













REVIEW



Exploring the epigenetic drug discovery landscape

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ABSTRACT

Introduction: Epigenetic modification has been implicated in a wide range of diseases and the ability to modulate such systems is a lucrative therapeutic strategy in drug discovery.

Areas covered: This article focuses on the concepts and drug discovery aspects of epigenomics. This is achieved by providing a survey of the following concepts: (i) factors influencing epigenetics, (ii) diseases arising from epigenetics, (iii) epigenetic enzymes as druggable targets along with coverage of existing FDA-approved drugs and pharmacological agents, and (iv) drug repurposing/repositioning as a means for rapid discovery of pharmacological agents targeting epigenetics.

Expert opinion: Despite significant interests in targeting epigenetic modifiers as a therapeutic route, certain classes of target proteins are heavily studied while some are less characterized. Thus, such orphan target proteins are not yet druggable with limited report of active modulators. Current research points towards a great future with novel drugs directed to the many complex multifactorial diseases of humans, which are still often poorly understood and difficult to treat.

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1. Introduction

Nature modulates the expression or activity of proteins and genes using three basic mechanisms: mutation/copy number variation/translocation collectively called genetic events [1], modulation via natural substrates (both small and large molecules) [2], and epigenetic regulation, primarily via posttranslational modifications (PTMs) such as acetylation and methylation of histones and DNA [3]. Epigenetics is a term coined by Conrad Hal Waddington to describe ‘the branch of biology which studies the causal interaction between genes and their products, which bring the phenotype into being’ [4]. Particularly, Waddington’s developmental landscape diagram metaphorically describes how an environmental stimulus may exert its influence on the inheritance of acquired characteristics [5,6]. This landmark event and other milestones in the history of epigenetics are summarized in Figure 1. Thus, epigenetic modification is pertinent for the regulation of gene expression and differentiation and as heritable changes in gene activity or cellular phenotype without alteration of the DNA sequence [3]. Such epigenetic modification encompasses three major epigenetic markers (e.g. writers, readers, and erasers) acting on three major substrates (e.g. DNA, histones, and noncoding RNAs). It is worthy to note that various components of the epigenetic machinery are highly interconnected and influenced by various factors (e.g. gender, nutrition,

environmental and chemical factors, social and economic status, aging and stress) as summarized in Figure 2.

DNA methylation directly affects the genomic DNA and is accomplished by DNA methyltransferases (DNMTs), which add methyl donor groups from the S-adenosylmethionine (SAM) to the 5’ position of the cytosine ring within 5’-cytosine-phosphate-guanosine-3’ (CpG) sites [7,8]. DNA methylation plays an important role in genomic imprinting (i.e. for conveying parent to offspring gene control), suppression of retrotransposons, maintenance of genome stability, X-chromosome inactivation, as well as other types of gene regulation [9,10]. An equally important mechanism aside from DNA methylation is DNA demethylation [11], which is the removal of the methyl group making such a process necessary for the reprogramming of genes.

Histone associates with DNA to form a complex known as nucleosome [12]. The nucleosome is composed of pairs of histone proteins H2A, H2B, H3, and H4 that are assembled in an octameric core with 147 base pairs of DNA wrapped around it. Repeating nucleosomes are linked and stabilized by histone H1 that is folded up to form the chromatin structure [13]. A myriad of histone PTMs exists for the epigenetic regulation of gene expression and these can be classified on the basis of their catalytic mechanisms and the substrates that they read, write, or erase [14]. Mechanisms employed by PTMs and currently explored in drug discovery research are illustrated in Figure 3. A large proportion of the epigenetic target space is PTM enzymes involved in reading,

Article highlights

- Epigenetic events act as a mediator between the external/environmental factors (cause) and the manifested genetic landscape regulation (effect). Therefore, lifestyle choices and environmental factors affect gene expression thereby influencing the etiology of various diseases.
- In a nutshell, epigenetic events allow a reversible and robust switching on and off of gene expression via the attachment and processing of chemical tags on DNA and histones thereby regulating the euchromatin state that is required for active transcription.
- Dysregulation of targets mediating epigenetic events can give rise to pathological deterioration associated with cardiovascular diseases, neurological disorders, metabolic disorders, and cancer development. Epigenomic targets thus represents an attractive avenue for pharmacological intervention by small molecules.
- Epigenetic drugs have primarily been studied for their use in treating cancer, however research on their use in Alzheimer's, Asthma and a myriad of other CNS, CVS, inflammatory/immune diseases is steadily rising.
- Great interest has been invested in the discovery of inhibitors or modulators against several key epigenetic drug targets. However, current epigenetic modulators lack isoform selectivity and produce global epigenetic changes. Advances in structural/functional genomics and chemical biology have provided insights into the isoform selectivity requirements and sequence-specific epigenetic chemical probes.
- Drug repositioning or drug repurposing represents an interesting route for the rapid discovery of novel drugs targeting the epigenetic machinery and for expanding the therapeutic indication profiles of existing epigenetic drugs.

This box summarizes key points contained in the article.

writing, and erasing the epigenetic marks [15]. While methylation and acetylation of histone residues are well-known writers of epigenetic marks, there are however a number of other modifications that can be classified into those that neutralize the positive charge of lysine and arginine (e.g. acetylation, butyrylation, citrullination, crotonylation, and propionylation) and those that retain (e.g. methylation) or add one or more negative charges (e.g. succinylation, malonylation, ADP ribosylation, and phosphorylation) [16]. The chromatin structure is highly dependent on the integrity of the nucleosome complex, which is mediated by the highly basic histone N-terminal tail that protrudes from the nucleosome and makes contact with adjacent nucleosomes [7]. Charge neutralization or gain in negative charge via PTM of these tails would not only affect internucleosomal interactions but also the nucleosomal-DNA complex structure and thus affect the overall chromatin structure and the expression levels of the corresponding genes [16].

In addition to the aforementioned histone and DNA modifiers, the epigenetic machinery is also composed of noncoding RNAs (ncRNAs). ncRNAs are functional RNA molecules that do not encode proteins. ncRNAs can bind DNA and alter its conformation, and in effect regulate gene expression, mRNA stability at the posttranscriptional level. Although important in its own rights, this review will not delve deeper into the topic as emphasis will be placed on DNA and histone modifications [17].

2. Factors influencing epigenetics

The epigenome situates itself at the interface between the genome and the environment [18] as summarized in

Figure 2. Susceptibility to epigenetic alterations is life-time dependent [19] where gametogenesis and early embryogenesis are considered to be critical periods with high genome plasticity [18]. Epigenetic memory conveys inheritance from generation to generation [19], meaning that memory can be transferred across generations [18] without the need of re-exposure to the same epigenetically driven factors [19]. The effect of altered epigenetic markers in early life stages is not only displayed as acute adaptive responses, but can also gradually manifest as adult-onset diseases [18] upon secondary triggers (e.g. aging and hormonal changes) [20]. The plasticity and reversibility of the epigenome render the hosts susceptible to reprogramming when exposed to external factors [19]. Thus, studies toward understanding the role of external factors in epigenetic alterations could at least partially demystify the rather complex etiology of multifactorial diseases, and may provide a promising strategy for the prevention and treatment of many complex multifactorially driven diseases [21].

3. Drugging epigenetic targets

Owing to its broad involvement in a wide range of diseases, epigenetics have received great attention for the discovery of novel therapeutic agents. **Figure 3** summarizes the various enzymes that mediate covalent modifications of DNA and histones as a collective mechanism for epigenetic control of gene transcription. The intense efforts in the field have already bore fruits as several FDA-approved drugs targeting the epigenetic machinery have been developed. Moreover, efforts on drug repositioning or repurposing has also led to the discovery of novel indications for existing FDA-approved drugs in the field. In this review, a few drug repositioning case studies are discussed in **section 5** under the heading 'Drug repurposing opportunities for epigenetic drug discovery.' Chemical structures of these drugs are shown in **Figure 4** while protein structures of epigenetic modifiers as determined by X-ray crystallography are illustrated in **Figure 5**.

Table 1 illustrates the available bioactivity data for epigenetic drug targets from the recent Release 22 of the ChEMBL database [22]. The availability of bioactivity data can be taken as a relative measure of their druggability and importance for drug discovery research. **Table 2** summarizes the available crystal structures and bioactivity data of epigenetic protein-ligand complexes. Resources such as this, as well as other manually curated bioinformatics database of epigenetic enzymes (e.g. dbEM) [23] and chemogenomics databases of epigenetic protein-ligand interactions (e.g. HEMD) [23,24] are great starting points for ligand and structure-based drug design efforts [25]. It is implied that some epigenetic enzymes are more popular among scientists than others, and as a result some may be more heavily studied while others may remain as orphan targets in need of attention. Computational chemogenomics and proteochemometrics [26,27] are promising approaches for suggesting potential ligands for orphan target proteins on the basis of the molecular similarity concept in which similar ligands are implied to bind to similar target proteins, as well as *vice versa* where potential target proteins could be suggested for a ligand of interest.

