Review

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HDAC6: Physiological function and its selective inhibitors for cancer treatment

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ABSTRACT: Acetylation and deacetylation of histones are important in regulating gene expression and play a key role in modification of gene transcription. Specific HDACs isoforms can be regarded as a target for cancer therapy avoiding side-effects, HDAC6 with a unique physiological function and structure has become a hot issue recently. The unique isoform HDAC6 is involved in tumorigenesis, development and metastasis through tubulin, HSP90, invasin and ubiquitin-protein. Here we review the structure elements, biological function, and recent selective inhibitors of HDAC6, and study the structure-activity and structure-selectivity relationship.

Keywords: Histone deacetylases, HDAC6, cancer, inhibitor

1. Introduction

Histones are one of the important components which constitute the chromosome of eukaryotes. Acetylation and deacetylation of histones regulate gene expression through key transcriptional modifications (*1,2*). Histone acetyltransferases (HATS) and histone deacetylases (HDACS) regulate post-translational modifications by the acetylation and the decetylation of the ε-amino group of lysine residues in histone tails and some non-histone proteins (*3*). Relevant research shows that acetylation and deacetylation of histones are of significant importance in tumor genesis and progression. Inhibition of HDACS has become a promising direction for cancer therapy (*4*).

HDACS consist of four classes (I, II, III, and IV) based on their homology to yeast histone deacetylases. Class I consists of four different isoforms (HDAC1, 2, 3, and 8). Class II with six isoforms is grouped into two subclasses named Class IIa (HDAC4, 5, 7, and 9) and class IIb (HDAC6 and 10). Class IV consists of only one isoform, HDAC11. Seven isoforms, Sirt1-7, are referred to as class III. The enzymatic activity of HDACs plays its role by two mechanisms which include the Zn^{2+}-dependent mechanism of class I, II, and IV and the NAD^{+}-dependent mechanism of class III.

Most HDACS target histone proteins, as mentioned, some HDACS also target non-histone proteins with one important example, tubulin. To date, only HDAC6 (*5-7*) and Sirt2 (*8*) can use tubulin as a substrate and regulate the balance of tubulin acetylation and deacetylation. These effects play an important role in the microtubule network.

HDAC6, a unique cytoplasmic deacetylase, targets tubulin, HSP90 and cortactin, hence it can regulate cell adhesion, motility and chaperone function (*9*). Another uniqueness of HDAC6 is that it has two homologous tandem catalytic domains, DD1 and DD2 (*10*). Previous work shows that DD2 plays the greatest role for tubulin deacetylase rather than DD1 *in vitro*. The nuclear export signal (NES) and the Ser-Glu-containing tetrapeptide (SE14) (*11,12*) of the HDAC6 functional domains cause it to localize in the cytoplasm (Figure 1) (*14*). In the C-terminal region of HDAC6, there is a unique ubiquitin-binding zinc-finger domain named the ZnF-UBP domain or BUZ domain (*13*).

Some structurally diverse compounds have been considered as HDACS inhibitors, such as TSA (trichostatin A, **3**), SAHA (suberoylanilide hydroxamic acid, **4**), CHAP31 (**1**), TPXB (trapoxin B, **2**), and MS-275 (**5**) (Figure 2). Hydroxamates are usually pan-HDAC inhibitors, such as TSA, SAHA (*15*), and CHAP31 (*16*). Compounds, such as TPXB (*17*) and MS-275 (*18*), with other Zn^{2+} chelating groups are now being studied, and some of these types of HDACS inhibitors are class or even isoform selective.

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Figure 1. Structure of HDAC6.

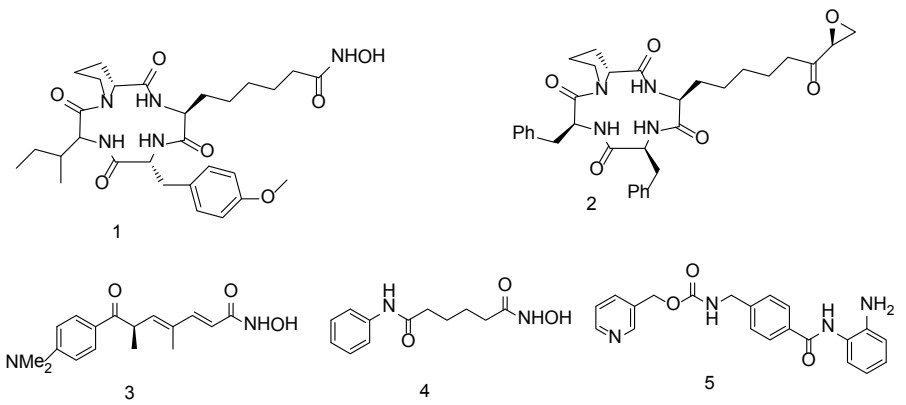


Figure 2. Structure of trichostatin A (TSA, 1), suberoylanilide hydroxamic acid (SAHA, 2), CHAP31 (3), trapoxin B (TPXB, 4), and MS-275 (5).

