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REVIEW ARTICLE

# Quinazolines as inhibitors of chromatin-associated proteins in histones

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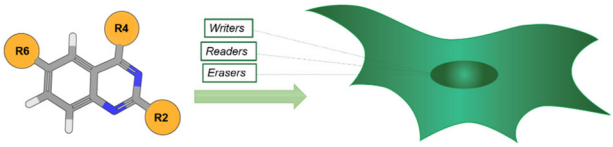
## Abstract

It is increasing the evidence that quinazolines are inhibitors of chromatin-associated proteins in histones. Quinazolines have a broad structural diversity among the structural classes that have been designed. Herein, we review the development of selective and potent quinazolines highlighting the current state of these molecules with an emphasis on the structural requirements for the interaction within the target. Chemical synthesis and results of the biological assays in vitro or in vivo of these compounds are also discussed. There is extensive evidence that support quinazoline derivatives as inhibitors of histone methyltransferase (G9a) and G9a-like protein (GLP). There is one quinazoline analogue that inhibits an extra-terminal bromodomain motif (BET) and that is on clinical trials as potential treatment for different chronic diseases. There is also clinical evidence that quinazolines act as dual inhibitors targeting histone deacetylases (HDACs) $Zn^{2+}$-dependent and kinase receptors for the potential treatment of cancer. Additional proposals of quinazoline structures are being evaluated as inhibitors targeting two or more chromatin-associated proteins simultaneously. Therefore, further improvements in synthetic methods, computational studies, and additional biological assays in vitro and in vivo remain to be addressed.

## Graphical Abstract




**Keywords** Quinazoline chromatin-associated proteins · Epigenetic · Histone modifiers · Inhibitors

## Abbreviations

| | |
|---|---|
| AML | acute myeloid leukemia |
| ApoA1 | apolipoprotein A1 |
| BRDs | bromodomains |
| CRDs | chromodomains |
| $CL_{int}$ | intrinsic clearance |
| DNA | deoxyribonucleic acid |
| $EC_{50}$ | half maximal effective concentration |
| $FAD^{+}$ | flavin adenine dinucleotide |
| G9a | euchromatic histone-lysine N-methyltransferase 2 |
| GLP | G9a-like protein |
| HDACs | histone deacetylases |
| HER1 | epidermal growth factor receptor |
| HER2 | human epidermal growth factor receptor-type2 |
| H3K9Me2 | dimethylated lysine 9 histone 3 |
| HKMT | histone lysine methyltransferases |
| $IC_{50}$ | half maximal inhibitory concentration |
| *iPS* | reprogramming induced pluripotent stem cells |
| *Kac* | acetylated lysine |
| LSD1 | lysine-specific demethylase 1 |
| MAO | monoamine oxidases |

**Supplementary information** The online version of this article (https://doi.org/10.1007/s00044-019-02300-0) contains supplementary material, which is available to authorized users.

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| | |
|---|---|
| MBT | malignant brain tumor |
| *Myc* | proto-oncogenes that code for transcription factors |
| $NAD^+$ | nicotinamide adenine dinucleotide |
| PTMs | post-translational modification, |
| PPARγ | peroxisome proliferator-activated receptor gamma |
| PHKMT | *Plasmodium* histone lysine methyltransferase |
| VEGFR-2 | vascular endothelial growth factor receptor |

## Introduction

Epigenetic involves heritable features that are not related to DNA sequence alterations, but with dynamical and reversible modifications. Most of these modifications occur in histones, modulating DNA transcription and hierarchical covalent modifications. Epigenetic regulation is vital for the maintenance of the phenotype, development, and differentiation in normal cells (Arrowsmith et al. 2012; Jones and Baylin 2007). Emerging evidence has shown that deregulations in these types of mechanisms imply localized or global variations that occur at different levels in the epigenome, such as in DNA and histones (Feinberg 2007). The disruption of the phenotypic plasticity would lead to chromatin remodeling causing deregulation in gene expression or repression. These alterations in the cell behavior are a consequence of the response to internal or external factors, resulting in the onset and progression of several diseases. New proposals of quinazoline analogues and added structure–activity relationship studies are being focused principally in cancer, neurological and inflammatory disorders, and cardiovascular diseases, among others (Esteller 2007; Feinberg 2007; Shortt et al. 2017).

Histones are key macromolecules involved in the regulation of gene expression due to its interaction and embroilment of DNA segments around them leading to the formation of nucleosomes. Four histone isoform pairs i.e., $2H_2A$, $2H_2B$, 2H3, and 2H4 are required for the histone–DNA complex formation, which association with other transcription factors and enzymes constitute a supramolecular structure known as chromatin. Structural changes in this complex for gene expression or repression are guided by specific epigenetic pathways (Esteller 2007; Helin and Dhanak 2013; Kwa 2011). These epigenetic mechanisms comprise several chemical modifications that occur at specific amino acids in histone sequences known as "*marks*". Chromatin-associated proteins are responsible of adding these *marks* that will later transduce into expression or repression of genes (Arrowsmith et al. 2012).

Chromatin-associated proteins are classified into three groups based on their functions: (1) *writers*, (2) *readers*, and (3) *erasers*. *Writers* are enzymes that add methyl and acetyl chemical groups in specific residues occurring mostly in the *N*-terminal region (see Fig. 1). These modifications are carried out mainly in lysine and arginine residues by histone lysin (HKMT) and protein arginine methyltransferases (HRMT), respectively. Both enzymes regulate gene expression or repression depending on the residue they modify and how many groups are added to the *N*-terminal region (Ganesan 2016; Helin and Dhanak 2013).

*Readers* are structural domains within several types of proteins, some of the most relevant are: bromo (BRDs) (Filippakopoulos 2010, 2014), chromo (CRDs), tudor, malignant brain tumor (MBT), and PWWP domains. These

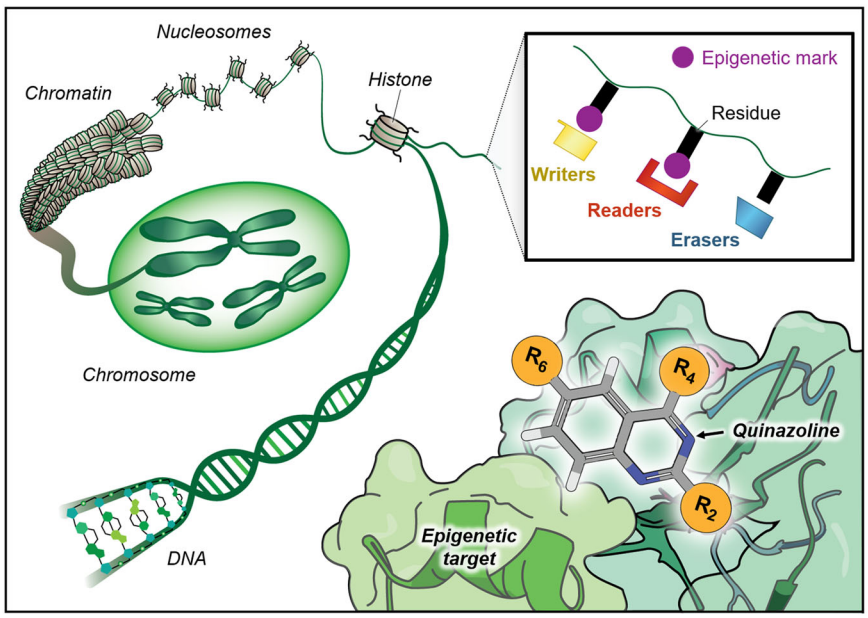


**Fig. 1** Depiction of chromatin complex (heterochromatin-euchromatin) histones octamers showing in the *N*-terminal region just a small representation of these histone modifiers (i.e., *writers, readers*, and *erasers*) in a simplified manner, highlighting quinazoline´s structure

domains recognize certain type of *marks* translating in a direct effect on the epigenome. The recruitment of protein complexes would enable the effect of specific marks that have been added, and consequently the imposed changes in chromatin structure (Filippakopoulos et al. 2014; Ganesan 2016; Maurer-Stroh et al. 2003; Vedadi et al. 2012).

Finally, marks added are removed by enzymes known as erasers, which are classified into two types: histone deacetylases (HDACs) and lysine demethylases (LSDs/KDMs and JMJDs) that remove acetyl and methyl groups from lysine and arginine residues, respectively (Arrowsmith et al. 2012; Helin and Dhanak 2013; Micelli and Rastelli 2015).

Different structural classes of small molecules have been used as inhibitors of BRDs, HKMT, LSD, and HDACs for the treatment of chronic diseases (Prachayasittikul et al. 2017). Examples of some of these compounds are benzamides derivates (Belinostat®), suberoylanilide hydroxamic acid (SAHA®), triazolothienodiazepine (JQ1®), valproic acid (Depakine®), and bicyclic depsipeptide (Romidepsin®) (Ganesan 2016; Valdespino 2015). Nowadays there are fewer than 16 approved drugs by the Food and Drug Administration (FDA) and 21 in clinical trials, mainly for HDACs and BRDs (De Lera and Ganesan 2016; Ganesan 2016; Prachayasittikul et al. 2017; Shortt et al. 2017). Further studies have reported a large diversity of chemical structures classified on the nature of their obtaining source, in which only few derivates preserve particular substituents (Naveja and Medina-Franco 2018). In this regard, different molecular libraries of compounds that target a diverse number of epigenetic targets have been published (Gortari and Medina-Franco 2015; Prieto-Martínez 2018a, b).

Quinazoline structure has been reported as a scaffold that is present in multiple analogues that act as chromatin-associated proteins inhibitors in order to be used as broad re-programmers or in targeted therapies. It is worth mentioning that the quinazoline analogue BRD inhibitor **RVX-208** that is known for its selectivity toward BD2 domain, is in clinical trials phase II as promising treatments for atherosclerosis, cardiovascular diseases, and diabetes mellitus (De Lera and Ganesan 2016; Ganesan 2016; Noguchi-Yachide 2016; Rudolf et al. 2005; Wang and Gao 2013). Similarly, the quinazoline derivative **CUDC-101** has shown dual activity targeting against HDACs and kinases. Nowadays, **CUDC-101** is on clinical trials (phase I) as an alternative treatment for preventing cancer progression (see Table 1). However, it is still unclear either the quinazoline scaffold or the substituents contributes to the interaction within HKMTs, HDACs, and BRDs.