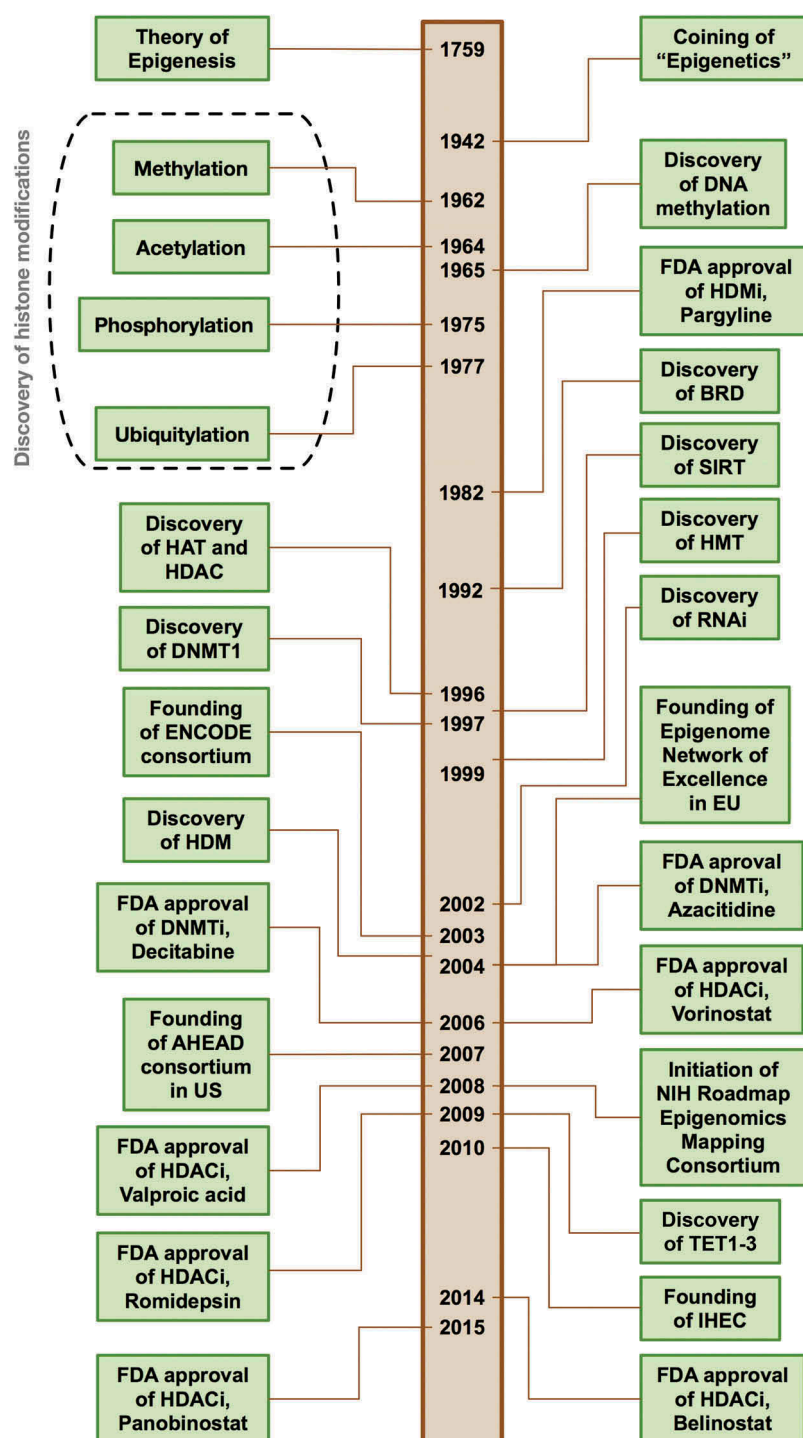


Figure 1. Timeline of milestones in the history of epigenetics.

3.1. DNA methyltransferase inhibitors (DNMTi)

DNA methylation is controlled by the family of DNMTs comprising of DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3 L [28]. The DNMTs share a common catalytic domain referred to as the AdoMet-dependent MTase fold [29]. Within this family, DNMT1 maintains methylation patterns after DNA replication while DNMT3A and DNMT3B together with its regulatory factor DNMT3L regulates *de novo* DNA methylation during the early embryonic development of mammals. Finally, DNMT2 is

involved in cytoplasmic RNA methylation [30–33]. Alterations of DNA methylation (e.g. hypomethylation and hypermethylation) have been found to be correlated with cancers, genetic disorders, neurological and autoimmune diseases. Hence, DNMTs have gained prominence as drug targets and as such several small-molecule inhibitors targeting the DNMT family have been reported [34,35]. Two nucleoside-based DNMT inhibitors belonging to the cytidine chemotype, 5-aza-cytidine (Azacitidine or Vidaza) and 5-aza-2'-deoxycytidine (Decitabine or Dacogen), have been approved by the FDA for the

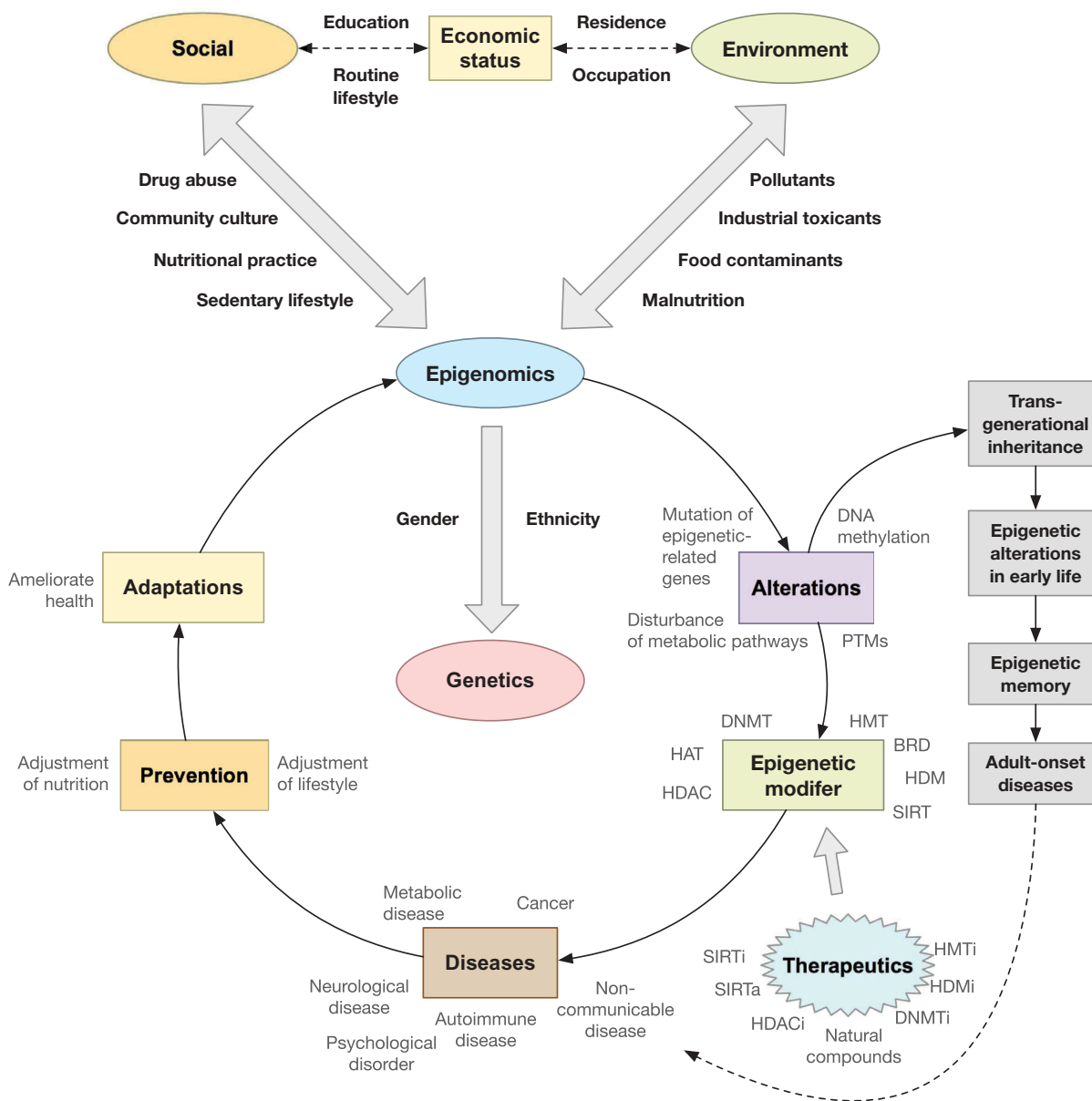


Figure 2. Epigenetics acts as a mediator between genetic and external factors in the development of diseases and its adaptations highlight its potential in therapy and disease prevention. Economic status may be considered to be a key factor that influences both social and environmental factors. An individual's income may govern their chance of toxic substance exposure as it governs their area of residence, education opportunity, occupation, workplace and nutritional practice.

treatment of myelodysplastic syndrome. These inhibitors intercalate between DNA base pairs and suppress the methylation of CpG islands that are generally enriched in transcriptionally relevant regulatory sequences also known as the promoter regions of genes [34,35]. However, DNMTi cytidine analogs are chemically unstable and have a highly promiscuous target association profile. Given these concerns, this calls for the need of more specific and selective DNMT inhibitors [36].

In recent years, the number of compounds tested as DNMT inhibitors have increased as reflected in the public databases. As summarized in Table 1, a query from the ChEMBL database [22,37] revealed that there were 502 compounds tested for DNMT1 (ChEMBL1993), 62 compounds tested for DNMT3A (ChEMBL1992), and 68 compounds for DNMT3B (ChEMBL6095). A number of potent non-nucleoside analogs targeting DNMT have been explored, including

SGI-110, procainamide, epigallocatechin 3-gallate (EGCG), RG108, and hydralazine. SGI-110 (also known as guadecitabine or S110) is a CpG dinucleotide derivative of 5-azadeoxycytidine (5-aza-dC or decitabine) [36]. Particularly, it is an oligonucleotide consisting of decitabine linked to the endogenous nucleoside deoxyguanosine via a phosphodiester bond. It is considered to be an efficient prodrug of decitabine [36] as the dinucleotide configuration provides protection against drug degradation by cytidine deaminase while maintaining the effect of its active metabolite, decitabine [38,39]. Thus, SGI-110 is considered to be a potent inhibitor of DNA methylation [38]. To date, SGI-110 is undergoing a phase III clinical trial for myelodysplastic syndrome and acute myeloid leukemia and a phase II clinical trial for hepatocellular carcinoma (<http://clinicaltrials.gov>). In addition to the identification of non-nucleoside analogs for