As pan-HDACS inhibitors often cause side effects, study of selective-inhibitors is necessary. HDAC6 is the only Zinc dependent isoform which deacetylates tubulin (*19*), because of the unique structure and function of HDAC6, there are some selective inhibitors for this subtype, and HDAC6 has been regarded as a therapeutic target for cancer without side effects. HDAC6-selective inhibitors with diverse structures are being studied by chemists.

2. Physiological function

HDAC6 regulates diverse important intracellular biological processes, it effects the growth, migration and death of cells. It also deacetylates HSP90 (heat shockprotein 90), tubulin and cortactin. A recent study found that HDAC6 can also deacetylate peroxiredoxin which is involed in regulation of redox reactions *in vivo* (*20*).

HDAC6 which plays a great role in misfolded protein degradation can be regarded as a target for protein conformational disorders (*21*). As a misfolded protein is harmful, cells can clear away it by the way of a molecular chaperon, ubiqutin-proteasomes system (UPS) and autophagy-lysosome pathway (ALP). For quite a long time, UPS and ALP have been regarded as two parallel degradation pathways, but recent research demonstrates that HDAC6 can form a tripolymer through the Znf-UBP combined with the ubiquitin-misfolded protein and dyneinmotor binding motif (*22*). The tripolymer can be degraded by ALP. So HDAC6 can control misfolded proteins through regulation of ALP and UPS.

HDAC6 is involved in neurological diseases, through some unclear mechanism. It is commonly accepted that HDAC6 affects the occurrence and development of neurological diseases by diverse pathways, such as the formation of aggresomes, autophagy increase, and clearing away misfolded protein.

Neurological diseases consist of Alzheimer disease, Huntingtons disease, Parkinsons disease (PD), and Oculopharyngeal muscular dystrophy which are familiar to us. Related work reveals that PD is related with graceful degradation and death of dopamine (DA) neurons, HDAC6 can promote the formation of α-synuclein complex, and the latter can prevent DA neurons from the damage of oligomers (*23*). Recent research demonstrates that aggresomes combined with misfolded protein is the mainly pathologic feature of neurological diseases. Now we review the definition and function of aggresomes and misfolded protein in neurological diseases. Misfolded protein (*24*) usually forms a poisonous polymerand is one of the elements causing the death of neurons. Aggresome (*25*), a temporary structure, is formed by the remaining misfolded protein without being cleared away. It can prevent cells from poisoning caused by misfolded protein through autophagy, and suspend the occurrence and development of neurological diseases. The formation of aggresome is a specific and active cellular protective response. HDAC6 can regulate either the formation of aggresome or autophagy as a component of aggresomes. Central nervous system (CNS) injury (*26*) is another neurological disease characterized by insufficient axonal regeneration and oxidative stress-induced neurodegeneration (*27,28*). Genetic and pharmacological approaches are employed to demonstrate the role of HDAC6 in CNS injury, and this fact reveals that inhibition of HDAC6 can promote regeneration of neurons in CNS injury (*29*). So HDAC6 can also be regarded as a target for a potential nontoxic therapy of CNS trauma.

Heart failure induced by pulmonary arterial hypertension has been studied recently. Catalytic activity of HDAC6 is consistently increased in myocardium and cultured cardiac myocytes and fibroblasts (*30*). This research describes a role in the heart for HDAC6, and HDAC6 can be regarded as a therapy for heart disease. In addition DNA damage caused by selective inhibition of HDAC6 was also reported recently.

3. Relationship with cancer

HDAC6 participates in tumorigenesis, development and metastasis by way of tubulin, HSP90, invasin and ubiquitin-protein (*14*). HDAC6 is mainly distributed in cytoplasm (*31*), and highly expressed in heart, liver, kidney, and pancreas (*32*).