The goal of this review is to discuss the role of quinazoline analogues and derivatives as inhibitors of chromatin-associated proteins. Herein, we provide a concise summary of the research published over the past 15 years discussing the advantages and disadvantages of quinazoline's substitution pattern or structure–activity relationships (SAR) studies toward their selectivity profile, and potency determined from the in vitro and in vivo testing (see Table 1 or online resource S1). Moreover, the synthetic routes of the most representative inhibitors of each target are shown to have a general view of the process for its production and further optimization. (see online resource S1). Collectively, we expect that this work allows the continuous research of quinazolines as promising scaffolds that target chromatin-associated proteins acting as further for the treatments of chronic diseases (Filippakopoulos and Knapp 2012).

## Structural features of the molecular targets

In the next subsections, key features will be mentioned about the structure of chromatin-associated proteins, focusing especially on the hotspot regions to which different reported small inhibitors bind. Thus, our principal interest relies on the structural requirements in order to understand SAR studies of quinazoline analogues. For more information, comprehensive reviews of the three-dimensional structures of epigenetic targets have been published elsewhere (Arrowsmith et al. 2012; Filippakopoulos et al. 2014; Micelli et al. 2015).

### Structural features in HKMTs (*writers*)

The mono- and di-methylated states of lysine residues in histones favor the relaxed form of the chromatin (euchromatin); these modifications are mostly done by G9a and GLP. The enzymes involved in this modification are characterized by five conserved domains known as: pre-SET, N-SET, SET, I-SET, and Post-SET (see Fig. 2a). Overall, stability within all these regions is maintained through non-bonded interactions, such as hydrogen bonds, hydrophobic interactions, and few covalent interactions such as disulfide bonds. A higher-stability state of these structures is obtained through the binding of a peptide substrate or an inhibitor in the I-SET and Post-Set domains. Therefore, it is important to study these regions for the optimization of new inhibitor molecules. I-SET is well-known as a rigid docking platform due to its well-established conformation that does not depend on the presence of any type of substrate or stabilizer. This region is formed by an α-helix followed by two anti-parallel β-sheets, linked by loops with variable lengths (Chang 2009). It is worth mentioning that hydrogen bond interactions established between two aspartic acid residues may play a key role in its binding substrate affinity. On the contrary, post-SET domain is considered a flexible region involved in the recognition of the small inhibitors known to be fully structured only in the presence of these small molecules (see Fig. 2a). The factors that contribute its

**Table 1** Potent quinazoline analogues and derivatives from each series published

| Epigenetic target | | Compounds name | Reference | Remarks |
|---|---|---|---|---|
| Writers | GLP | BIX-01294 | (Kubicek et al. 2007)<br>(Liu et al. 2009) | Synthesis yield: 80 % (2 steps).[a]<br>Selective against GLP ($IC_{50}$: 34 nM) compared to G9a ($IC_{50}$: 180 nM).<br>Diminishes H3K9Me2 *mark* in several cell lines targeting 8 genes.<br>High-toxicity ratio ($EC_{50}$ > 4.1 μM) |
| | G9a | UNC0224 | (Liu et al. 2009) | Overall synthesis yield: 46% (9 steps).[a]<br>Selective against G9a ($IC_{50}$: 16 nM) towards GLP ($IC_{50}$: 58 nM). |
| | G9a | UNC0321 | (Liu et al. 2010) | Synthesis yield: 44% (9 steps).[a]<br>Most potent compound in biochemical assays.<br>Selective against G9a ($IC_{50}$: 6 nM) towards<br>GLP ($IC_{50}$: 23 nM).<br>Less potent tan BIX-01294 in cellular assays.<br>Poor permeability. |
| | G9a/GLP | UNC0642 | (Liu et al. 2013) | Synthesis yield: 46% (9 steps).[1]<br>Non-selective inhibitor against G9a and GLP ($IC_{50}$: < 2.5 nM).<br>Potent in biochemical assays ($IC_{50}$: < 10 nM).<br>Low-toxicity ratio ($EC_{50}$: > 16 700 nM).<br>In vivo pharmacokinetic properties: ($CL_{int}$: $8.3 \times 10^{-9}$ $m^3/s$), half-life ($T_{1/2}$: > 6400 s) and permeability brain/ plasma (0.33). |
| | BRD4 | PFI-1 | (Fish et al. 2012) | Synthesis yield: 34% (3 steps).[a]<br>Selective and potent inhibition (biochemical and biophysical assays) towards BRD4 0.22 μM.<br>Low toxicity ratio ($EC_{50}$: > 1.89 μM).<br>In vivo pharmacokinetic properties: ($CL_{int}$: 3^$10^{-7}$ $m^3/s$), half-life ($T_{1/2}$: > 7200 s), and oral bioavailability (32%) due to the suboptimal solubility in the gut of the rat and binding plasma proteins (69%).<br>Possible mechanism of action: cell cycle arrest in G1, and downregulation in aurora B kinase. |
| | BRD3 | RVX-208 | (Picaud et al. 2013) | Clinical trials phase II as treatment of: diabetes mellitus, atherosclerosis, cardiovascular diseases, and dyslipidemia.<br>Synthesis yield: 31% (7 steps).[a]<br>In vivo pharmacokinetic properties: ($CL_{int}$: $8 \times 10^{-8}$ $m^3/s$), half-life ($T_{1/2}$: 5400 s), and oral bioavailability (44%).<br>Selective towards BD2 domain ($IC_{50:}$ 195 nM). |
| | BRD3 | RVX-297 | (Hansen 2011; Kharenko et al. 2016)<br>(Kharenko et al. 2016) | Patented synthesis (no reported yield).<br>~ 2 times more selective than RVX2018 towards BD2 of BRD4 ($IC_{50:}$ 20 nM). |

**Table 1** (continued)

| Epigenetic target | | Compounds name | Reference | Remarks |
|---|---|---|---|---|
| Erasers | HDACs $Zn^{2+}$-dependent class I and IIb | LF12 | (Lin et al. 2016) | -Identified from an in-house library (no reported yield). |
| | | | | Decreased the activity of HDACs class 1 and IIb being more effective the inhibition towardsHDAC3. |
| | | | | Possible mechanism of action: regulation of cholesterol and mevalonate biosynthesis related in the progression on prostate cancer. |
| | | FWP03 | (Peng et al. 2015) | Synthesis yield: 45% (5 steps).[a] |
| | | | | Potent inhibitors against HDACs class I and II ($IC_{50:}$ 2.2 nM), and VEGFR-2 ($IC_{50:}$ 7.4 nM). |
| | | CUDC-101 | (Cai et al. 2010) | Overall synthesis yield: 84% (4 steps).[a] |
| | | | | Potent inhibitors against HDACs class I and II ($IC_{50}$: 4.4 nM), EGFR ($IC_{50:}$ 2.4 nM), and HER2 ($IC_{50:}$ 16.7 nm). |
| | | | | Induces tumor regression in Hep-G2 liver cancer model and inhibits tumor growth in a dose dependent manner. |
| | | | | Phenotype revertion in MDA-MB-468 breast cancer model, overexpressing EGFR. |
| | | | | This compound is on clinical trials phase I as a novel anticancer agent. |
| | Kinase receptors/HDACs $Zn^{2+}$-dependent class I and IIb | SM05 | (Beckers et al. 2012) | Synthesis yield: 82% (7 steps).[a] |
| | | | | Potent inhibitors against HDACs class I and IIb ($IC_{50}$: 7-16 nM), to lesser extent EGFR ($IC_{50:}$ 1200 nM), and HER2 ($IC_{50:}$ 3400 nM). |
| | | | | Its potency is 10 times higher than SAHA. |
| | | SM09 | (Beckers et al. 2012) | Overall synthesis yield: 62% (7 steps).[a] |
| | | | | Potent inhibitors against EGFR ($IC_{50:}$ 18 nM), HER2 ($IC_{50:}$ 11 nM), and to lesser extent against HDACs class I and IIb ($IC_{50}$: 86-630 nM). |
| | | CD11 | (Ding et al. 2017) | Synthesis yield: 91% (8 steps) [a] |
| | | | | Very potent compound against HDAC1 ($IC_{50}$: 0.12 nM), HDAC6 ($IC_{50}$: 0.72 nM), and EGFR ($IC_{50}$: 3.2 nM). |
| | | | | $IC_{50}$: 8.76 μM reported in five tumor cell lines. |
| | | | | Possible mechanism of action: regulates phosphorylation of kinase receptors and hyperacetylation of H3 inducing apoptosis. |
| | BRD4/HDACs | MS01 | (Shao et al. 2017) | Synthesis yield: 50% (5 steps).[a] |
| | | | | Potent inhibitors against BRD4/BD2 ($IC_{50:}$ 401 nM), and HDAC1 ($IC_{50:}$ 204 nM). |
| | | | | Selective binding to BD2 (BD1 $IC_{50}$: > 5000 nM). |
| | SIRT1 | DR16 | (Rambabu et al. 2013) | Synthesis yield: 78% (1 step).[a] |
| | | | | Potent inhibitor against Sir2 in a dose dependent manner ($IC_{50:}$ 1 μM) inhibiting 9% of the cells that expressed Sir2. |
| | LSD1/KDM1 | E11 | (Chang et al. 2010) (Speranzini et al. 2016) | Synthesis yield: 92% (2 steps).[a] |
| | | | | Potent inhibitor against KDM1 (Ki: 440 nm). |

[a]The number of steps considered do not take into account the purification steps involved in the methodology and the reported yield corresponds to the last step of the synthetic route