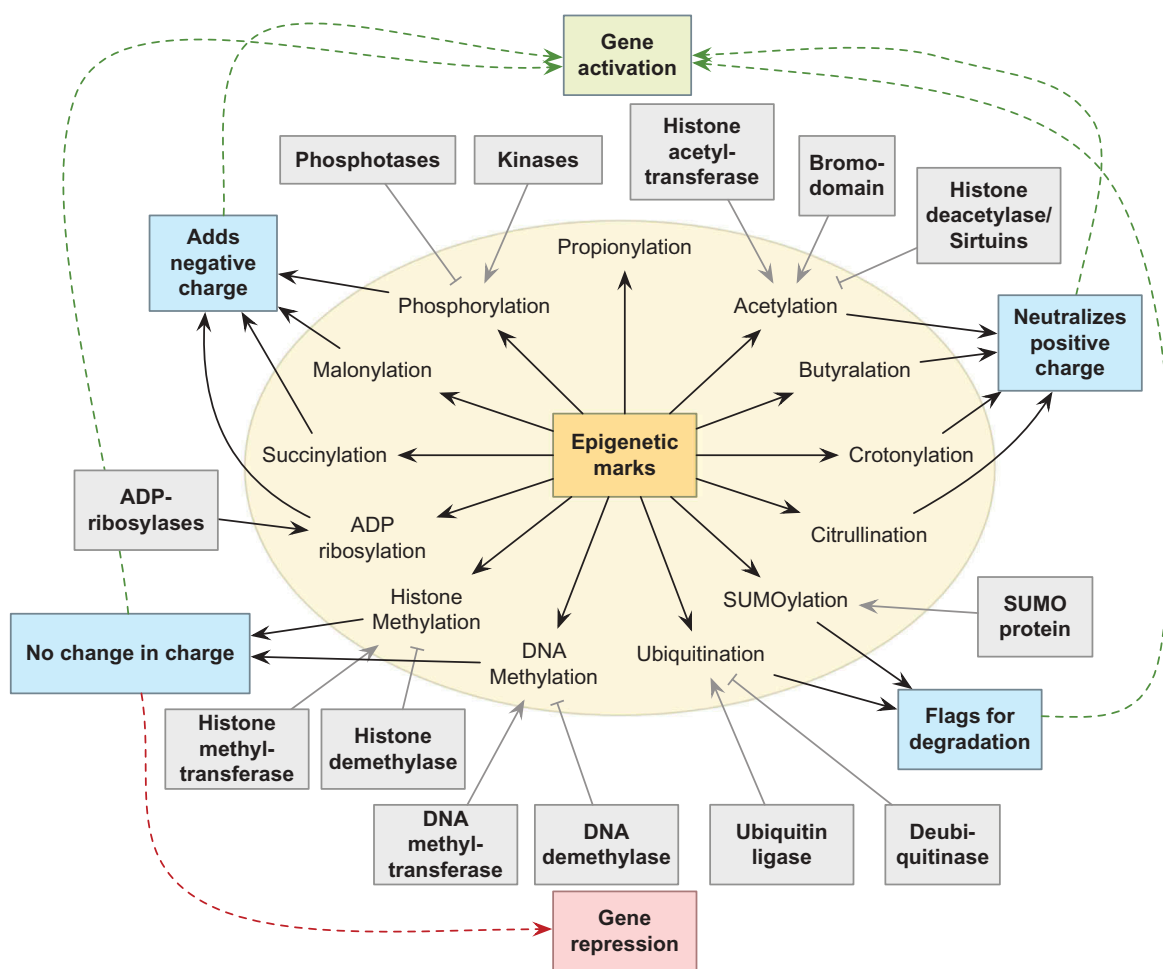


Figure 3. Illustration of the comprehensive spectrum of various DNA and PTM-mediated epigenetic marks. Effects of these marks on the charge and activation/repression of gene expression via an open chromatin structure is shown schematically. The open chromatin structure is characterized by the loss of complementary interactions between negatively-charged DNA and positively-charged residues in histone tails. The neutralization of positive charges or gain of negative charges leads to disruption of complementary interactions and results in an open chromatin structure and facilitates activation of gene expression.

DNMT targets, several strategies have been proposed including the development of allosteric inhibitors, SAM analogs for DNMT, DNA substrate competitors, combining two DNMT substrates SAM and cytosine/deoxycytidine in a single structure, and molecules for disruption of protein-protein interactions [36].

3.2. Histone acetyltransferase inhibitors (HATi)

Histone acetyltransferases (HATs) were first identified as regulators of tumor suppressors and were implicated in several diseases, including cancer progression, viral infection, and certain respiratory disorders [40]. Three naturally occurring small molecules have been described as HAT inhibitors: curcumin, garcinol, and anacardic acid [41]. Curcumin is an EP300- and CREBBP-specific inhibitor capable of repressing EP300-mediated p53 acetylation *in vivo* [42]. Its antitumor activities in a wide variety of cancers included, respectively, the downregulation and upregulation of CCND1 (cyclin D1) and CASP8 (caspase-8), as well as the inhibition of constitutive nuclear factor- κ B (NF- κ B) activation [43]. Garcinol and anacardic acid are both EP300 and KAT2B HAT inhibitors. Although, garcinol exhibits a much better cell

permeability than anacardic acid, both may improve cancer therapy. Whereas, garcinol has been shown to induce apoptosis in HeLa cells while anacardic acid can sensitize cancer cells to ionizing radiation. A few other small molecules have been described as HAT inhibitors, but to date only a series of isothiazolones affecting EP300 and KAT2B activity were found to inhibit the growth of colon and ovarian cancer cells.

3.3. Histone deacetylase inhibitors (HDACi)

To date, 18 HDACs have been identified in mammals and categorized into four structurally and phylogenetically distinct classes, namely class I, IIA, IIB, and III. Class I is homologous to yeast Rpd3 deacetylase, IIA and IIB are homologous to yeast Hda1 deacetylase, and III is homologous to yeast Sir2. Interestingly, HDAC11 shows homology to enzymes of both classes I and II but is classified as a class IV enzyme. Class I and II HDACs as well as HDAC11 are zinc-dependent hydrolases whereas class III sirtuins are NAD-dependent enzymes. These enzymes are implicated in a wide variety of biological processes, such as apoptosis, differentiation, proliferation, and senescence [44]. Referring to

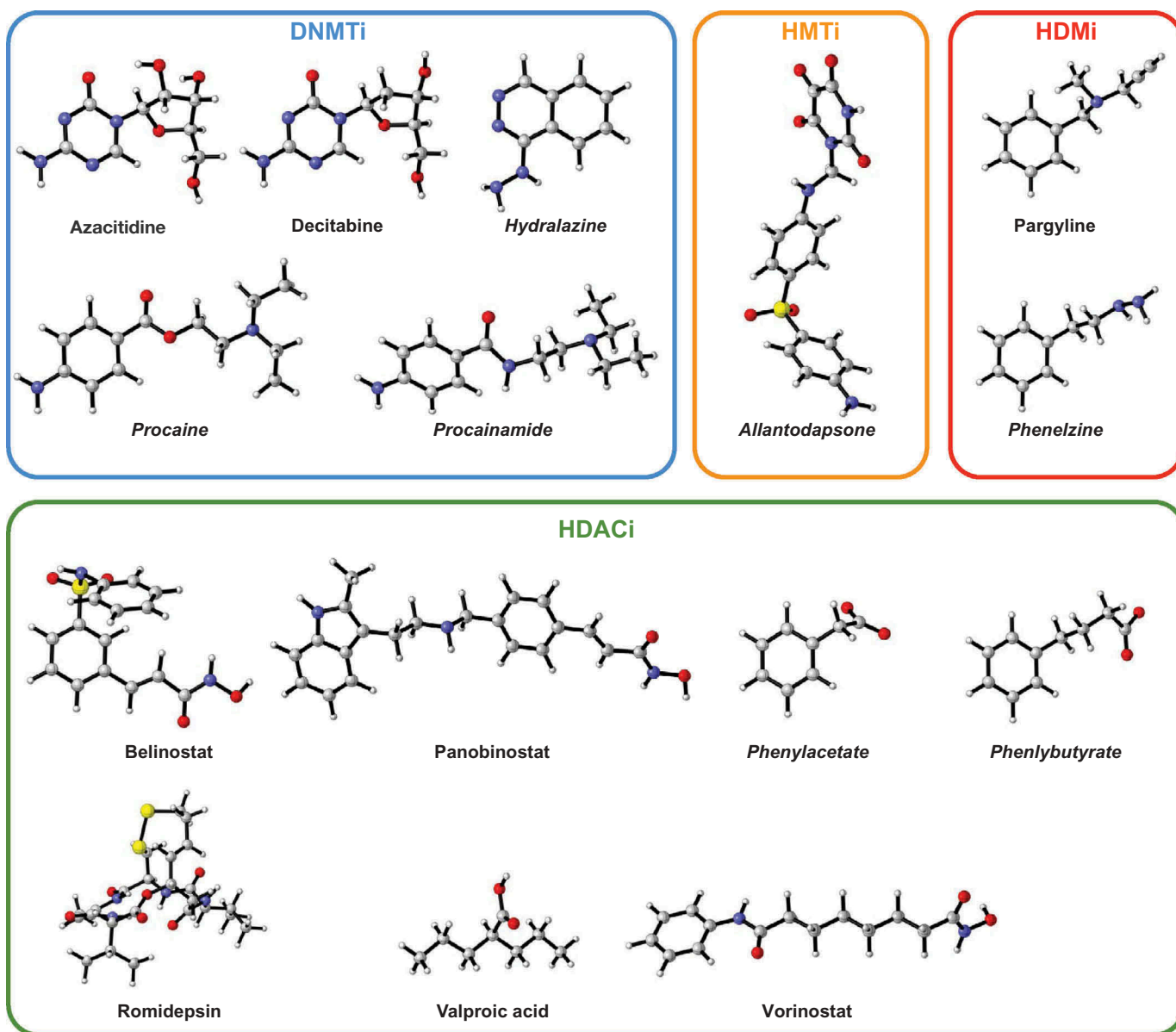


Figure 4. Chemical structures of FDA-approved drugs targeting the epigenetic machinery. Drugs that are FDA-approved for epigenetic targets are indicated by bold text whereas those that were approved for other indications and repurposed for epigenetic targets are highlighted as bold italic text. Epigenetic targets consisted of DNA methyltransferase inhibitors (DNMTi), histone methyltransferase inhibitors (HMTi), histone demethylase inhibitors (HDMi) and histone deacetylase inhibitors (HDACi).

Table 1, it is interesting to note that much of the current efforts have been directed toward HDAC1, HDAC6, HDAC8, and SIRT1 and SIRT2 with reported number of compounds showing bioactivity amounting to 3822, 2117, 1371, 1240, and 1260, respectively, while other HDACs have accumulated less than 1000 compounds.

The essential ligand-based pharmacophoric requirements for HDAC can be summarized as follows: (i) a capping group that interacts with residues at the active site entrance, (ii) a Zn-binding group (ZBG) that coordinates with the catalytic metal atom within the active site, and (iii) a linker group that binds with hydrophobic tunnel residues and positions the ZBG and the capping group for interaction in the active site [45]. Several HDACi chemotypes have been developed consisting of short-chain fatty acids (e.g. sodium butyrate, phenylbutyrate, pivanex,

and valproic acid), cyclic tetrapeptides and natural compounds as well as the newer and more selective classes consisting of hydroxamic acids (e.g. vorinostat, belinostat, panobinostat, and dacinostat), benzamides (e.g. entinostat and mocetinostat), and bicyclic depsipeptide (e.g. romidepsin) [46].