Research demonstrates that intracellular ubiquitination and deubiquitination of HDAC6 are involved in tumorigenesis. Ubiquitin-HDAC6 with deacetylation activity disturbs normal gene transcription and protein expression in the course of chromosome condensation. So ubiquitination and deubiquitination of HDAC6 are of great importance in therapy of malignancy caused by a disorder of ubiquitination (*32*). HSP90 is an important regulator in cellular signal transduction, Aoyagi (*33*) and coworkers found that HSP90 is directly regulated by HDAC6. A decrease of HDAC6 expression induces acetylation of HSP90 and α-tubulin, then inhibits the combination with HSP90 and ATP, in this course the combination of chaperone and oncogene is reduced. Targeted inhibition of HDAC6 acetylates HSP90, and destroys the function of chaperone (*34*). It is of great importance in biological cancer treatment.

Inhibition of HDAC6 shows a synergistic role with known anticarcinogens in some cancers. Combined implication of bortezomib and tubocurarine chloride inhibiting proteasome and invasin can enhance the therapeutic effect on multiple myeloma (*32*). Estradiol can enhance the expression of mRNA and protein with respect to HDAC6, and remarkably enhance cell motility (*35*). While tamoxifen, a kind of antiestrogen, can obviously inhibit tubulin deacetylation, and consequently reduces cell motility which reveals the relationship of HDAC6 and tumor metastasis (*35*). HDAC6 can be regarded as one of the significant prognostic indicators through estrogen signaling. The therapeutic effect on malignancy can also be enhanced through the combination of Taxanes and endocrinotherapy.

Research shows that HDAC6 also plays a rolein ovarian cancer, ER+ breast cancer, esophageal cancer and gastric cancer. Consequently HDAC6 draws the attention of scientists for cancer therapy without cytotoxicity.

4. Selective inhibitors of HDAC6

To date, there are many structurally diverse HDACs inhibitors synthesized, such as hydroxamic acid derivatives, benzamides, carboxylates (short chain fatty acids), cyclic peptides, and electrophilic ketones. Most of them are pan-inhibitors without selectivity, such as SAHA, tri-chostatin A (TSA), trapoxin B, and MS-275 and are identified as the earliest inhibitors of HDACS. Cytotoxicity causing side-effects for patients is observed during cancer therapy, so isoform-selective inhibitors avoiding severe side-effects have been studied. With its unique physiological function and structure HDAC6 has been regarded as a new target for cancer therapy. With scientists' efforts, such structurally diverse selective inhibitors of HDAC6 have been described. According to the structure of the zinc binding group (ZBG), HDAC6 inhibitors are grouped into five types.

4.1. *Hydroxamic acid*

This type has been studied as the ZBG in the beginning, it is regarded as the most potent inhibitor for cancer therapy. Following the authorization of the first HDAC inhibitor SAHA by FDA, a number of structurally diverse inhibitors of this type have been described.

4.1.1. *Tubacin*

Tubacin (Figure 3) is identified as the first HDAC6 inhibitor which targets tubulin acetylation. It was screened by Haggarty *et al*. (*36,37*) through a high-throughput screening which is a multidimensional chemical genetic process. It is characterized by a 1,3-dioxane structure. To identify its tubulin acetylation and selectivity, Western blot analysis targeting tubulin and histone H3 was carried out with human A549 lung carcinoma cells. The study revealed that tubacin caused a dose-dependent increase of tubulin acetylation compared to H3 acetylation (*39*). It was also proved that tubacin decreased the level of cell motility in lymphocytes and showed (*39*) no effect on stability of microtubules.

A synergistic effect of tubacin and bortezomib was studied in multiple myeloma (MM) cell lines, cytotoxicity was not observed in normal noncancerous peripheral blood mononuclear cells. So a synergistic effect with tubacin and bortezomib is of great significance for cancer therapy.

4.1.2. *Isoxazole containing hydroxamic acids*

A series of new HDAC6 inhibitors characterized by

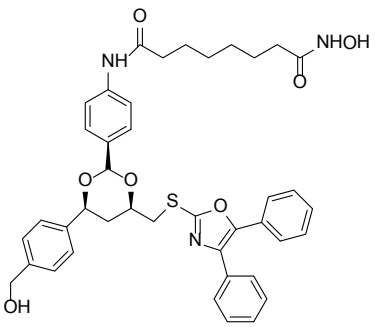


Figure 3. Structure of tubacin.

the CAP group with arylisoxazole (*38*) have been synthesized through the chemistry of nitrile oxide cycloaddition.