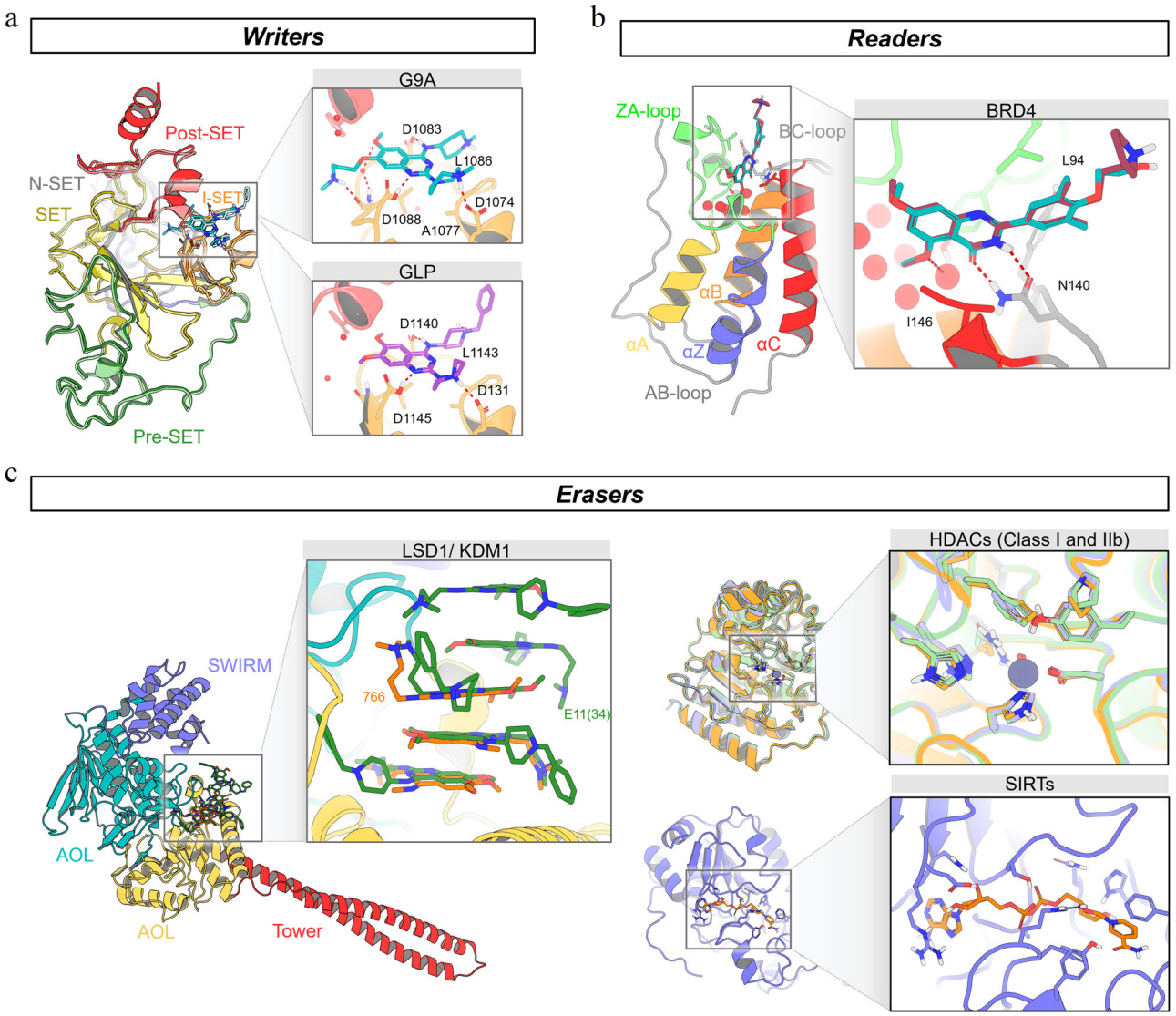


**Fig. 2** Structural features and binding mode in chromatin-associated proteins. **a** Binding mode of the most potent quinazoline derivatives (**UNC0224** y **UNC0638**) and the two principal *writers enzymes* **G9a** and **GLP** (PDB ID: 3K5K and 3RJW) (Liu et al. 2009; Vedadi et al. 2011). **b** *Readers* structure with RVX-208 and RVX-297 binding mode in BRD4 pocket, noting the importance of the grid of five structural water molecules in BRDs structure (PDB ID: 4MR4 and 5DW2) (Kharenko et al. 2016; Picaud et al. 2013). **c** *Erasers* structure within five E11 and 766 molecules for the inhibition of LSD1 (PDB ID: 5LBQ and C767) and HDACs superimposed structures of members of class I, IIb, and III centering in their catalytic binding pocket (PDB ID: 4BKX, 4LY1, 4A69 and 4I5I) (Millard et al. 2013; Prachayasittikul et al. 2017; Speranzini et al. 2016; Watson et al. 2012)

stability of this domain is the presence of the cofactor that is known as a contributor of its partial folding. In addition, the electrostatic potential has shown to be a key structural feature since most of the inhibitors have shown a positive total charge within the structure, for example, quinazoline derivatives and spiro [cycloalkyl-1,3-indol]-2′-amines (Wu et al. 2010).

G9a and GLP enzymes are not functionally redundant, implying that they differ in terms of their biological implication as well as in their expression profile in distinct tissues (Battisti 2016; Kramer 2016). Pharmacologic inhibition of G9a and GLP have been reported to be related with full reprogramming of induced pluripotent stem cells (*iPS)* (Shi et al. 2008, 2008), as well as in cocaine addiction (Maze et al. 2010) and mental retardation (Schaefer et al. 2009). Furthermore, deregulation of these enzymes has also been implicated in the reliance of ailments; for example (Rea and Thomas 2000; Schneider et al. 2002) overexpression of G9a is observed in leukemia (Goyama et al. 2010), prostate cancer, hepatocellular carcinoma (Kondo et al. 2008, Kondo et al. 2007)), and parasitic diseases (Malmquist et al. 2012, Malmquist et al. 2015; Sundriyal et al. 2017). Recent reports indicate that regulation of these enzymes is involved in the amelioration of a few symptoms

involved in Alzheimer's disease, due to the adjustment of mRNA expression levels of a neurotrophic factor (*BDNF*) that plays a crucial role in the progression of this disease ((Butkiewicz et al. 2012; Cacabelos and Torrellas 2015; Chen et al. 2002; Sharma et al. 2017).

## Structural features of BRDs (*readers*)

Bromodomains are structural domains that recognize acetylated modifications in lysine (Kac) residues. Further studies state that BRDs family and BET subfamily are characterized for preserving some structural features implying their involvement in different physiological pathways (Fu et al. 2015; Gallenkamp et al. 2014). In general, this BET subfamily is integrated by BRD2, 3, 4, and BRDT, all of these members containing tandem domains (i.e., BD1 and BD2). Therefore, it is important to mention that BD2 is one of the most targeted domains in BRD4 due to its relevance in different physiopathologies (Brown et al. 2018; Filippakopoulos et al. 2014). In most cases, deregulation of BRDs is not directly linked to a physiopathological state; nevertheless, prominent levels of histone acetylation and the consequent BRDs sensing promotes the expression of oncogenes, associated to tumorigenesis or deregulated cellular growth. Also, acetylated proteins have been proposed as co-regulators of adipogenesis enhancing the expression of PPARγ, which is a receptor involved in the regulation of gene expression during the progression of metabolic diseases (Brown et al. 2018; Gallenkamp et al. 2014; Wang et al. 2016; Zhang et al. 2018).

These domains are highly conserved within all these members and are constituted approximately by 110 amino acids (Gallenkamp et al. 2014). These BRDs display secondary structure key elements, such as β-hairpin structure and four α-helices: αZ, αA, αB, and αC (Zhang et al. 2018). This association leads to the formation of the three principal hotspots (see Fig. 2b): Kac pocket, ZA channel, and BC loop or WPF shelf (Prieto-Martínez et al. 2018a, b). Further studies toward the selectivity of the BET subfamily have shown that the pocket size may be an important feature during the targeting different isoforms (Gallenkamp et al. 2014) (see Fig. 2b). The loop known as BC has become of particular interest because of its known activity as a common hotspot for different compounds, such as amentoflavones (Ember et al. 2014), finasteride (Filippakopoulos et al. 2014), xanthines (Atkinson et al. 2014), and quinazoline analogues (Jahagirdar et al. 2011; Picaud et al. 2013). The principal interactions of the latter hotspot are guided by the hydrophobic interaction with the gatekeeper residue Ile146. An additional hotspot called ZA channel has shown to enable a polar interaction with the recently identified "P-binding" compounds (Noguchi-Yachide 2016; Prieto-Martínez 2018a, b). In addition to these hotspots, there is a grid of five structural water molecules (Aldeghi et al. 2018) that augment the number of hydrogen bond interactions within the inhibitors. Based on these structural features, the inhibitor's structure proposals have included a rigid hydrophobic core linked to a hydrogen bond donor or acceptor groups with an added bulk substituent (Filippakopoulos et al. 2012).

## Structural features of HDACs (*erasers*)

HDAC isoforms have been classified into four classes depending on their cellular localization, and the cofactor needed to remove the acetyl from an specific residue (see Fig. 2c) (De Ruijter et al. 2003; Micelli et al. 2015). Deregulation of HDACs expression is associated with cancer subtypes of acute myeloid leukemia, neurological diseases, and immune disorders (Falkenberg and Johnstone 2014; Zhao et al. 2013). Principal differences among these enzymes have been found principally in the acquired conformations of the most mobile regions (i.e., loops), length, and residue composition. Previous studies have shown that dependence on the cofactor required is the type of conformation found in the catalytic cavity, for example, in class I, II, and IV members or zinc-dependent enzymes that are defined by eight-stranded parallel β-sheets and several α-helices. On the other hand, class III or nicotinamide adenine dinucleotide ($NAD^+$)-dependent members are described by a Rossman fold type (Micelli et al. 2015). In addition, studies revealed that the catalytic pocket is preserved among all HDACs members, this pocket has a tube-like shape due to its mostly hydrophobic residues composition (Pro, Gly, Phe, Leu) (Micelli et al. 2015). Although, the residues that play a key role in the catalysis reaction are mostly polar (Asp-His or Tyr-Asp) interacting with the cofactor. Added interactions within the cofactor are also catalytic water molecules (see Fig. 2c) (Bolden et al. 2006; Micelli and Rastelli 2015).