Particularly, the majority of compounds under clinical trials are hydroxamic acid analogs [47]. The clinical success of hydroxamic analogs has been demonstrated first for the FDA-approved drug vorinostat [48]. HDAC inhibitory activity of compounds in this class can be attributed to the crucial polar hydroxamic group that interacts with the Zn-binding protein or chelates the Zn ion located at the catalytic site of the enzyme pocket, thereby leading to the inhibition of deacetylation [48]. Romidepsin, a cyclic tetrapeptide, is a naturally derived FDA-approved drug that blocks HDAC activity via the reduction of thiol released from the cell

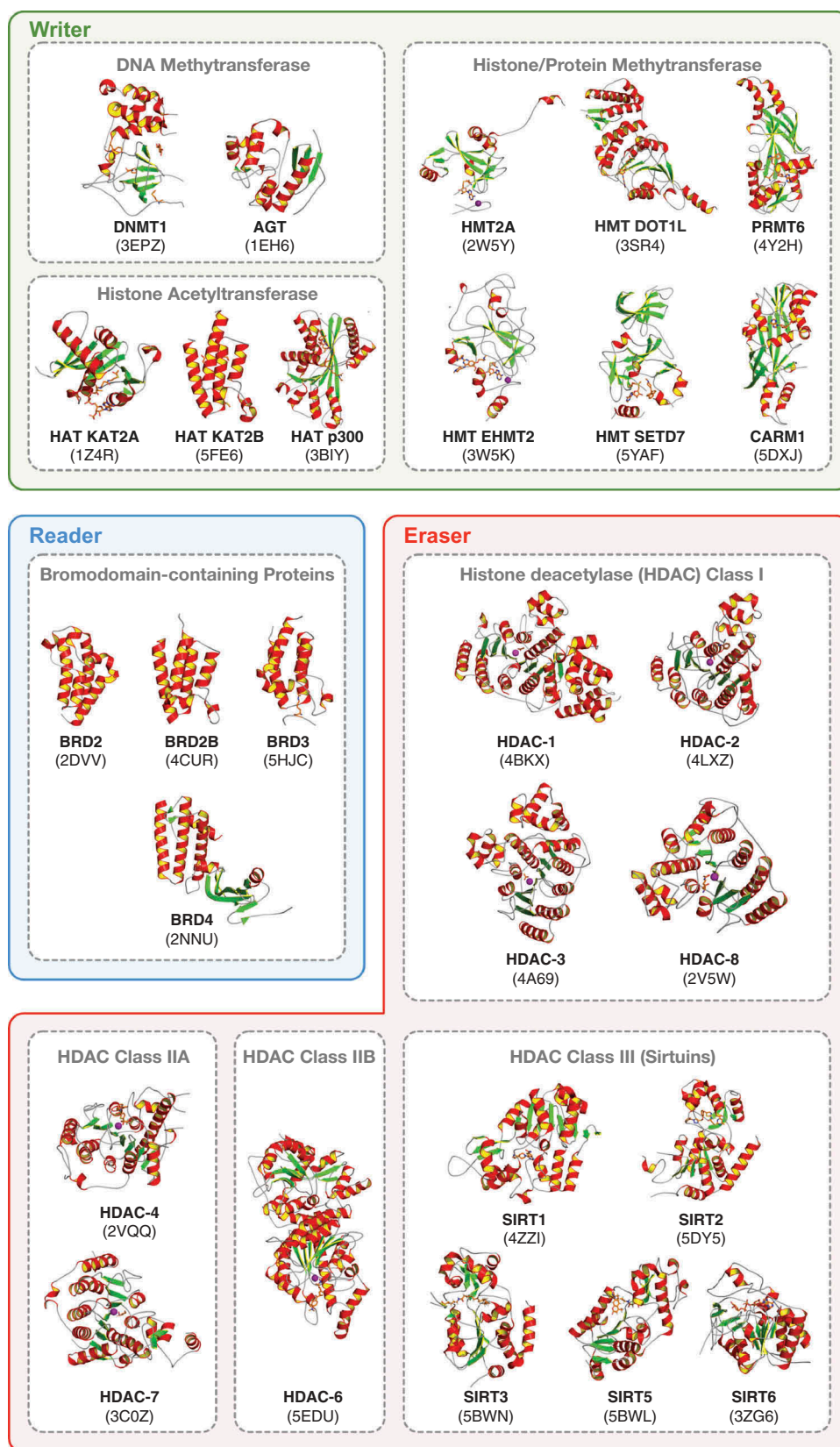


Figure 5. Protein structures of epigenetic drug targets. Proteins are classified into the three classes of epigenetic modifiers consisting of writers, readers and erasers. Alpha-helices are represented as a red ribbon (inner face shown in yellow), beta-strands are shown in green, loops are in gray and Zn ions are depicted as a purple sphere. Each structure is labeled by its acronym followed by the PDB ID in parenthesis on the subsequent line. Protein names and their acronyms are listed as follows: DNA (cytosine-5)-Methyltransferase-1, DNMT1; O-6-Alkylguanine-DNA Alkyltransferase, AGT; Histone-Lysine *N*-Methyltransferase 2A, HMT2A; Histone-Lysine *N*-Methyltransferase, H3 Lysine-79 specific (H3K79) DOT1L, HMT DOT1L Histone-Lysine *N*-Methyltransferase EHMT2, HMT EHMT2; Histone-Lysine *N*-Methyltransferase SETD7, HMT SETD7; Protein Arginine Methyltransferase PRMT6, PRMT6; Histone-Arginine Methyltransferase CARM1, CARM1; Histone Acetyltransferase KAT2A, HAT KAT2A; Histone Acetyltransferase KAT2B, HAT KAT2B; and Histone Acetyltransferase p300, HAT p300; Bromodomain-containing Protein-2, BRD2; Bromodomain containing Protein-2B, BRD2B; Bromodomain containing Protein-3, BRD3; Bromodomain containing Protein-4, BRD4; Histone Deacetylases 1 to 8, HDAC1 to HDAC8; and Sirtuins 1 to 6, SIRT1 to SIRT6. Full color available online.

Table 1. Summary of bioactivity data for compounds targeting the three classes of epigenetic modifiers.

CHEMBL ID	Target Name	UniProt	Number of compounds	Number of end points
Writer				
CHEMBL2864	6-O-methylguanine-DNA methyltransferase	P16455	167	387
CHEMBL1993	DNA (cytosine-5)-methyltransferase 1	P26358	502	597
CHEMBL3784	Histone acetyltransferase p300	Q09472	268	406
CHEMBL5500	Histone acetyltransferase PCAF	Q92831	393	500
CHEMBL5501	Histone acetyltransferase GCN5	Q92830	13857	14188
CHEMBL2189110	Histone-lysine N-methyltransferase EZH2	Q15910	338	589
CHEMBL6032	Histone-lysine N-methyltransferase, H3 lysine-9 specific 3	Q96KQ7	90127	92115
CHEMBL1293299	Histone-lysine N-methyltransferase MLL	Q03164	17174	17203
CHEMBL1795117	Histone-lysine N-methyltransferase, H3 lysine-79 specific	Q8TEK3	121	185
CHEMBL5523	Histone-lysine N-methyltransferase SETD7	Q8WTS6	166	188
CHEMBL5406	Histone-arginine methyltransferase CARM1	Q86X55	144	202
CHEMBL5524	Protein-arginine N-methyltransferase 1	Q99873	329	544
CHEMBL2093861	Menin/Histone-lysine N-methyltransferase MLL	O00255, Q03164	44110	47950
Reader				
CHEMBL1293289	Bromodomain-containing protein 2	P25440	237	346
CHEMBL1741220	Bromodomain adjacent to zinc finger domain protein 2B	Q9UIF8	55612	55612
CHEMBL1795186	Bromodomain-containing protein 3	Q15059	190	285
CHEMBL1163125	Bromodomain-containing protein 4	O60885	873	1646
Eraser				
CHEMBL325	Histone deacetylase 1	Q13547	3822	5453
CHEMBL1937	Histone deacetylase 2	Q92769	933	1334
CHEMBL1829	Histone deacetylase 3	O15379	862	1124
CHEMBL3524	Histone deacetylase 4	P56524	912	1274
CHEMBL2563	Histone deacetylase 5	Q9UQL6	333	416
CHEMBL1865	Histone deacetylase 6	Q9UBN7	2117	2969
CHEMBL2716	Histone deacetylase 7	Q8WUJ4	413	524
CHEMBL3192	Histone deacetylase 8	Q9BY41	1371	1664
CHEMBL4145	Histone deacetylase 9	Q9UKV0	234	295
CHEMBL5103	Histone deacetylase 10	Q969S8	285	359
CHEMBL3310	Histone deacetylase 11	Q96DB2	245	302
CHEMBL4506	Sirtuin 1	Q96EB6	1240	2133
CHEMBL4462	Sirtuin 2	Q81XJ6	1260	1921
CHEMBL4461	Sirtuin 3	Q9NTG7	373	467
CHEMBL2163183	Sirtuin 5	Q9NXA8	277	317

Briefly, writers, readers and erasers adds, reads and removes epigenetic marks such as methyl and acetyl. Data was obtained from ChEMBL database (version 22)[22] where only those with a compound count exceeding 100 are shown.

through the formation of a disulfide bond [47]. Thiol is essential for the interaction with the Zn-dependent pocket of HDACs and therefore the decreased availability of thiol leads to HDAC inhibition [47]. Following the discoveries of vorinostat and romidepsin, analogs of clinically potent second-generation inhibitors have been developed to improve their specificity and toxic profiles [48]. Emphasis has been paid on compounds belonging to the classes of hydroxamic acids (e.g. panobinostat, givinostat, and belinostat) and benzamides (e.g. entinostat and mocetinostat) [48]. Compounds in this generation exhibit improved profiles (i.e. improved efficacy, pharmacodynamic and pharmacokinetic properties with decreased toxicity). However, their mechanisms of action are the same as the clinically used ones and their ability to produce more effective clinical outcomes has yet to be seen [48]. Some promise can be found in classes I (e.g. RG2833, PCI-34051) and II (e.g. trifluoromethyloxadiazole (TFMO)) HDACi that are currently under preclinical development [48]. Of particular note is that adamantane and noradamantane are crucial scaffolds where compounds possessing these moieties exhibited HDAC inhibitory effects in the picomolar range [48]. Furthermore, natural compounds (e.g. diallyl disulfide, resveratrol, and spiruchostatin A) and other scaffolds (e.g. thioesters, epoxides, and electrophilic ketones) were also reported as HDACi [49].

