNA methylation	2 tested	ChEMBL

Continued

Table 2 Representative Confirmatory Assays in PubChem Related to DNMTs—cont'd

AID	Name	Title	Assay Data	Source
657293	Inhibition of human DNMT1 using oligonucleotide 2 as substrate after 5000 s by micro plate reader based real-time break-light assay	Development of rationally designed DNA N6 adenine methyltransferase inhibitors	1 active, 1 activity ≤1 μM, 3 tested	ChEMBL
257909	Inhibitory activity against DNA methyl transferase in leukemic NALM6 cells	Discovery of two novel, small-molecule inhibitors of DNA methylation	2 tested	ChEMBL
631009	Inhibition of human recombinant DNMT1 expressed in Sf9 cells assessed as remaining activity at 2 mM after 3 h by scintillation counting in presence of [3H] *S*-adenosyl methionine	Synthesis and biochemical evaluation of delta(2)-isoxazoline derivatives as DNA methyltransferase 1 inhibitors	26 tested	ChEMBL
1073761	Cytotoxicity against human U937 cells after 48 h by trypan blue exclusion assay	Selective nonnucleoside inhibitors of human DNA methyltransferases active in cancer including in cancer stem cells	6 active, 6 tested	ChEMBL
602386	Dose response confirmation of DNMT1 inhibitors in a fluorescent molecular beacon assay	Dose–response confirmation of DNMT1 inhibitors in a fluorescent molecular beacon assay	179 active, 1 activity ≤1 μM, 200 tested	Burnham Center for Chemical Genomics
613112	Inhibition of DNMT in cell-free system	Epigenetic profiling of the antitumor natural product psammaplin A and its analogs	1 active, 1 activity ≤1 μM, 1 tested	ChEMBL
890	Confirmation concentration-response assay for epigenetic modulators	Confirmation concentration-response assay for epigenetic modulators	24 active, 1 activity ≤1 μM, 51 tested	NCGC
597	qHTS assay for epigenetic modulators	qHTS assay for epigenetic modulators	59 active, 68401 tested	NCGC