HDACs inhibitors are classified according to their chemical structure as follows: short-chain fatty acids, hydroxamic acids, benzamides, ketones cyclic peptides, and quinazoline derivatives (Lin et al. 2010). The mechanisms involved in the inhibition of these enzymes are: (1) chelating the zinc ion, (2) through the displacement of $NAD^+$, and (3) by occupying the cofactor's cavity, resulting in major structural changes turning out in enzyme's inactivation (Silvestri et al. 2012; Zhao et al. 2013). Based on the first mechanism, a pharmacophoric model has been proposed based on the presence of three main structural components: (1) Zn-binding group, (2) a hydrophobic spacer, and (3) a hydrophobic capping group (Silvestri et al. 2012). Further studies of the displacement of $NAD^+$ mechanism propose two structural requirements: an hydrophobic core

and a substituent that acts as a hydrogen bond donor (Zhao et al. 2013).

### Structural features of LSDs (*erasers*)

The importance of this epigenetic target relies in the overexpression of LSD1/KDM1 linked to the development of leukemic stem cells that are mostly present in acute myeloid leukemia (AML), glioblastoma stem cells, and numerous cases of neoplasms, such as the prostate, lung, breast, bladder retinoblastoma, and neuroblastoma (De Lera and Ganesan 2016; Niwa and Umehara 2017).

Shi and Jenuwein identified a well-known domain related to lysine demethylase activity called JmjC domain that utilize flavin adenine dinucleotide ($FAD^{+}$) as a cofactor for the removal of the methyl groups. This JmjC domain is present in mostly all LSDs or KDMs enzymes (Lombardi et al. 2011). These enzymes are classified in two groups according to the required substrate they need to catalyze their reaction: (1) $Fe^{II+}$ and (2) α-ketoglutarate-dependent dioxygenase families. In the following section, structural highlights will be discussed, focusing on regions that are crucial for the interaction with small molecules that act as inhibitors (Shi 2013). In addition, it is important to mention the homology within the catalytic domain of monoamine oxidases (MAO) and LSD1/KDM1 implying that MAO inhibitors (i.e., pargyline, phenelzine, and tranylcypromine) can also target this enzyme (Prachayasittikul et al. 2017).

JmjC domain is constituted by different regions, such as a *C*-terminal region, an amine oxidase (AOL) domain, an *N*-terminal SWIRM domain that is known due to its high preservation among species, and the tower domain (see Fig. 2c). The AOL domains have two characteristic subdomains, the FAD-binding site and the substrate-binding site. The substrate-binding subdomain comprises three fragments defined by a six-stranded mixed β-sheet surrounded of six α-helices. The catalytic pocket within AOL domains shows that it is defined by its variable length resulting in a variable space in between these subdomains that will define its selectivity (see Fig. 2c). Other structural features that might help to design new inhibitors, is the different nature of surface potential within the two catalytic cavities, first left side cavity is constituted mostly by hydrophobic residues and on the right side pocket the composition is mainly hydrophilic (Niwa et al. 2017). One of the most important polar interaction preserved in the cofactor is a hydrogen bond with a Lys661 residue; studies suggest that a single mutation on this residue abolishes its demethylating activity. A couple of studies propose LSD1/KDM1 as potential epigenetic target due to its self-renewal in several types of cancer (Niwa et al. 2017; Speranzini et al. 2016). To date, most LSD1/KDM1 inhibitors have been focused on the repurposing of known inhibitors of other chromatin-associated proteins, such as quinazoline derivatives discussed in the following subsection.

## Quinazoline derivatives and analogues as modulators of epigenetic targets

Since 2009, different quinazoline analogues and derivatives have been proposed as potential inhibitors for different chromatin-associated proteins or as dual inhibitors (see Fig. 3). Some of these inhibitors have been considered as potential polypharmacological inhibitors, consisting in one "multicomponent" molecule that is able to interact selectively with several targets at the same time (De Lera and Ganesan 2016). In addition, drug repurposing has been applied to these quinazoline derivatives (for more information see Online Resource S1 or Table 1) (Speranzini et al. 2016). The repurpose of small molecules might be a plausible solution for the better understanding of their mechanism of action (Naveja et al. 2018). In the following sections, previously reported analogues and derivatives are described as HKMTs, BRDs, HDACs, and LSD1 inhibitors.

### Quinazoline derivates as inhibitors of HKMTs

#### Non-competitive cofactor-SAM-inhibitors

The first inhibitor **BIX-01294**, a diazepinquinazolinamine derivative, was identified by Jenuwein and collaborators (Kubicek et al. 2007) in a high-throughput screening study in which a minor inhibition of GLP was observed with respect to its homolog G9a (around three- to fivefold), and a competitive inhibitor toward the peptide substrate. Additionally, the research group of Shi proposed a new technique for the generation of induced pluripotent stem cells (*iPS*) (Shi et al. 2008), in which **BIX-01294** was used as a stand-in of viral transduction used for reprogramming *iPS*. Likewise, this molecule has been involved in the reactivation of latent HIV-1, further analogues proposals based on **BIX-01294** structure have been related to DMNT3A inhibition (Imai et al. 2010; Rotili et al. 2014; Shi et al. 2008). The benign effects of **BIX-01294** were reported by Yang et al., in which they observed that chronic administration in cloned mouse embryos amend the effects in a wider genome level of the anomalous high levels of H3K9Me2 (Huang et al. 2017). However, the concentration values of the latter activity and the toxicity range were akin one to the other (Huang et al. 2017; Kubicek et al. 2007).

Further crystallographic studies based on the complex of GLP-BIX-01294 (PDB ID:3FPD), allowed the proposals of G9a/GLP inhibitors, such as **UNC0224** (PDB G9a 3K5K) (Liu et al. 2009), **UNC0321** (Liu et al. 2010), and **E72** (Chang et al. 2010). However, these last three compounds

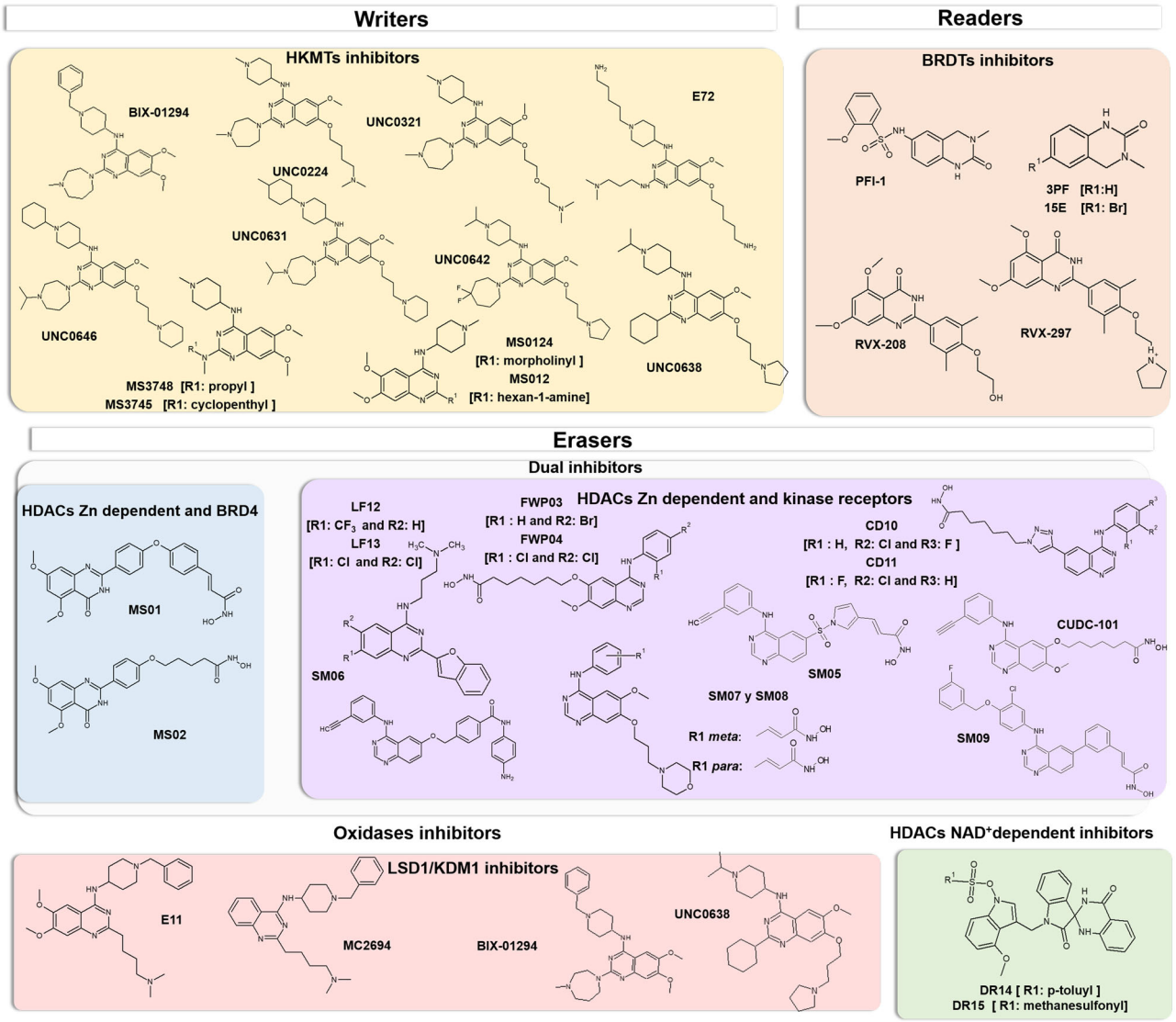


**Fig. 3** Quinazoline derivatives and analogues chemical structures that target chromatin-associated proteins

mentioned were less potent than **BIX-01294** in cellular assays (Chang et al. 2010). Furthermore, new structures were identified, **UNC0646** (Szczepankiewicz et al. 2006) and **UNC0631** (Szczepankiewicz et al. 2006), with favorable potency in different cells lines, the structural changes were implemented in order to improve cellular membrane permeability and lipophilicity. Nevertheless, these inhibitors were not suitable for in vivo studies due to its related pharmacokinetic constants. Further optimization of these pharmacokinetic properties led to the discovery of potent and selective G9a/GLP dual inhibitors **UNC0642** (Liu et al. 2013) and **UNC0638** (G9a PDB ID: 3RJW) (Liu et al. 2013), whose properties were adequate for in vivo pharmacokinetic studies. Amid all seven compounds previously mentioned, the only reported inhibitor that was more selective toward GLP in comparison with G9a was **BIX-01294** (GLP PDB ID: 3FPD). For this reason, Jin's and coworkers design and synthesize selective compounds, such as **MS0124** ((Vedadi and Jin 2017a; Xiong et al. 2017) (GLP PBD ID: 5TUZ and G9a ID: 5TUY) and **MS012**, which were >30-fold more active against GLP. Notwithstanding a poor permeability and a high-reflux ratio was associated to **MS0124**. Added SAR studies revealed **MS3748** (Vedadi and Jin 2017b; Xiong et al. 2017) (GLP PBD ID: 5VSD and G9a PDB ID: 5VSC) and **MS3745** (GLP PDB ID: 5VSF and G9a PDB ID: 5VSE) (Xiong et al. 2017) two inhibitors highly selective toward GLP (59–65-fold), revealing that this substituents may favor the disposition within the GLP-binding site. Overall, these results suggest that these attempts still require improvement and modification in the substitution pattern of the structure for further biological assays.