Based on the analysis of existing HDACi and active compounds [50], several important issues could be considered in the design of HDAC inhibitors. First, as illustrated in Figure 6(a),

isoform selective inhibition of HDACs could achieve beneficial efficacy profiles. With the exception of a few reports from the Bradner lab [51] and a few other groups, very few structure–activity relationship (SAR) studies have been reported aiming to improve the isoform selectivity. Second, the majority of HDACi have hydroxamic acid as a Zn-binding group. Due to concerns regarding the toxicity of the hydroxamic acid substructure and the general nature of Zn-chelating fragments, the identification of alternative Zn-binding groups or non-chelating fragments complementary to residues of the Zn-containing pocket are highly desirable [52]. Third, most computational studies of HDAC enzymes have not discussed or adequately compared the ionization states of HDAC enzymes and bound ligands. This limits the insights gained from most pharmacophore modeling studies that have only characterized metal groups as hydrogen bond acceptor/donor groups but not in terms of ionizable features, which are commonly seen in most metal chelators [50]. This has in turn critically limited the utility of pharmacophore models for virtual screening endeavors in the identification of HDACi with novel Zn-chelating fragments. Fourth, since HDACi is predominantly a metal chelator, the creation of a more effective scoring function that can effectively deal with molecular recognition events (i.e. the coordinate covalent bond formation) is needed. In particular, such scoring functions are required to advance HDACi design and development while generally advancing docking efforts against metal-containing proteins [50].

Table 2. Summary of X-ray crystal structures of epigenetic drug targets and their bound active ligand.

PDB ID	Target name	UniProt ID	Compound name	PDBeChem code	Bioactivity type	Bioactivity value (nM)
Writer						
4NVQ	Histone-lysine N-methyltransferase, H3 lysine-9 specific 3 (EHMT2)	Q96KQ7	A-366	2OD	IC ₅₀	3.3
3QOX	Histone-lysine N-methyltransferase, H3 lysine-79 specific (DOT1L)	Q8TEK3	S-adenosyl-L-homocysteine	SAH	K _i	270
Reader						
4UYG	Bromodomain- containing protein 2 (BRD2)	P25440	I-BET726 (GSK1324726A)	73B	K _D	4.4
4NRB	Bromodomain adjacent to zinc finger domain protein 2B (BRD2B)	Q9UIF8	N01197	2LX	IC ₅₀	38000
3LQJ	Histone-lysine N-methyltransferase 2A (MLL)	Q03164	H3(1-9)K4me3 peptide	-	K _D	4300
2YEL	Bromodomain- containing protein 4 (BRD4)	O60885	GW841819X	WSH	IC ₅₀	15.5
Eraser						
3MAX	Histone deacetylase 2 (HDAC2)	Q92769	N-(4-aminobiphenyl-3-yl)benzamide	LLX	IC ₅₀	27
2VQO	Histone deacetylase 4 (HDAC4)	P56524	2,2,2-trifluoro-1-(5-{3-phenyl-5H,6H,7H,8H-imidazo[1,2-a]pyrazine-7-carbonyl}thiophen-2-yl)ethane-1,1-diol	TFG	IC ₅₀	317
3ZNR	Histone deacetylase 7 (HDAC7)	Q8WU14	TMP269	NU9	K _i	36
2V5X	Histone deacetylase 8 (HDAC8)	Q9BY41	(2 R)-N~8~-hydroxy-2-[(5-methoxy-2-methyl-1H-indol-3-yl)acetyl]amino-N~1~-[2-(2-phenyl-1H-indol-3-yl)ethyl]octanediamide	V5X	IC ₅₀	100

Only druggable targets discussed in Table 1 were processed. The bioactivity data were extracted from the BindingMOAD [55].

3.4. Sirtuin inhibitors (SIRTi) and modulators

Development of sirtuin (SIRT) modulators is an ongoing research where most compounds are still under preclinical investigation. Among all human SIRTs, the discovery of modulators has been driven toward SIRT1 and SIRT2. Specific inhibitors against SIRT1 have been suggested for cancer treatment [47]. SIRTi can be classified by their scaffolds as β -naphthols (e.g. sirtinol, splitomicin, salermide, and cambinol), indoles (e.g. EX-527 and oxyindole) and ureas (e.g. suramin and tenovin) [53]. In addition, other types such as chalcone and 1,4-dihydropyridine have been reported to inhibit SIRTs [53]. Great attention has been given to SIRT1 activators for conveying neuroprotection [47]. In addition, phenol derivatives such as resveratrol, quercetin, and piceatannol have been reported as SIRT1 activators [47]. Of note, resveratrol and its synthetic derivatives (e.g. SRT1720 and SRT2183) are promising compounds undergoing clinical trials [47]. These resveratrol-based compounds have been suggested to act as allosteric enzyme activators [47]. Furthermore, a different mechanism of SIRT1 activation has been reported for isonicotinamide whereby it interacts competitively with an endogenous SIRT1 inhibitor (e.g. nicotinamide) in order to promote deacetylation [47].

3.5. Histone demethylase inhibitors (HDMi)

In humans, the demethylation of N-methyl lysine residue is catalyzed by two distinct subfamilies of demethylases (KDMs), the flavin-dependent KDM1 subfamily and the 2-oxoglutarate (2OG)-dependent JmjC subfamily, both of which employ oxidative mechanisms [54]. Modulation of the histone methylation status is proposed to be important in epigenetic

regulation and has substantial medicinal potential for the treatment of diseases including cancer and genetic disorders. Demethylases of the LSD1/KDM1 family share some sequence and structural similarities to amine oxidases and monoamine oxidase. Consequently, inhibitors of monoamine oxidases (MAOi) such as pargyline, phenelzine, and tranylcypromine can also inhibit the HDM KDM1A (Figure 4) [56]. Increasing the arsenal of inhibitors against the many HDMs involved in cancer will be a major challenge in the coming years. Furthermore, studies on the selective inhibition of the catalytic domain from both human KDM1/LSD and JmjC families of KDMs are progressing rapidly. Although these studies are at a relatively early stage, the signs suggest that with sufficient medicinal chemistry efforts, it will be possible to make highly potent and selective inhibitors against the catalytic domains from both families of human KDMs. To date, most KDM1 and JmjC KDM inhibition efforts have been focused on the extension of known inhibitors for other family members (i.e. mechanism-based inhibition of KDM1s and active site iron chelators for the JmjC KDMs). It is likely that the extension of those methods (i.e. by competing with histone substrate binding interactions) will lead to highly selective inhibitors of the catalytic domains [57].

3.6. Histone methyltransferase inhibitors (HMTi)

Histone/protein methyltransferases (HMTs/PMTs) catalyze the transfer of methyl groups from SAM to the side chains of lysine or arginine on the target protein. PMTs can be classified into lysine and arginine methyltransferases (PKMTs and PRMTs, respectively) [58]. All PKMTs contain the conserved catalytic 'SET' (Su(var)3-9, Enhancer-of-zeste, and Trothorax) domain whereby cofactors and substrates bind, with the exception of DOT1L [59,60]. The binding pocket of SAM and

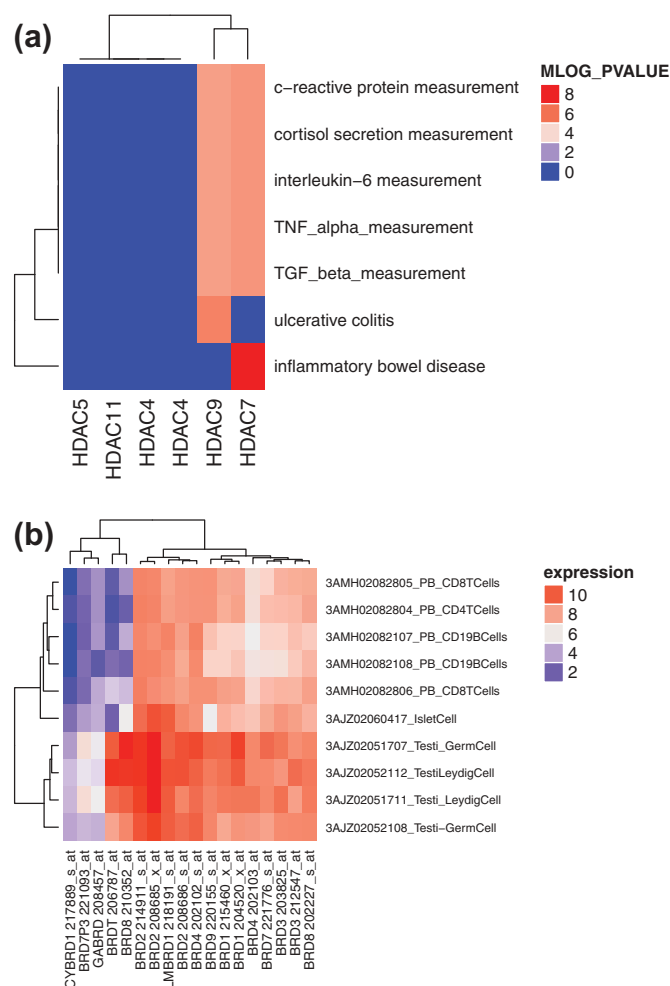


Figure 6. Analysis of the GWAS catalog and BioGPS data to illustrate the advantages of isoform selective modulation of HDACs and BETs respectively. (a) HDAC7 and HDAC9 show high association (in terms of MLOG_PVALUE) with various immune response phenotypes. The HDAC sub-family gene-disease associations were extracted from the GWAS catalog database and analyzed using the Bioconductor package 'gwascat' under the R programming environment. (b) High expression of BRDT in testis cells in comparison to inflammatory-like cells suggests that BRDT inhibition may play no role in anti-inflammatory effects and further reduces the development of testis causing temporary infertility-like effects. The BRD sub-family gene-tissue expression values were extracted from the BioGPS gene expression data set. The BioGPS expression data, GSE1133, was downloaded using the Bioconductor package 'GEOquery' and heatmaps were generated using the 'complexHeatmap' package in R.

amino acid provides structural features for inhibitor interaction and thus makes these enzymes attractive targets for intervention by small-molecule inhibitors [58].