The typical compounds of this series consist of **3**, **4**, **7**, **8**, and **11** (Figure 4), the activity of such compounds has been evaluated in order to understand the structure-activity relationship and structure-selective relationship, and their activities have been tested with isolated enzymes and pancreatic cancer cell lines *in vitro* (*38*). The results showed that the best compound **7** displayed an IC_{50} value of 2 pM towards HDAC6 and high selectivity, and its antiproliferative activity is nearly 11-fold more potent than SAHA (*38*). Compound **7** is characterized by a linker of 6-methylene units and a Boc protected on arylisoxazole.

Compounds characterized by only the position of the BOC-protected group different from compound **7** also showed high potency and selectivity. Compound **11** synthesized by replacement of the BOC group with an acetyl group induced a considerable drop in enzyme inhibition activity (*38*). When we compared the activity of compound **4** and **8** with the relative compounds **3** and **7**, the same drop in potency was observed. Above all, the results demonstrate that the bulky lipophilic group and 6-methylene units are of great importance in enzyme assays.

As observed, compound **7** of this series is the most potent and selective for HDAC6 and more potent than SAHA, so it is highly potent for exploring HDAC6 biology and cancer treatment.

4.1.3. *Arylalanine acid*

Arylalanine containing hydroxamic acids have been identified as other HDAC6 selective inhibitors. In order to identify favorable structural elements of this type (*41*), structurally diverse arylalanine containing inhibitors have been synthesized from inhibitor SW55 (*39,40*). In the beginning compound **3** was synthesized, then series 4 (Figure 5) as the analoges of **3** were synthesized. *In vitro* selectivity and activity towards HDAC6 of the **4** series were tested through immunoprecipitated HDACD1 and HDAC6, and compound **4e** with a linker of 7 methylene spacers length and bromophenylalanine **4n** with a 6 methylene linker showed high potency and selectivity, and both compounds were also evaluated by Western blot through histone and tubulin acetylation (*42*). High selectivity of both compounds was revealed with hyperacetylation in the low micromolar range towards tubulin.

Pyridylalanines compound **8b** and phenylalanine compound **8c** (Figure 5) as analogues of compound **4n** have been synthesized for structural elements of this series. Activity and selectivity of them were also evaluated through immunoprecipitated HDACD1 and HDAC6 and Western blot (*42,43*). Both of them showed high selectivity and activity. Configuration of this series has also been studied in pyridylalanines compounds, and the research showed us a higher selectivity in compound **8b** with an S configuration (*42*). The spacer lengh of 6 methylenes was regarded as the best linker for pyridylalanines compounds.

Arylalanine diverse compounds have specific traits for highly potent selectivity towards HDAC6. Bromophenylalanine with a 6 methylene linker, pyridylalanines with a 6 methylene linker and phenylalanine with an 8 methylene linker are considered as the best selective HDAC6 inhibitors. The structure-activity relationship and structure-selectivity relationship have been identified. An azelaic acid spacer, with a 3-heteroaryl substituent were revealed as favorable structural elements. The research reveals a significative way of designing compounds with high potency and selectivity for HDAC6.

4.1.4. *Cyclic tetrapeptides*

Compounds containing cyclic peptides have been studied as inhibitors of HDACS, such as trapoxin B and CHAP31 containing different ZBG. Both of the compounds were tested for HDAC6 selectivity, but results were illusionary. However, research of cyclic peptides are still in the progress. With the effort of chemists, a cyclic tetrapeptide scaffold is used for selective HDAC6 inhibition (*44*). The compound containing the Tyr–Arg motif at aa3–aa4 positions was regared as a leading compound. Further study has been done on the modification of the aa2 position, and compounds **22** and **23** (Figure 6) with different ZBG were synthesized by introducing an Asp residue at the aa2 position, the latter containing hydroxamate is the most potent and selective for HDAC6 in *in vitro* enzyme assays (*44*). Confirmation of the *in vitro* activity of compound **23** was exhibited with the result of higher tubulin acetylation compared to histone 3 (*44*).