It is important to understand all the interactions within the chemical groups present in the inhibitors and structural features of G9a or GLP (see Fig. 2a). The analysis advocates the main interactions required for G9a, discussed in the following paragraphs based on what previous studies reported (Xiong et al. 2017). In general terms, preserved interactions within the quinazoline structure are salt-bridge formed due to the proton transfer with N1 and Asp1088, forming the protomer of the quinazoline. As well as hydrophobic interactions were established between the two rings of the quinazoline and Leu1086. In addition, methoxyl substituent in C-6 serves as a hydrogen bond acceptor, which interacts with Asp1083 and a structural water molecule. The difference relies on substituents present in C-7 and C-2, such as in **UNC0638**, a hydrophobic interaction within the cyclohexyl in C-2 and the residue of Ala1077 also in C-7, the pyrrolidine contacts with Leu1086. Another possibility is that this substituent might be protonated, acting as a hydrogen bond donor interacting with the carbonyl of the main chain of Leu1086. Added interactions on **UNC0224** consisted in substituents located C-2 of the quinazoline an amine group in the diazepane may be protonated interacting via a salt bridge with an Asp1074 residue and the alkyl chain in C-7 interacts with the Phe1168.

In addition, GLP residues within the binding pocket interact in an analogous way to what has been mentioned, the N1 in the quinazoline core forms a salt bridge with Asp1176. Likewise, a hydrophobic interaction is established between the quinazoline and Leu1174 present in the binding cavity. Interaction in C-4 may differ on which substituents are located, for example, in **BIX-0194** and **MS012** the amino substituent is protonated interacting via hydrogen bonding with Asp1140, in the same manner, the amino present in C-2 diazepane substituent of **BIX-0194** acts as a hydrogen donor bridging with Asp1131. Recently, **MS3748** (Xiong et al. 2017) and **MS3745** (Xiong et al. 2017) were reported as selective inhibitors toward GLP, this may be due the small substituents in C-2 that might favor certain disposition of these compounds that bulkier substituents present in other derivatives may have, although crystallographic data may not fully explain the selectivity observed in the biological assays (see Table 1).

Further studies made by Jin's and collaborators reported other quinazoline derivates that were based on the structure of the 12 compounds previously mentioned that inhibit covalently to SETD8. This enzyme is implicated in broad biological processes where DNA damage is placed; further studies report that it may be involved in regulating the expression level of *N-cadherin*, considered a biomarker in breast cancer metastasis (Butler et al. 2016, Ma et al. 2014a, b). Added studies done by Fuchter et al.; consisted on the design and synthesized quinazoline derivatives based on the reported crystallographic data of human's *writers*. Biological results reported that these compounds were not that potent against *Plasmodium falciparum*, which might be due to the difference between species in terms of residues present on the binding site (Lubin et al. 2018; Malmquist et al. 2012, Malmquist et al. 2015; Sundriyal et al. 2017).

Further synthetic studies have reported several routes to obtain these quinazoline derivatives, starting from raw materials or the pre-formed quinazoline core (see Fig. 4). Despite the adequate yield of these procedures, further synthetic optimization may still be required to obtain these quinazoline derivatives.

To sum up, based on the previous crystallographic data reported, it is observed that interactions are preserved within the quinazoline structure and GLP or G9a. Adding different substituents in C-2, C-4, and C-7 positions of the quinazoline may favor the selectiveness within these targets. Quinazoline derivatives reported until now are very potent, but further biological assays may be assessed.

## Quinazoline analogues as inhibitors of BRDs

### Competitive inhibitors toward Kac

Nowadays, a major part of the discovery and design of small molecules as potent inhibitors is centered in BET subfamily members (Arrowsmith et al. 2012; Fish et al. 2012; Gallenkamp et al. 2014). Their main difference within every member relies in their binding preference to acetylated histone motifs (Gallenkamp et al. 2014).

First proposals of quinazolinones were made by Conway's and Chung's research groups as an interesting scaffold, whose activity was confirmed by a virtual screening study (Fish et al. 2012). The first complex crystallized was BRD2 with a known inhibitor **3PF** (PDB ID: 4A9E), in which the observed interactions were hydrogen bonding within the amino group placed on C-3 and the carbonyl on C-4 in the quinazoline with the backbone of Asn166 residue. Later on, they reported the crystal structure complex BRD4-**16E** (PDB ID:4HBV) (Fish et al. 2012) with a better resolution, in which the interaction with the quinazoline analogue were preserved hydrogen bond interactions with the carbonyl group and the Asn140 residue, resolution improvements enable to observe additional interactions with structural water molecules (Filippakopoulos et al. 2012, Filippakopoulos et al. 2010). Hydrophobic interactions were preserved among the two crystallized quinazolinones and the interactions with the residues Ile and Leu from chain A of BRD2 and BRD4 were entrenched.

Subsequently, a potent and selective compound called **PFI-1** was reported to target BRD4. The mechanism of action related to the inhibition of BRD4 through **PFI-1** was toward the inhibition of cellular proliferation and apoptosis, which could be quantified through a related transcription

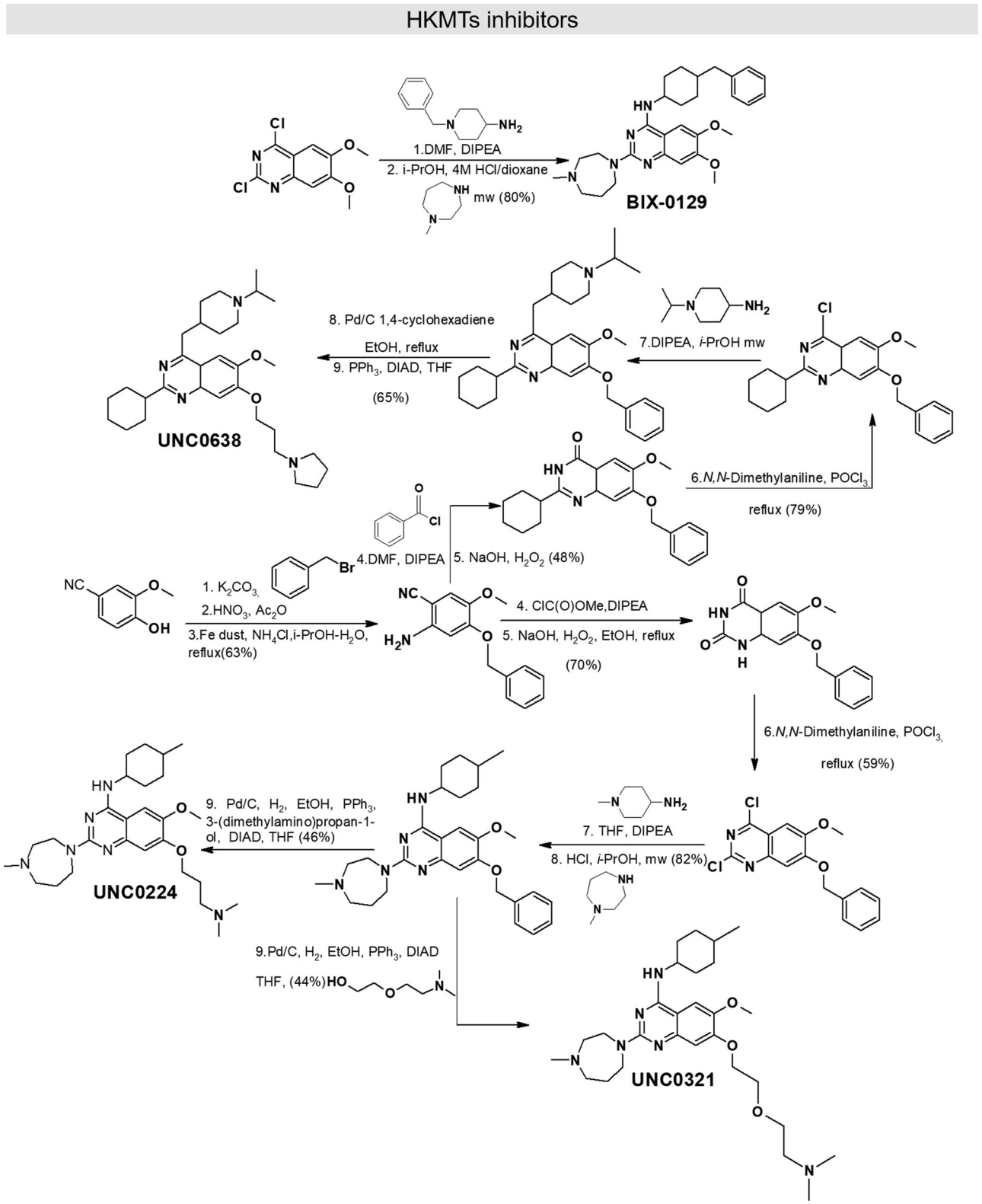


**Fig. 4** Synthetic routes of the most representative inhibitors of *writers*

factor *HOXA9* and aurora B kinase, which are known upregulated biomarkers in different types of leukemia (Fu et al. 2015; Picaud et al. 2013; Thorsteinsdottir et al. 2017). In the crystallized complex BRD4-**PFI-1** (PDB ID: 4E96)