Among many PKMTs, DOT1L and EZH2 are examples of attractive targets for epigenetic therapy. DOT1L is involved in inappropriate methylation of H3K79 and expression of HOX genes that drive leukemia [58]. EPZ004777 was one of the first SAM-competitive inhibitors intended to alter DOT1L, but has poor pharmacokinetic properties. Thus, second-generation DOT1L inhibitors with improved properties were made, such as EPZ-5676, which has successfully completed a phase I clinical trial [61]. In addition, hypermethylation of H3K27 by EZH2 promotes transcriptional silencing whereas high expression of EZH2 is associated with many types of cancer [58]. The first-generation EZH2 inhibitor 3-deazaneplanocin-A (DZNep) targets the S-adenosyl

homocysteine activity and leads to alterations of methionine metabolism [62]. Furthermore, several potent SAM-competitive inhibitors including CPI-1205, GSK2816126, and EPZ6438 have been discovered and are currently undergoing clinical trials for treatment of hematological malignancies (<http://clinicaltrials.gov>). In addition, a growing number of compounds have been tested against other families of lysine methyltransferase including histone-lysine N-methyltransferase, H3 lysine-9 specific 3 (CHEMBL6032), and histone-lysine N-methyltransferase MLL (CHEMBL1293299) (Table 1).

PRMTs are structurally distinct with a conserved methyltransferase domain, a β -barrel specific to PRMTs, and a dimerization domain [59]. Although a number of PRMTs families have been associated with cancer, neurodegenerative diseases, and inflammatory diseases [63], the development of small-molecule inhibitors targeting PRMTs are still limited. An attempt was directed to identify a potent inhibitor targeting CARM1 (PRMT4) that catalyzes the methylation of H3R17. Compound RM65 is a drug-like inhibitor that induced hypomethylation in HepG2 cells [64]. Later, an inhibitor derived from plants, namely TBBD (ellagic acid) has been identified as a specific inhibitor of CARM1 [65]. Recently, a potent and selective inhibitor of PRMT5 with anti-proliferative activity has been characterized. This compound, EPZ015666 (GSK3235025), has entered phase I clinical trial for the treatment of solid tumor and non-Hodgkin's lymphoma [66]. DZNep induces apoptosis in breast cancer MCF7 and colorectal HCT116 cells, where it promotes the depletion of the polycomb-repressive complex-2 proteins (e.g. EZH2) and inhibits methylation of H3K27 [41]. Additionally, the arginine-specific HMT inhibitor AMI-1 (arginine N-methyltransferase inhibitor-1) is believed to inhibit PRMT1, PRMT3, PRMT4, and PRMT6 [67]. The fact that PRMT4 is overexpressed in hormone-dependent cancers may encourage research on these particular inhibitors [68]. Owing to structural similarities, analogs of the AMI-1 derivative AMI-5 can inhibit not only lysine and arginine-specific HMTs but also some HATs and sirtuins with the same potency, thus giving rise to the term 'epigenetic multiple ligands' [69].

3.7. Bromodomain inhibitors (BRDi)

BRD2, BRD3, and BRD4 are among the well-studied proteins of the bromo and extra terminal (BET) family with bioactivity data of 237, 190, and 873 compounds as reported in the ChEMBL database. This class of proteins bind to the acetylated lysine of histones and have been associated with a range of diseases spanning from cancer to inflammation and cardiovascular diseases. Currently, ten compounds capable of blocking the protein-protein interactions of BET bromodomains have entered clinical trials [70]. A phase III clinical trial candidate, RVX-208, developed by Resverlogix Corp. has been evaluated in a total of seven clinical trials for the treatment of atherosclerosis and associated cardiovascular disease. RVX-208 increased the levels of HDL-cholesterol and apolipoprotein A1 as well as decreased the incidences of major adverse cardiac events (MACE) in patients with diabetes mellitus. However, in a phase IIb, randomized, double-blind, multicenter, ASSURE trial, RVX-208 showed no significant increase in either apoA-I

or HDL-C, nor an incremental regression of atherosclerosis than that observed with administration of a placebo [71]. OTX015, BMS-9861158, and GSK525762 have also reached phase II clinical trials [72]. OTX015, developed by OncoEthix and Merck, is involved in four different clinical trials for the treatment of acute leukemia and hematologic malignancies, advanced solid tumors (NCT02259114), recurrent *multiforme glioblastoma* and in combination with azacitidine for the treatment of patients with newly diagnosed acute myeloid leukemia that are not candidates for standard intensive induction therapy. BMS-986158 (i.e. structure undisclosed) has been tested for multiple cancer indications alone and in combination with paclitaxel. Finally, GSK525762, also known as I-BET762, is involved in two clinical trials: one to investigate the safety, pharmacokinetics, pharmacodynamics, and clinical activity in patients with NUT midline carcinoma and other cancers, and a second one directed toward patients having solid tumors and hematologic malignancies [70]. In addition to these four molecules, six other BET inhibitors have recently entered phase I clinical trials and are being studied for both solid tumors and hematological malignancies: two compounds with a very similar structure to (+)-JQ1, TEN-010 [73] and CPI-0610 [74]; GS-5829 (i.e. structure undisclosed); BAY1238097 (i.e. structure undisclosed); ABBV-075 (i.e. structure undisclosed); and INCB054329 (i.e. structure undisclosed) [70].

Despite the fact that several compounds are in clinical trials, the development of BET inhibitors having selectivity for individual BET proteins has remained a major challenge. Major motifs associated with bromodomain-containing proteins are made up of a conserved Asn, a conserved Tyr, the WPF motif, the ZA-loop and the BC loop. These motifs are conserved in most BET family isoforms including the testis-specific transcriptional regulator, BRDT [70]. Existing BET inhibitors are critically limited by the lack of isoform selectivity especially against BRDT, which is a source of unwanted adverse effects on male fertility. Benzodiazepines are a major class of high-affinity BRD inhibitors that suffer from numerous adverse effects associated with the promiscuity of this class of inhibitors.

4. Isoform selective modulation of epigenetic targets

A majority of the aforementioned HDAC and BET inhibitors nonselectively govern the activities of distinct classes of human HDAC and BET isoforms [75]. The reason for the lack of selectivity is certainly due to the high conservation of active site residues. Furthermore, the absence of isoform selective compounds (i.e. along with its corresponding phenotypic readouts and adverse effects data) that can be used as lead compounds, hampers further development. In the absence of sufficient chemical perturbation data, we used the SNP and mRNA expression data present in the GWAS catalog [76] and BioGPS [77], respectively, as a proof of concept for identifying isoform selective modulation of epigenetic targets with improved efficacy and toxicity profiles against existing drugs. The epigenetic drugs discussed in the above sections generally produce global epigenetic changes (i.e. acetylate both

disease relevant or adverse effect-associated genes), which could lead to unintended toxicities or reduced efficacy. In the following section, we discuss recent developments in chemical biology, especially the use of chemical probes to produce sequence-specific epigenetic changes in order to alleviate some of the toxicities associated with global epigenetic changes.

4.1. Selective isoform inhibition can achieve better efficacy and toxicity profiles

A recent trend in epigenetic drug discovery has been the discovery and development of isoform selective inhibitors [75]. Although the absence of a critical number of epigenetic drug-target-phenotype profile leads to an over-optimistic view of the current isoform selective inhibitors, they are of substantial interest as part of efforts to translate the findings obtained from human genetics into novel therapeutic strategies. A major promise provided by genomics in drug research is the elucidation of a set of protein targets so as to achieve efficacy and reduce toxicity. Hence, in the absence of sufficient small-molecule-induced readouts, we illustrate the utility of genetic data (i.e. GWAS and BioGPS) to highlight the relevance of selective isoform inhibition for improving efficacy and toxicity profiles. A genome-wide association study (GWAS) is an approach to rapidly scanning genetic variants (markers) across the genome (~0.5 M or 1 M) of many people (>2 K) to find genetic variations that are associated with a specific disease or trait. Such studies are particularly useful in finding genetic variations that contribute to common complex diseases such as asthma, cancer, diabetes, heart disease, and mental illnesses. GWAS studies are a source of target validation in humans. An analysis of the GWAS catalog data of the HDAC class of epigenetic targets reveal that HDAC7 and HDAC9 can synergistically produce relevant immune responses (Figure 6 (a)) [78]. Similarly, BioGPS is an mRNA expression data set encompassing a panel of 79 human tissues that can be used to analyze off-targets with unfavorable expression profiles. An analysis of the BioGPS data reveals that BRDT is an anti-target that should be avoided to reduce male contraceptive-like adverse effects of pan-BRD inhibitors that are currently being investigated as anti-inflammatory agents (Figure 6(b)). These analyses highlight the importance of isoform selective modulation of epigenetic targets for efficacy enhancement while reducing adverse effects.

4.2. Sequence-specific targeting of epigenetic switches

Recently, Pandian et al. developed a novel class of epigenetically active small molecules called SAHA-PIPs by conjugating selective DNA-binding pyrrole-imidazole polyamides (PIPs) with the histone deacetylase inhibitor SAHA, a pan-HDACi [79]. Through microarray studies and functional analysis, they demonstrated the remarkable ability of several SAHA-PIPs to trigger transcriptional activation of exclusive clusters of genes and noncoding RNAs, rather than inducing a whole genome-wide transcriptional regulation. These compounds called SAHA-PIPs can serve as chemical biology tools and help gain

insight into unresolved mechanisms and may also be able to assign functions to uncharacterized genes. Since selected disease relevant gene clusters can be precisely targeted, the design and development of cell permeable sequence-specific epigenetic switches like SAHA-PIPs represents a major advance in epigenetic drug discovery [79].