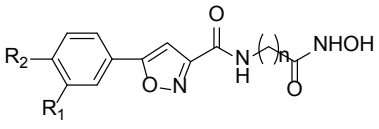


Figure 4. Structure of compounds containing isoxazole and triazole. 3: R1 = NHBOC, R2 = H, n = 6. **4**: R1 = NHBOC, R2 = H, n = 4. **7**: R1 = H, R2 = NHBOC, n = 6. **8**: R1 = H, R2 = NHBOC, n = 4. **11**: R1 = H, R2 = AcNH, n = 6.

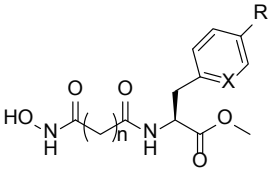


Figure 5. Structures of compounds 3 series and 4 series. Compound **3**: n = 6, R = phenyl, X = H; **4e**: n = 7, R = 3-thienyl, X = H; **4n**: n = 6, R = Br; **8b**: n = 6, X = N, R = -H; **8c**: n = 8, X = CH, R = -H.

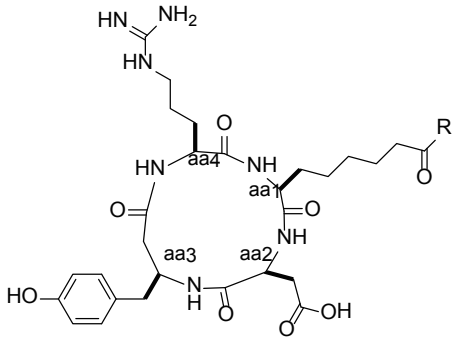


Figure 6. Structure of compound 22 and 23. Compound **22**: R = OH; **23**: R = NHOH.

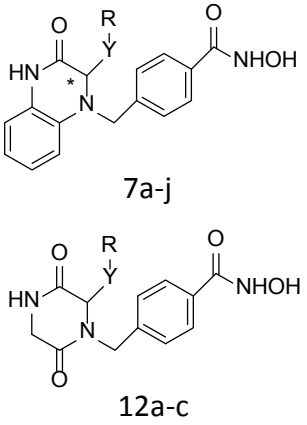


Figure 7. Structure of compounds 7 series and 12 series.

With the appearance of compound **23**, compounds with cyclic tetrapeptides are not non-selective any more. Modification at the aa2 position of the cyclic tetrapeptide structure displayed a promising improvement for HDAC6 selectivity.

4.1.5. *Chiral 3,4-dihydroquinoxalin-2(1H)-one and piperazine-2,5-dione aryl hydroxamates*

Novel 3,4-dihydroquinoxalin-2(1H)-one and piperazine-2,5-dione aryl hydroxamates displaying selectivity and potency for HDAC6 have been designed and synthesized, they are evaluated to have about a 40-fold selectivity for HDAC6 over HDAC1 (*45*). *In vitro* enzyme assays with compounds of the 7 series with dihydroquinoxalin displayed higher potency than the 12 series with piperazine (*45*), the result showed that cap dihydroquinoxalin had a performance increase for greater selectivity than that of series 12.

The structure-selectivity relationship of both series was studied. Achiral compound **7a** displayed no preferential a-tubulin acetylation over H3 acetylation than other chiral compounds (*45*), consequently the chiral center of such compounds is of great importance in selectivity and potency. By comparison with compounds **7b**, **7c**, **7d**, **7e**, **7i**, **7j** and **12a**, **12b**, **12c** (Figure 7), the conclusion is that the most selective and potent compounds with such structural elements are characterized by the configuration of the chiral centre rather than substituent 'R'.

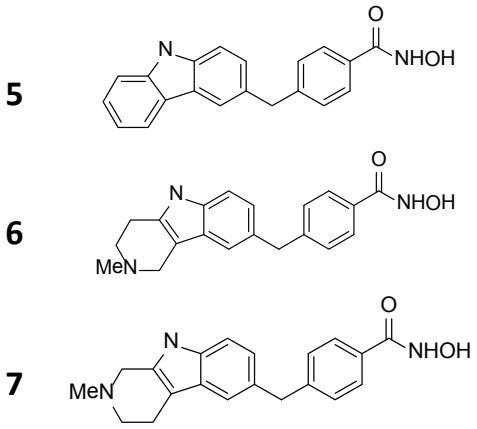


Figure 8. Structure of compound 5, 6, and 7.

Structural features of compounds **7b**, **7d**, **7i**, **7j**, and **12a** can be used for designing more potent selective inhibitors, as well as probing the biology of specific HDAC isoforms.