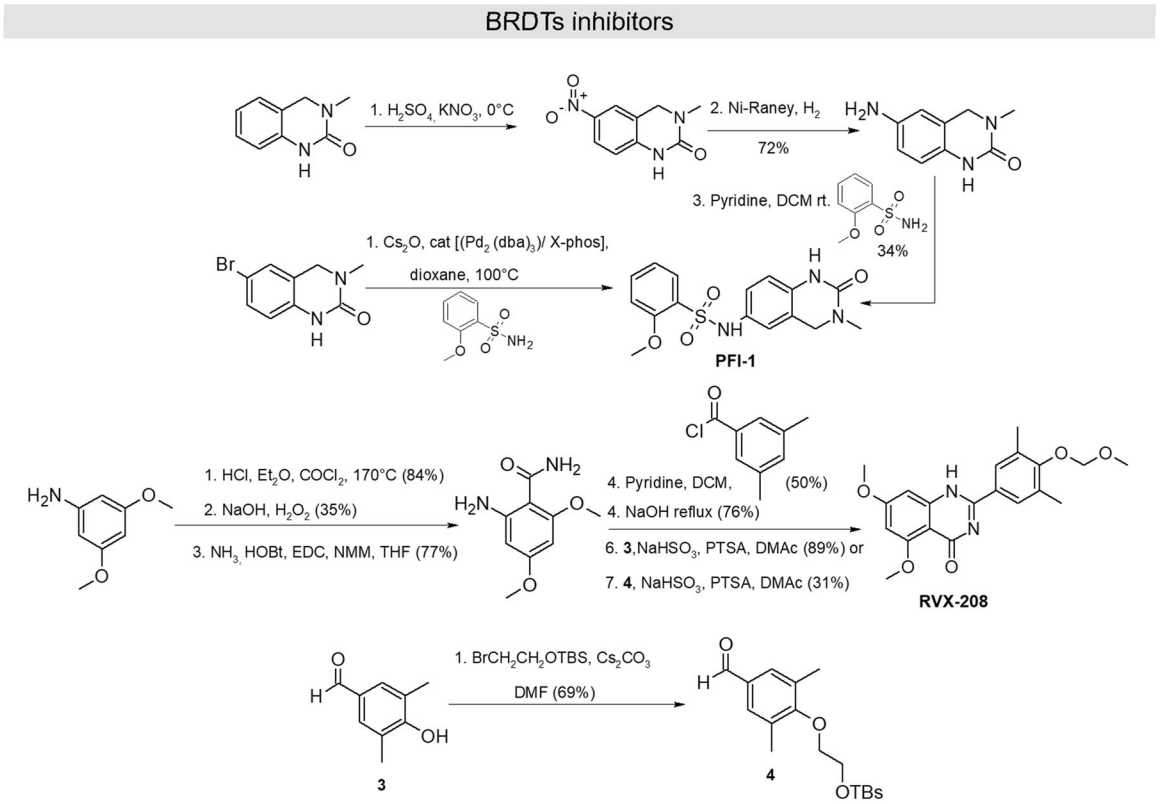


**Fig. 5** Synthetic routes of the most representative inhibitors of *readers*

(Picaud et al. 2013), it was observed the preservation of the interactions within the carbonyl groups and the nitrogen N1 in the quinazolinone as acting as hydrogen acceptors and donors, respectively, with the residue Asn140. Moreover, the carbonyl showed a second interaction of hydrogen bond with a water molecule coordinated with Tyr97, surrounded with a grid of five molecular waters that together they interact with each other, found gathered near by the pocket were the substrate is recognized, mimetizing the substrate union. Added to this, hydrophobic interactions entrenched within the quinazolinone and Leu94 as well as with the sulfonamide substituent and Ile146, resulted to agree with the previously crystallographic data.

Further studies targeting BRD4 were reported by Grupta et al. who proposed a new derivate of **RVX-208** as a modulator of ApoA1, which is in clinical trial in phase I/II to treat cardiovascular diseases. It's selectivity for BRD4 has been proved in different biological assays, such as in clinical trials phase III studies which revealed that these compounds may act as a potential treatment of diabetes mellitus type 2 by inhibiting BRD4 (Gosh et al. 2017; Noguchi-Yachide 2016). Crystallographic studies revealed that BRD4-**RVX-208** (PDB: 4MR4) (Picaud et al. 2013) complex preserve weak interactions with this quinazoline analogue, which agreed with previous report (see Fig. 2b). Principal differences rely in the binding mode because of the substituents in C-5, C-7-dimethoxy groups, and the carbonyl in the quinazolinone serves as a hydrogen bond donor and acceptor that interacts with added structural water molecules. Based on **RVX-208** structure, new molecule proposal was made known as **RVX-297** differing in the pattern substitution of C-4 in the quinazolinone (see Fig. 2b). Its biological activity seem to differ from **RVX-208**, associating this new compound as an inhibitor related to autoimmune disease models of multiple sclerosis and arthritis (Attwell et al. 2012; Jahagirdar et al. 2011). Nonetheless, their binding modes and established interactions are very similar within BRD4, to confirm these additional studies of X-ray crystallography were done. Co-crystallization with **RVX-297** (PDB: 5DW2) (Kharenko et al. 2016) permitted to observe that the interactions are preserved among these two quinazoline analogues (Kharenko et al. 2016).

Previous synthetic reports of the most representative BRDs inhibitors have seek to simplify the number of steps and yields during the reaction pathways (see Fig. 5 and online resource S1).

In summary, in this part we had reviewed quinazoline analogues, such as quinazolinones as potential inhibitors of BRD2 and BRD4. Crystallographic data suggest that the

weak interactions (i.e., hydrophobic and hydrogen bonding) are preserved among mostly all the reported analogue quinazolines. Apparently, the selectivity in this target might be due to the mechanism, in which these modifiers recognize its sequence within the tandem domain, and it's different binding mode.

## Quinazoline analogues and derivative as dual inhibitors

### Quinazoline analogues as dual inhibitors of BRD4 and HDACs $Zn^{2+}$ dependent

Atkinson's earlier studies proposed to target HDACs and BRDs by modifying the profile expression in related genes and biological effects. Studies reveal that the repression of *Myc*-induced murine lymphoma is due to the synergic effect of inhibiting these two enzymes. Additional studies prove that co-administration of two inhibitors of BRD4 and HDACs relate to synergic response toward human acute myelogenous leukemia cells (Alaei-mahabadi et al. 2016).

For this reason, Chen's research group proposed incorporating these two chemical portions on a single molecule based on the structure of BRD and HDACs known inhibitors (Atkinson et al. 2014). SAR studies of 5,7-dimethoxy-2-phenylquinazolin-4(3 *H*)-one were focused mainly in modifying C-2 phenyl substituent in positions *meta* and *para*. The latter results in **MS02** and **MS01** that were the most active compounds of the series, having inhibitory effects against BRD4 and HDAC class I. The range of concentrations reported were nanomolar from these two compounds, being **MS01** the most potent of the two. Cytotoxicity activity was evaluated using three different AML cell lines: MV4-11, OCI-AML2, and OCI-AML3, in the second cell line **MS01** showed a major inhibitory effect. To bear out the effect on AML cells, they measured the concentration of c-*Myc* (oncogene related to leukomogenesis) and its bioactivity resulting in a diminished concentration of c-*Myc* (Shao et al. 2017).

As an added part of the investigation, they performed some docking studies of the compound **MS01** with the proteins BRD4 (PDB: 5U2C) and HDAC1 (PDB: 4KBX). Fortunately, the interactions within BRD4 and **MS01** were very similar to the quinazoline analogues previously discussed, in which carbonyl group interacts through hydrogen bonding with Asn433. On the other hand, interaction between HDAC1 and the substituent phenyl hydroxamic acid in C-2 which was located in the terminal part may contribute in its movement restriction allowing the stronger coordination within the $Zn^2$ ion (see Fig. 6). Additional hydrophobic interaction may be established between this phenyl hydroxamic substituent through π-stacking with Phe160 (Shao et al. 2017).

The synthetic route to obtain this quinazoline analogues consists of five steps with an acceptable yield; however, further improvements in these methods may still be required in order to simplify the generation of these derivatives (see Fig. 6).

To sum up, new chemotypes are being proposed combining substituents that act as HDACs and BRDs inhibitors. Additional SAR studies may help to identify the type of substituent resulting in polyepimolecules, such as in these two derivatives. No significant difference was observed in the computational study in terms interactions established or its binding mode that could relate to the results reported in the biological assays, in which one compound is more potent than the other.

### Quinazoline derivatives as dual inhibitors of HDACs $Zn^{2+}$ dependent and kinase receptors

**HDACs (class I and IIb)/VEGFR-2** Polypharmacology treatments are based on addressing several targets simultaneously or not being selective toward a specific target, considering this to be a limitation that can be put aside (De Lera and Ganesan 2016). For an in-depth review of epidrugs in clinical trials (Nebbioso et al. 2012).

Based on this premise, Lei Shi's group evaluated the activity in vitro of a set of quinazoline derivatives against the vascular endothelial growth receptor isoform 2 (VEGFR-2) and HDACs class I. The SAR studies that were carried out, were based on a proposal variation made principally on position C-4 of the quinazoline. The most active compounds were **FWP03** and **FWP04**, which differ in the substitution pattern in C-4 aniline, the first compound had a *p*-bromide, and the second compound had a 2,5-dichloride group (see Fig. 3 and online resource S1). However, the most potent compound was **FWP03** in which tested concentration range was in the nanomolar in terms of inhibition of HDACs class I and IIb and VEGFR-2 inhibition. This effect seemed to decrease in growth inhibition of MCF-7 cell line whose concentration was in the micromolar range.