5. Drug repurposing opportunities for epigenetic drug discovery

Drug repositioning or repurposing refers to the association of known authority-approved drugs to new indications (i.e. new diseases). Before the advent of the genomic era, epigenetic drug repurposing for specific targets was performed using computational ligand- and structural-based approaches [80]. By contrast, advances in bioinformatics techniques and the availability of numerous genome-wide measurement data sets has presented a more general, automated, and unbiased approach to drug repurposing [81]. The availability of drug–drug similarity, protein activity–drug, gene expression–drug, protein–protein interactions and gene/protein–disease data sets makes it possible to statistically prioritize new epigenetic drug–disease associations [82,83]. Genomics-based approaches seem really interesting as it has been shown to afford promising results for drug repurposing [81]. Still, cheminformatics and structural bioinformatics techniques are relevant and add value to genomic approaches. In fact, there exist many success stories for epigenetic drug repurposing [83]. Méndez-Lucio et al. [85] utilized cheminformatics analysis for identifying olsalazine (i.e. a drug that was previously approved by the FDA as an anti-inflammatory agent) as a DNA hypomethylating agent. Using a known hypomethylating agent (NSC14778) as a reference molecule, a similarity search approach was conducted by comparing the structure of the reference with the structures of 1582 FDA-approved compounds from the DrugBank database. The analysis led to the identification of Olsalazine (i.e. due to affording a high Tanimoto Combo score of 1.032 with NSC14778) as a good candidate for DNMT1 inhibition. The Tanimoto Combo score, as implemented in the Rapid Overlay of Chemical Structures (ROCS) package obtained from the OpenEye scientific software, is the sum of the Shape Tanimoto and the Chemical Functionalities Tanimoto (color Tanimoto). The range of the Tanimoto Combo score varies from 0 to 2 with a score exceeding 1.4 representing a high degree of similarity for a pair of compounds. An *in vivo* study conducted on HeLa cells adapted to report gene expression visually via the green fluorescent protein was used to experimentally establish the DNA hypomethylation ability of olsalazine by virtue of its interaction with DNMT1. The binding mode of olsalazine against DNMT1 and DNMT3b was further elucidated using a detailed docking study. In another interesting study by *de La, Cruz-Hernandez et al.* [86] also made use of cheminformatics for epigenetic drug repurposing of 3-deazaneplanocin A, a known inhibitor of SAM-dependent methyltransferase that targets the degradation of EZH2 and leads to apoptosis in various malignancies, was used as a reference compound for a chemical similarity search against FDA-approved and experimental drugs. The

cheminformatic analysis identified ribavirin, a nucleoside drug approved for the treatment of hepatitis C virus infection, as having high structural similarity with 3-deazaneplanocin A. Experimental assays in various cell lines revealed that ribavirin could inhibit the expression of EZH2 and two other cancer-associated epigenetic targets.

Structural bioinformatics approaches also have great potential for drug repurposing via disease-associated epigenetic targets. Among several success stories of structure-based drug design approaches, the proteochemometric approach utilized by Dakshanamurthy et al. [87] was the most interesting in which they developed a new computational method called 'TMFS' that consisted of a docking score, ligand and receptor shape/topology descriptor scores and ligand–receptor contact point scores to predict 'molecules of best fit' and filter out most false-positive interactions. Using this method, they reprofiled 3671 FDA-approved/experimental drugs against 2335 human protein targets with a good prediction accuracy of 91% for the majority of drugs. Amongst the several novel associations, they experimentally validated that the anti-hookworm medication mebendazole could inhibit VEGFR2 and angiogenesis activity. Furthermore, they also found that the anti-inflammatory drug celecoxib and its analog DMC could bind CDH11 (i.e. a biomolecule that is very important in rheumatoid arthritis and poor prognosis malignancies, for which no targeted therapies currently exist). The advantages of the proteochemometric approach (i.e. analysis concomitantly involving both protein and chemical structural features) over a traditional cheminformatics approaches are multifold such as, the possibility to gain detailed insight into binding modes in addition to the discovery of novel drug–target associations.

The integration of genomics-assisted approaches to drug repurposing along the lines described by the above methods can address some of their drawbacks such as the limited applicability domain and the non-immediate disease relevance of ligand-based cheminformatics approaches and target-based structural bioinformatics approaches, respectively. The genomics-assisted approach to drug repurposing has some success stories in epigenetic drug discovery [88,89]. Drug repurposing through the genomics approach generally involves either correlating drug–drug gene expression profiles or drug–disease expressions using a range of statistical procedures to find useful patterns. An example is using the Kolmogorov–Smirnov statistical test as implemented in the connectivity map (CmAP), which is a searchable chemogenomic database containing thousands of gene-expression signatures of various cultured cancer cells as exposed to a large collection of small-molecule compounds, to find a pattern indicating a possible repurposing [90]. The database and statistical procedure represent a useful tool for the discovery of hitherto unexplored connections amongst small molecules with diseases in terms of Anatomical Therapeutic Chemical (ATC) codes. By comparing expression signatures, CmAP serves as a proxy to search for novel indications of all surveyed compounds. The correlation between a given gene expression profile and the various ranked gene expression profiles in the CMap is presented as the signed enrichment score. The signed enrichment score varies from +1 to –1.

Table 3. Known ATC associations of some epigenetic drugs mentioned in Figure 4.

DB ID	Name	Class	ATC code	ATC code description
DB00721	Procaine	DNMTi	C05AD05	Cardiovascular System, Vasoprotectives, Local Anesthetics
DB00721	Procaine	DNMTi	N01BA02, N01BA52	Nervous System, Anesthetics, Esters Of Aminobenzoic Acid
DB00721	Procaine	DNMTi	S01HA05	Sensory Organs, Ophthalmologicals, Local Anesthetics
DB00928	Azacitidine	DNMTi	L01BC07	Antineoplastic And Immunomodulating Agents, Antineoplastic Agents, Pyrimidine Analogues
DB01035	Procainamide	DNMTi	C01BA02	Cardiovascular System, Cardiac Therapy, Antiarrhythmics, Class Ia
DB01262	Decitabine	DNMTi	L01BC08	Antineoplastic And Immunomodulating Agents, Antineoplastic Agents, Pyrimidine Analogues
DB01275	Hydralazine	DNMTi	C02DB02	Cardiovascular System, Antihypertensives, Hydrazinophthalazine Derivatives
DB01275	Hydralazine	DNMTi	C02LG02	Cardiovascular System, Antihypertensives, Antihypertensives And Diuretics In Combination
DB02546	Vorinostat	HDACi	L01XX38	Antineoplastic And Immunomodulating Agents, Other Antineoplastic Agents
DB05015	Belinostat	HDACi	L01XX49	Antineoplastic And Immunomodulating Agents, Other Antineoplastic Agents
DB06176	Romidepsin	HDACi	L01XX39	Antineoplastic And Immunomodulating Agents, Other Antineoplastic Agents
DB06603	Panobinostat	HDACi	L01XX42	Antineoplastic And Immunomodulating Agents, Other Antineoplastic Agents
DB06819	Phenylbutyrate	HDACi	A16AX03	Alimentary Tract And Metabolism, Other Alimentary Tract And Metabolism Products, Various Alimentary Tract And Metabolism Products
DB00752	Tranylcypromine	HDMi	N06AF04	Nervous System, Psychoanaleptics, MAO Inhibitors, Non-Selective
DB00780	Phenelzine	HDMi	N06AF03	Nervous System, Psychoanaleptics, MAO Inhibitors, Non-Selective
DB01626	Pargyline	HDMi	C02KC01	Cardiovascular System, Antihypertensives, MAO Inhibitors
DB01626	Pargyline	HDMi	C02LL01	Cardiovascular System, Antihypertensives, MAO Inhibitors And Diuretics
DB00250	Allantodapson	HMTi	D10AX05	Dermatologicals, Other Anti-Acne Preparations For Topical Use
DB00250	Allantodapson	HMTi	J04BA02	Antiinfectives For Systemic Use, Antimycobacterials, Drugs For Treatment Of Lepira

ATC is the WHO recommended Anatomic Therapeutic Chemical classification system for drugs. As demonstrated in various sections of this article and the connectivity map analysis presented in Table 4, there are numerous indications that epigenetic drugs' expression signatures can be linked with various disease gene expression signatures. For instance, while known associations presented in this table links HDACi with L01 (antineoplastic drugs), the connectivity map analysis presented in Table 4 associates a functionally similar second generation HDACi with numerous other ATCs. The DrugBank data set was downloaded as an XML file and parsed using 'xmlstarlet' package to extract the DB_ID and ATC codes to generate the table. Reproduced with permission from [93].

Table 4. Correlation of diseases and drugs on the basis of gene signature associations with 'ST7612AA1,' a novel second-generation oral HDAC inhibitor.

Name of drug, cell line or ATC code	Signed enrichment score
Vorinostat – MCF7	0.985
Trichostatin A – PC3	0.959
Trichostatin A – HL60	0.952
Trichostatin A – MCF7	0.931
Pioglitazone – PC3	-0.913
Sirolimus – PC3	0.911
Wortmannin – MCF7	0.821
Tanespimycin – HL60	0.805
Trifluoperazine – MCF7	0.796
LY-294002 – PC3	0.727
N05AB (Antipsychotic and Anxiolytic drugs)	0.647
N03AG (Fatty acid derivatives as Antiepileptic drugs)	0.47
L04AA (Selective immunosuppressants)	0.426
R06AX (Other antihistamines for systemic use)	0.411
A07DA (Intestinal anti-infectives)	0.854
N05AC (Hypnotics and sedatives drugs)	0.45
C01AA (Cardiac glycosides list)	0.636
L01CB (Plant alkaloids and other natural products as antineoplastic drugs)	0.831
B02AA (Antifibrinolytics)	-0.616
N05AG (Antipsychotic drugs)	0.591

ST7612AA1's gene expression dataset was downloaded from the NCBI GEO using id 'GSE62460'[94]. Connectivity map analysis on the drug-induced gene signatures from CMap database [90] was used to identify drugs and their ATCs whose expressions correlate with the top 250 up- and down-regulated genes of 'ST7612AA1.' The differentially expressed up- and down-regulated genes were extracted after Limma [95] analysis of 'GSE62460.' These types of *in silico* drug repurposing studies of epigenetic drugs can propose novel disease indications for experimental verification. Signed enrichment score were computed using the Kolmogorov–Smirnov (KS) test.

Using the CMap analysis, Zerbini et al. [91] presented a case study for the identification of compounds whose gene expression signatures were negatively enriched with the gene signatures of metastatic clear cell renal carcinoma (ccRC). The consensus top-scoring 8 drugs (those with a negative enrichment correlation between -0.7 and -1.0 in more than 50% of

the patients) were selected to be tested *in vitro* and *in vivo*. Five of these drugs exhibited a strongly incremental rate of apoptosis in cancer cells; however, they did not affect the survival of normal cells. They also demonstrated that the status of VHS gene (whose mutation is known for causing ccRC) was strongly associated with the response. The best responses were observed in cells deficient in VHC. Furthermore, amitriptyline was seen to induce multiple myeloma apoptosis through the inhibition of cyclin D2 expression and also via repression of HDAC and consequently, its activity.

Using microarray technology, Claerhout et al. [88] generated a gene expression profile of human gastric cancer-specific genes from human gastric cancer tissue samples. They used this profile for CMap analysis as to identify candidate therapeutic compounds for gastric cancer. The histone deacetylase inhibitor vorinostat, emerged as the lead compound and thus a potential therapeutic drug for gastric cancer. Vorinostat has been experimentally shown to induce both apoptosis and autophagy in gastric cancer cell lines and it was further suggested that combination of vorinostat with autophagy inhibitors may be therapeutically synergistic. Moreover, gene expression analysis of gastric cancer identified a collection of genes (e.g. *ITGB5*, *TYMS*, *MYB*, *APOC1*, *CBX5*, *PLA2G2A*, and *KIF20A*) whose expressions were elevated in gastric tumor tissues and downregulated by more than twofold upon treatment with vorinostat in gastric cancer cell lines. In contrast, *SCGB2A1*, *TCN1*, *CFD*, *APLP1*, and *NQO1* manifested a reversed pattern.