In addition to this, computational studies were done in histone deacetylase-like protein *"HDLP"* an homolog of HDAC (PDB: 1C3S) (Finnin et al. 1999) for the better understanding of the interactions within these targets (Peng et al. 2015). The results of the binding mode were compared with a known inhibitor suberoylanilide hydroxamic acid (SAHA or Vorinostat®), which were very similar to the other compared with **FWP03**. These studies suggest that the length of substituent *N*-hydroxy-7-phenoxyheptanamide in C-6 of the quinazoline is appropriate to coordinate $Zn^{2+}$ ion. Hydrophobic interaction was as well present within the core of the quinazoline and the residue Pro22, added

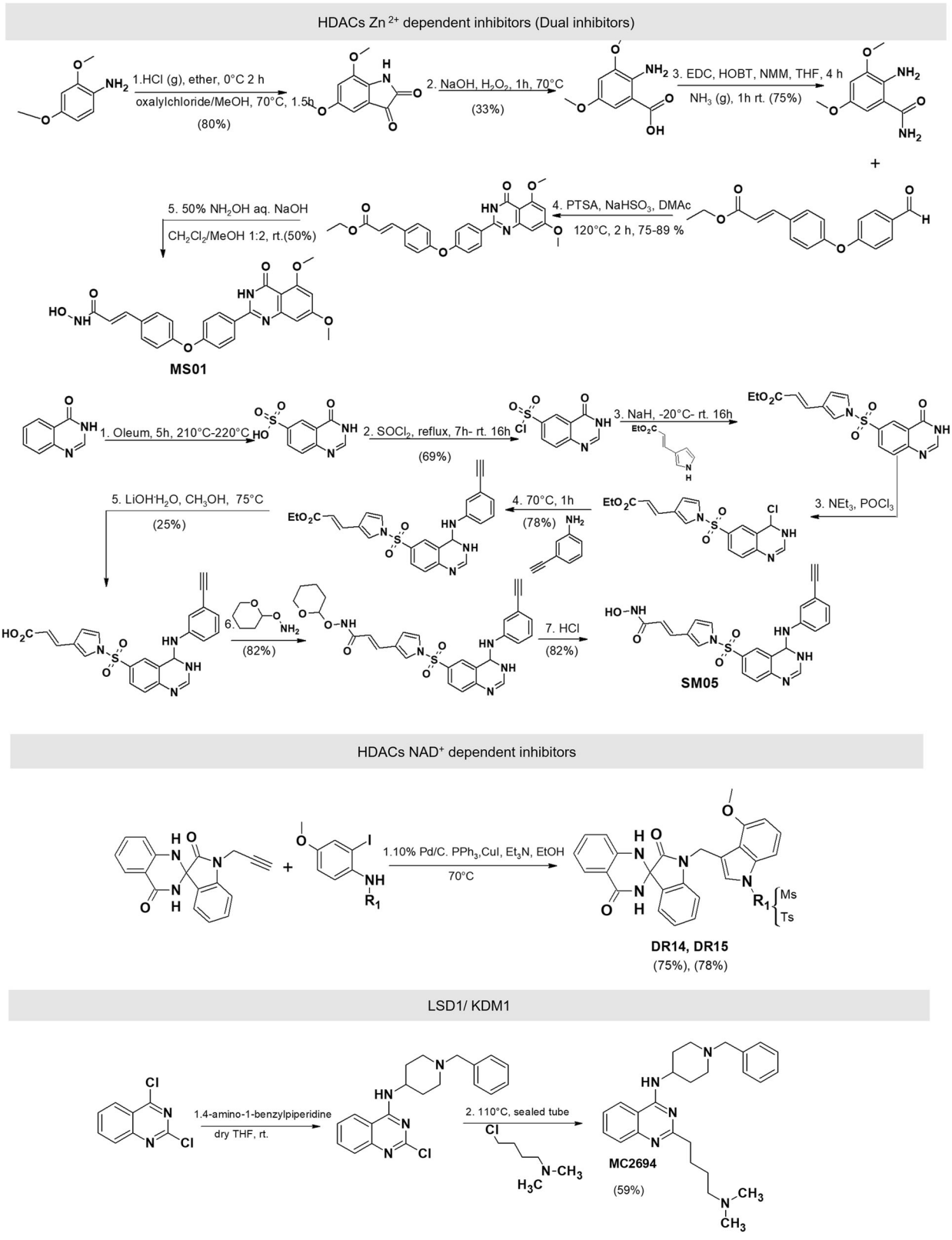


**Fig. 6** Synthetic routes of the most representative inhibitors of *erasers*

parallel displaced stacking interaction was observed between the quinazoline and Tyr91 residue.

To sum up based on the computational studies, both compounds preserve the same interactions and the binding mode compared with the reference compound. No significant difference was observed because of the different pattern in C-4 that could relate to the difference in the potency obtained in the biological assays.

**HDACs (class I and IIb)/HER1/2** Based on previous reports, it is known that synergistic cross-talk is established between HDACs and EGFR/HER2 (Bali et al. 2005; Edwards et al. 2007). For this latter, Changgeng Qian et al. synthesized a set of chemical of hybrids, comparing the biological results with two reference compounds such as: Vorinostat® (HDACs inhibitor) and Erlotinib® (kinase inhibitor) and combining their substitution pattern of these compounds and mix them in only one derivative (Cai et al. 2010). The latter allowed the synthesis of **CUDC-101** (7-(4-(3-ethynylpehylamino)-7-methoxyquinazolin-6-yloxy)-N-hydroxyheptanamide), which resulted to have an inherited potency against these two enzymes compared with the reference drugs (see Table 1 or online resource S1). Furthermore, **CUDC-101** showed a relevant size tumor reduction in Hep-G2 (hepatocellular carcinoma), MDA-MB-468 (breast adenocarcinoma) cell lines, and a synergic effect in the last cell line mention in the co-administration with paclitaxel (Taxol®). This compound exhibited promising results in clinical trials phase I as well as in the pharmacodynamic analysis proving the interaction with both of the two targets predicted (Cai et al. 2010).

Based on the previous information and on the substitution pattern of **CUDC-101**, further changes were proposed such as the rigidity within the different linkers that connect quinazoline's structure to the hydroxamic acid chelating the cofactor within HDACs (Mahboobi et al. 2012).

Mahboobi and collaborators synthesized a set of quinazolines series, in which variations proposals were mainly on C-6 of the quinazoline resulting in **SM05** and **SM06** two promising compounds (see Fig. 3). Thus, the biological assays indicate that compound **SM05** was more selective to HDACs than EGFR/HER2 and the opposite happened with the compound **SM06**. The last second-generation of compounds were design, considering different chemical structures of known kinase inhibitors, such as Gefitinib® and Lapatinib®. Resulting in two Gefitinib®-based compounds **SM07** and **SM08**, which main variations were on phenyl substituent situated in C-4 of the quinazoline (see Fig. 3 and online resource S1). These groups positions in *p*-phenyl resulted in augmented selectivity against HDACs, although poor inhibition was observed against EGFR/HER2. On the other hand, substitution in *m-phenyl* resulted in the inverse pattern in terms of selectivity. Added derivatives based on Lapatinib® structure (i.e., **SM09**) resulted to be competent toward the reference compounds in both enzymes, suggesting that from all of this compounds the most important substitution may be in C-4 to obtain more potent compounds (Mahboobi et al. 2010).

Jiang's research group synthesized a few hybrid compounds based on the structure of compound **CUDC-101** and 4-phenylaminoquinazoline, which is an EGFR inhibitor with proven activity. They also studied the effect of 1,2,3-triazole as a linker between these chemical structures previously mentioned (Ding et al. 2017). Further biological evaluation of **CD10** and **CD11** as inhibitors of HDAC/EGFR/HER2, resulted in contrasting in vitro results within the two targets (see Fig. 3). First, HER2 results suggest that the smaller the substituent in C-4 of the quinazoline will result in a diminished activity, likewise less voluminous substituent in C-6 resulted in diminished potency (see online resource S1). In addition, they also studied the effect of the di-substitution pattern with different halogens positioned in C-4 phenyl group, resulting that this may contribute to the activity against HER2. Conversely, the inhibition of HDACs (1 and 6) indicate that a less voluminous substituent pattern favors the activity, preserving the di-substitution pattern within the phenyl group may contribute to the acquire potency.

Moreover, anti-proliferative activity was evaluated using two cell lines in which the overexpression of these two receptors varies one from the other (A549 cell line overexpresses EGFR, and BT-474 cell line expression of HER2 is enhanced) arising that in both cell lines growth inhibition was favored. The results were favorable for both compounds, although **CD11** was more potent than **CD10** (see online resource S1) and also induced apoptosis in BT-474 cell line. Further studies were carried out in order for better understanding of the mechanism of action that consisted of inhibiting the phosphorylation of EGFR and HER2, added to the hyperacetylation of histone H3. In summary, compound **CD11** may act as an anti-proliferative and proapoptotic effector in BT-474 cells because of its capacity to act as inhibitor against HDAC1/6, HER2, and EGFR (Ding et al. 2017).

Finally, docking studies were carried out on EGFR (PDB: 1XKK) (Wood et al. 2004) and HDAC2 (PDB: 4LXZ) (Lauffer et al. 2013) only for compound **CD11** (see Table 1). Allowing the identification of some key interactions such as: (1) in EGFR the N1 of the quinazoline establishes a hydrogen bond with the carbonyl of Met793 backbone, (2) substituent in C-4 fits on the hydrophobic pocket, and (3) the substituent on C-6 substituent extends toward the

hydrophilic region and the hydroxyl group forming a hydrogen bond interaction with Asp800. On the other hand, the interaction held with HDAC2 based on the crystallized structure of HDAC1-**Vorinostat** was not available, principal interactions consist on the chelation with $Zn^{2+}$ were the triazole linker played a crucial role due to its rigidity that may allow the entrance of this substituent into the binding pocket (Ding et al. 2017).