Oprea and Overington [92] suggested a robust classification scheme, DREL, that can be used to evaluate drug repositioning projects according to the level of scientific evidence. Based on this scheme, the study by Zerbini et al. [91] and Méndez-Lucio et al. [85] can be classified as DREL-2 (i.e. animal studies with hypothetical relevance in man) whereas the

studies by Claerhout et al. [88] and De la Cruz-Hernandez et al. [86] can be classified as DREL-1 (i.e. representing experimental validation in the form of *in vitro* studies with limited value for predicting *in vivo*/human situation).

To illustrate the utility of the CMAP approach for drug repurposing, we present a short case study involving the novel second-generation HDAC inhibitor ST7612AA1. The present study can be considered as an intermediate between DREL-0 and DREL-1 since it presents limited experimental validation in the form of *in vitro* drug-induced gene expression analysis and no phenotypic assays. As illustrated in Table 3, many epigenetic drugs map to multiple ATC classes, which classifies drugs according to the organ or system on which they act and their associated therapeutic, pharmacological and chemical properties. The ATC classification is hence a useful indicator of the drug's phenotype or its disease relevance. As illustrated in Table 3, the selected HDACi are primarily classified by ATC as Antineoplastic and Immunomodulating Agents (L01XX) and are presently widely used in anticancer therapies. However, our CMAP analysis correlating the ST7612AA1-induced gene expression signature in TMD8 and DOHH2 lymphoblastoma cell lines with various drug-induced gene expression values in the CMAP database reveals that HDACi could be repurposed for numerous other indications as anti-psychotics (N05AB with an enrichment score of 0.647), anti-infectives (A07DA with an enrichment score of 0.854) and cardiovascular agents (C01AA with an enrichment score of 0.636). The results presented in Table 4 corroborate the findings of numerous other studies including findings in clinical trials. In addition, the CMap analysis presented in Table 4 also shows the correlation of ST7612AA1-induced gene expression profiles with the expression signatures of other HDACi's such as vorinostat (enrichment score of 0.985) and trichostatin A (enrichment score of 0.959). The analysis reveals a positive correlation of ST7612AA1-induced gene expression profiles with anticancer compounds like sunitinib (also called rapamycin with an enrichment score of 0.911), wortmannin (enrichment score of 0.821), tanespimycin (enrichment score of 0.805), LY-294002 (enrichment score of 0.727), and antipsychotic compounds like trifluoperazine (enrichment score of 0.796). Interestingly, ST7612AA1-induced gene expression profile negatively correlates with pioglitazone (enrichment score of -0.913), which is a PPAR-gamma agonist that have been associated with a higher risk of cardiac events.

6. Conclusion

Epigenetics modulate the regulation of gene expression for the maintenance of homeostasis via the concerted actions of several epigenetic modifiers. The physiological functions of these modifiers are altered by external factors, which may lead to aberrant gene expression and diseases. Inherited (e.g. gender and racial), environmental (i.e. exposure to pollutants and chemicals, stress, etc.), and social (i.e. income, residence, occupation, education, culture, and malnutrition) factors are known to influence epigenetic regulations. Epigenomics has gained notable attention as a field that could provide answers on how external stimulus (e.g. environment, nutrition, and behavior) governs the development

and progression of multifactorial diseases, as well as providing an explanation on the differential susceptibility to diseases amongst individuals. Moreover, epigenetic alterations have been implicated in a wide spectrum of diseases. Great progress has been made on identifying disease-relevant epigenetic targets, which has contributed to a better understanding of the pathogenesis and management of many complex diseases (e.g. metabolic and cardiovascular diseases, autoimmune diseases, psychological disorders, neurodegenerative and neurodevelopmental diseases, and cancers). This has involved the use of many advanced genomics, epigenomics, bioinformatics, and cheminformatics technologies, all of which has facilitated the discovery of several novel classes of epigenetic modifiers for therapeutic applications. As an example, a widely cited study by Jones and Baylin [96] reviewed advances in understanding how epigenetic alterations participate in the earliest stages of neoplasia, including stem/precursor cell contributions, and discuss the growing implications of these advances for strategies to control cancer. Naturally derived compounds are in the spotlight as an excellent source of active scaffolds for epigenetic drugs, while drug repositioning/repurposing demonstrates a powerful strategy for the discovery of novel indications for existing FDA-approved drugs. Discovery of novel epigenetic drugs may pave way for fulfilling several unsolved problems in multifactorial diseases. The field is a highly challenging one indeed. Of particular note is the distinct characteristics of the epigenome, which include long-lasting memory, transgenerational inheritance and environmental adaptations. Awareness of maternal and early life exposures to predisposing factors may decrease the risk of developing adult-onset diseases and developmental disorders. In addition, understanding the environmental adaptations of the epigenome renders adjustment of lifestyle and nutritional behavior as a potential path for disease prevention and health promotion.

7. Expert opinion

Extensive chemical biology and genomic studies have revealed druggable and clinically relevant epigenetic targets (e.g. DNMTs, HDACs, HATs, SIRT, HDMs, BRDs, and PMTs). The clinical success of epigenetic modifiers has been demonstrated by the many drugs approved by the FDA. Therapeutic potential has been expressed most clearly in oncology where almost all types of epigenetic modifiers may have impact, whereas for cardiovascular and neurological disorders only a few modifiers have shown utility (i.e. BRDi, DNMTi, and SIRT modulators for the former; while HDMi for the latter).

The development of DNMTi has been primarily directed toward cytidine analogs. However, the CpG dinucleotide analogs (e.g. SGI-110) show promise as DNMTi owing to their superiority in resisting cytidine deaminase (i.e. a cytidine inactivating enzyme). Attention has also been directed toward compounds interfering with protein-protein interactions and compounds exhibiting DNMT inhibition via other mechanisms of action such as allosteric inhibitors, SAM mimicking compounds and DNA competitive substrates. For HDAC inhibitors, the discovery of new inhibitors has mostly been focused on hydroxamic acids and benzamides. However, the clinical

outcome of these compounds are still uncertain and future direction could be emphasized toward the discovery of HDACi with novel mechanisms of action. Moreover, the development of novel hybrid molecules targeting HDAC inhibition and other oncogenic/inflammatory pathways has provided interesting results, especially those bearing adamantane moieties. Furthermore, the discovery of SIRT1 activators and HDMi are in the spotlight for neurodegenerative diseases. The development of SIRT1 activators has been focused toward naturally occurring phenol derivatives, especially resveratrols. Scaffolds possessing an inhibitory effect toward monoamine oxidases such as pargyline, phenelzine, and tranylcypromine have been suggested to be within the potential chemical spaces for the discovery of HDMi. The development of PMTi and HATi is currently limited to the area of oncology and is still in their infancy. Further research regarding these two types of epigenetic modifiers may extend the area of therapeutic epigenetics. Selectivity is of high concern regarding the development of BRDi as most of the bromodomain-containing proteins share similar structures but possess distinct structural differences and functions in biological pathways. Thus, the development of selective BRDi for reduced side effects is a challenging opportunity. In addition, advanced approaches employing availability of genomic data derived from GWAS and BioGPS expression data sets are underlined for the discovery of isoform selective inhibitors with improved efficacy and side effect profiles. The development of an epigenetically active hybrid molecule as a chemical biology tool to unravel insights, mechanisms, and functionally relevant genes of complex diseases is also marked as an area with great potential.

Natural compounds cannot be overlooked as attractive sources of novel scaffolds for the development of epigenetic modulators. For example, naturally derived polyphenols (e.g. EGCG, curcumin, and caffeic acids), flavonoids (e.g. genistein and quercetin), quinones (e.g. hypericin and laccaic acid), lycopene, and boswellic acid have been reported as DNMTi. Moreover, naturally occurring phenol derivatives are in the spotlight as SIRT activators especially resveratrol derivatives, which are suggested to act as allosteric activators. However, the development of these natural compounds may also pose similar problems as those observed in clinically useful drugs (e.g. poor absorption, metabolic stability, and pharmacokinetics).

Although, epigenetic drug discovery is increasingly directed toward selective epigenetic modifiers (i.e. inhibitors or modulators), the discovery strategies of promiscuous or pan-modulators are currently the most viable. Greater phenotypic responses of pan-modulators as observed by the broad range of factors are implicated via the etiology, pathogenesis, and progression of disease and the expensive screening techniques used to discover isoform selective modulators, renders isoform selective discovery programs both medically and financially inefficient [97]. In the present review, 'class-selective' modulators are not necessarily those which modulate a single target but instead modulate a subset of targets to produce the required phenotypic responses.

Drug repurposing is currently considered an attractive strategy for the discovery of new indications of the existing drug space. Specifically, it reduces the need for the costly and

time-consuming preclinical pharmacology, formulation and toxicity testing, which are otherwise required for clinical trial approval. Computational approaches that play a crucial role in early stages of drug discovery have formed the core technology in drug repurposing. *In silico* analysis (e.g. cheminformatics, structural bioinformatics, and genomics) of relevant data sets (e.g. drug libraries, gene expression–disease, protein–drug, and protein–protein interactions) have proven capable of identifying novel epigenetic drug–disease associations.

The current interest in personalized medicine is largely due to recent insights into genomics and epigenomics. Epigenetic factors are responsible for phenotypic plasticity and are increasingly associated with individual specific disease etiologies and drug responses, and can be revealed by mining genomic and epigenomic data of individual patients. Hence, in an era of lifestyle-induced diseases where a complex myriad of individual and environmental factors exists that constantly modifies the individual's epigenetic landscape via external stimuli, there is an enormous potential for prevention and therapy. On top of this, the fields of nutritional and stress epigenomics that we have not covered here are on the rising trend for personalized diagnosis, prevention and control of cancer, cardiovascular, neurological and aging diseases. All of these points toward a great future for novel drugs directed to the many complex multifactorial diseases of humans, which are still often poorly understood and difficult to treat. In this regard, the redesign of routine lifestyle behaviors (i.e. involving alteration to nutrition, exercise and stress management) along with advanced studies in related areas (e.g. nutraceuticals and complementary medicine) should not be overlooked as key factors toward achieving good health and well-being.

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