## Quinazoline derivatives as inhibitors of HDACs $Zn^{2+}$ dependent

### Role of HDACs (class I and IIb) in the regulation in the expression or repression of certain genes associated with prostate cancer

Ferguson's and collaborators analyzed a library of compounds for the proposal and synthesis of a set of quinazoline derivatives, studying relevant substituents. Main appraises was to vary three positions of the quinazoline itself and studying the effect related to the inhibition of HDACs and their level of expression oncogenes (*c-Myc)*, inflammation-related (*interleukin 1*), and tumor suppressor genes (*p21* and *p53*). Resulting in two active compounds **LF12** characterized by a substitution on C-6 with a trifluoromethyl and **LF13**, which had di-substitution pattern in C-6 and C-7 with two chlorides (see Fig. 3 and online resource S1). Therefore, biological results suggested that **LF12** was the most active toward the repression of oncogenes, inflammation-related genes, and *p21*. Although it allowed the expression of *p53*, this particular was observed in compound **LF13**, which restored the activity of *p53*. In broader terms, both compounds inhibit HDACs of class IIb and in lesser extent class I enzymes this may be associated to the restriction on the transport through the nucleus membrane. HDACs inhibition may be the cause of changes in the expression level of these genes, which might be related to the acetylation in histones and no-histones proteins, diminishing the expression of *c-Myc*, *interleukin 1, p21*, and on the other hand it favored the expression of *p53* (Lin et al. 2010; Philpott et al. 2007).

Further studies of compound **LF12** were made by Ferguson, revealing that these compounds may influence the regulation in cholesterol biosynthesis and mevalonate pathways in androgen-dependent prostate cancer cell lines. **LF12** that might be a good candidate for the treatment of prostate cancer (Lin et al. 2016) (see Table 1).

Most of the synthetic routes to obtain these quinazoline derivatives were carried out with different polysubstituted anilines with acceptable yields. It is noteworthy that there are at least four steps involved in the synthetic methodology due to the different substitution pattern present in these derivatives (see Fig. 6).

## Quinazoline analogues as inhibitors of HDACs $NAD^{+}$ dependent (SIRTs)

Based on the structure of the Selisistat® (1,2,3,4-tetrahydroquinazoline) that is a known SIRTs inhibitor, Manojit Pal et al. designed a series of molecular hybrids fusing these last structures to form spiro heterocycles (Rambabu et al. 2013). The main variation for the SAR studies were mainly in *N*-indolyl methyl substituents evaluating them in *Sir2* of yeast due to its high structural homology with human isoform SIRT1.

The results suggested that the increasing volume on the substituent in C-4 of the quinazoline may be related to an increase in the inhibition of Sir2. The latter agreed with the substituent present in the most active compounds **DR14** and **DR16**, in which 4-methoxyl substituent in the indole structure was one of the most voluminous group proposed in this study (see Fig. 3 and online resource S1). These two compounds differed principally on substituent place in position 1 of the indole having a *p*-toluyl acid or a methanesulfonyl, respectively. Although methanesulfonyl group in **DR16** seemed to be more adequate for the inhibition of Sir2 (Rambabu et al. 2013).

As complementary part of this study, the authors reported a docking study in order to understand the interactions based on a SIRT1 crystallized complex (PDB ID:4I5I) (Zhao et al. 2013), observing that sulfoxide group present in both compounds forms a hydrogen bridge with the main alkyl chain in the residue Arg446. In addition, it was observed that quinazoline and the indole formed a π-stacking interaction with His363 and Phe414, respectively, occupying the same pocket in which the cofactor is positioned, suggesting that these compounds might act as competitive inhibitors toward the cofactor (Rambabu et al. 2013). Apparently, this variation in position 1 of the indole ring does not alter the binding mode and the number of interactions established within the two compounds and Sirt1. Therefore, this would not explain a significant difference in the activity measured within the two compounds and its binding mode to the epigenetic target of interest.

The synthesis route proposal reported elsewhere to obtain these spiro heterocycles consists of one reaction step with an acceptable yield for both quinazoline analogues (see Fig. 6).

## Quinazoline derivatives as inhibitors of oxidases

Drug repositioning or repurposing is a well-known technique that consists of finding an alternative mechanism of action of reference compound. The underlying principles of drug repurposing entail that drugs might have activity against more than one target (Méndez-Lucio et al. 2016).

Based on this concept, Mattevi et al. evaluated a set of quinazoline derivatives with known anticancer activity acting as G9a inhibitors. Nevertheless, in this study new proposals of these compounds were made as potential dual inhibitors of LSD1/KDM1and G9a. This study permitted the identification of **UNC0638**, **BIX-01294**, **E11**, and **MC2694** as dual inhibitors targeting G9a and LSD1/KDM1. In which, results reported that **UNC0638** apart from being well-known potent inhibitor of G9a the affinity against LSD1/KDM1 was diminished. In the same manner, **BIX-01294** as a potent compound against G9a showed poor affinity for this enzyme. Nevertheless, **E11** and **MC2694** are considered as poor inhibitors of the methyltransferase enzyme; however, they showed a high affinity for oxidase LSD1/KDM1 (Speranzini et al. 2016).

In order to understand the binding mode of **E11**, they used X-ray diffraction in complex with LSD1-CoREST (PDB: 5L3E) (Speranzini et al. 2016). The crystal showed that the inhibitor binds with a stoichiometry of 5:1 to the enzyme (five molecules of **E11** for each enzyme). Additional crystallographic structures with polymyxins (PDB: 5L3F and PDB: 5l3G) (Speranzini et al. 2016) corroborated that the inhibitor occupies the same site of polymyxins, explaining the number of quinazoline molecules required for the association to LSD1/KDM1 that occupy less area compared with the polymyxins molecules. Added hydrophobic interactions were observed within the five molecules of **E11** through a π-stacking interaction, occupying a distant pocket from the cofactor's binding site (see Fig. 2c). These multiple ligands are widely exposed to solvent that is why interactions are limited within the interphase of LSD1-CoREST heterodimer.

Other compounds were evaluated (not shown in this work), presenting differences in the substitution pattern, resulting in the identification of key groups in C-4 (benzylpiperidine) and C-2 (dimethylaminopropylamine) in the quinazoline, which was compared with compounds that did not have this substituent (PDB: 5LBQ, C767, and C768) (Speranzini et al. 2016), and the activity was diminished against LSD1/KDM1.

Complementary biological assays contribute to the analysis of the activity related to these compounds as potential inhibitors of the leukemic cell growth. Further studies consisted in quantifying the levels of mRNA of *Gfil-B*, which is a known gene involved in hematopoiesis pathway. The consequent cultures exposure (ranging 24 h at different concentrations), permitted to observe that prolonged exposure to **E11** and **MC694** show significant beneficial effects in the leukemic cells.

To sum up, repositioning is a good strategy to find alternative biological activities in certain compounds that have been previously biologically characterized. Furthermore, hydrophobic interactions of π-stacking between inhibitors might be a new trend in augmenting the potency inherited in oxidases as epigenetic targets. The importance of specific chemical groups is required for the interaction with LSD1/KDM1. It was shown that the presence of the quinazoline structure is not sufficient for the inhibition of LSD1/KDM1, additional substituents are needed such as the presence of C-6 and C-7 electron-donating substituents, such as methoxyl groups in **E11** and **MC2694**. Figure 6 shows the synthetic rout of the most representative LSD1/KDM1 inhibitors.

## Conclusions

Herein, we discussed the development over the past 15 years of quinazolines analogues and derivates as potential inhibitors of chromatin-associated proteins. All reviewed compounds were classified based on the interactions within the quinazoline and the specific epigenetic target i.e., *writers, readers*, and *erasers*. The presence of the quinazoline itself that seems to be required for the interactions in which nitrogen and carbonyl in the core act as hydrogen acceptors/donors, salt bridge or hydrophobic interactions surrounding all the bicycle. In addition to these interactions, the most prevalent substituents are located at positions 2, 4, 6, and 7 of the quinazoline nucleus displaying key electrostatic interactions. Quinazoline derivatives or analogues have shown activity against one or more chromatin-associated proteins, paving a way to fulfilling several problems due to multifactorial causes that results in a complex pathology. The importance related to this scaffold is due to that they are considered as broad re-programmers implicated in the indirect regulation of gene expression for the maintenance of the homesotasis via epigenetic modifiers. Moreover, quinazoline repurposing is a powerful strategy to identify multiple activities due to that these molecules target multiple epigenetic modifiers or enzymes. These might still require further studies to identify other activities in the quinazolines structures reported thus far. Further studies may still be acquired in order to improve the potency, synthesis yields, and biological activity for treatment of chronical diseases.

## Perspectives

Table 2 summarizes major perspectives. Overall, quinazoline analogues and derivatives studies are growing very fast in identifying more chromatin-associated proteins that these structures may target. Further biological assays are still required to elucidate their pharmacodynamics as pharmacokinetic properties. These assessments are important in maternal and early-life exposures as well as in chronical

**Table 2** Overall, perspectives that might still be required

| Target | Perspective |
|---|---|
| G9a and GLP | For **UNC0646** further biological assays in vivo should be driven. |
| | Require determining pharmacokinetic parameters for a few quinazoline derivatives. |
| | Plausible studies to dispose undesirable effects related to in vivo in long periods of co-administration and different concentrations. |
| BRDs | Further studies of efficacy and are required in order to identify adverse reactions, or drug interactions involved in the co-administration of **RVX-208**. |
| | Different synthesis strategies in order to increase the synthesis yield of **RVX-208**. |
| | Added biological studies in vivo and in vitro should be carried out for **RVX-297**. |
| Class III HDACs (SIRTs) | More computational studies should be used as guide for new molecules proposals based on a rational design. |
| | Further biological assays, for the estimation of their activity on human enzyme SIRT1 and other members of this class. |
| Dual inhibitors HDAC $Zn^{2+}$ dependent | For **CUDC-101** in human voluntaries might be require. |
| | Additional biological assays in vivo and in vitro should be driven for **FWP03** and **CD11**. |
| | Further computational studies for new proposals rational design may help increased potency in these compounds. |
| LSD1/KDM1 | Inhibitors previously mention still require additional studies in terms of more pharmacodynamics. |
| | New computational studies should be used as guide for new molecules proposals based on a rational design. |
| | New repurposing studies are needed to identify more inhibitors. |

treatments. Additional computational studies and crystallographic data is crucial for understanding the binding mode and interactions established within the quinazoline compounds.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.



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