4.1.6. *The tricyclic inhibitors tubastatin A*

HDAC inhibitors containing a tricyclic structure were reported highly selective for HDAC6. Tubacin (Figure 3), the first HDAC6 selective inhibitor, was usually a probe for biology and selectivity for HDAC6 as a result of its high lipophilicity. A series of compounds with carbazole group and alkylaryl linker were synthesized based on the specificity of HDAC6 with a wider and shallower channel.

In order to find drug-like compounds, the lipophilicity should be decreased with regard to tubacin with high lipophilicity by introducing a tertiary amino on the carbazole of compound **5** (Figure 8), the tertiary amino moiety can form salts improving the solubility of compounds. Compound **6** and **7** respectively characterized with tetrahydro-γ-carboline and tetrahydro-β-carboline were obtained through modification of the tricyclic, both of the compounds displayed higher selectivity for HDAC6 over HDAC1 compared to tubacin (*46*). Compound **6** named tubastatin A displayed an IC_{50} value of 15 nM towards HDAC6 and about 1000-fold selectivity for HDAC6 over HDAC1 (*46*). Tubastatin displayed neuroprotective action in a cell model of oxidative stress and is the first HDAC6 inhibitor with neuroprotection (*46*).

Some brand-name drugs have already been demonstrated that have high selectivity and potency for HDAC6. Bufexamac (Figure 9) usually regarded as a nonsteroidal anti-inflammatory drug was found during a high-throughput adaptation of chemoproteomics for selective HDAC inhibitors. Selective HDAC6 inhibition of Bufexamac was confirmed through tubulin immunofluorescence and Western blot (*47*).

A series of compounds were synthesized through modification at the C3 position of SAHA, these analogues also demonstrated high selectivity for HDAC6 (*48*). Compound **1e** (Figure 9) with a methyl introduced at the C3 position is the most selective and potent of this series.

The existing compounds should never be ignored in probing HDAC6 selective inhibitors, as it may be highly potent for isoform selectivity.

4.2. *Thiol based inhibitors and their ester prodrugs*

Under the attention of selective HDAC6 inhibitors with little side-effects, many researchers devote themselves to finding better compounds for HDAC inhibition, and therefore structurally diverse groups were used for substituent groups for ZBG and CAP in order to study their structural elements required for HDAC6 selective inhibitors. With the effort of Itoh and Suzuk, a series of compounds with thiol (Figure 10) and diverse CAP groups were synthesized for HDAC6 inhibition (*49,50*). It was the first time that compounds containing thiol were used as selective HDAC6 inhibitors. These compounds were evaluated by HDAC1, HDAC4, and HADC6, and their selectivity for HDAC6 was better than tubacin. Compound **11a**, IC_{50} of 23 nM towards HDAC6, was the most potent, and compound **13a**, 46-fold selectivity for HDAC6/HDAC1 and 51-fold selectivity for HDAC6/HDAC4 (*49*), was the most selective. With comparison research, bulky alkyl and tert-butylcarbamate groups are structural elements for selectivity of HDAC6 (*49,50*). As thiol analogues **9b-13b** with thioesters can enhance stability and lipophilicity, compounds **9b-13b** were used for Western blots instead of **9a-13a** in order to confirm activity observed in *in vitro* enzyme assays, and the result showed an increased acetylation of tubulin acetylation rather than histone 4 (*49*).

In a recent study, compound **9a** was used for synthesizing a series of analoges characterized with five methylenes, diverse ZBG, and CAP. The structure-selectivity relationship was investigated for these analoges. Compound **16** (Figure 10) containing hydroxamate with an IC_{50} value of 26 nM towards HDAC6 and 55-fold selectivity for HDAC6/HDAC1 in *in vitro* enzyme assays was the most potent and selective for HDAC6 (*51*). Through comparing and analyzing with other analoges which were structurally different in the ZBG and N-terminus groups, Boc and cyclopentyl groups on CAP were necessary for selective HDAC6 inhibitors containing thiol, and a high affinity ZBG was discovered unfit for isoform selectivity compared with a compound containing carboxyl, thiol, and hydroxamate.

Compounds containing thiol can be converted into thioesters with high stability and lipophilicity, the latter with good pharmacokinetic properties can reduce the paclitaxel dosage in cancer treatment and with less cytotoxicity. All in all, thiol analoges have already been regarded as a new strategy for cancer therapy to avoid side effects.

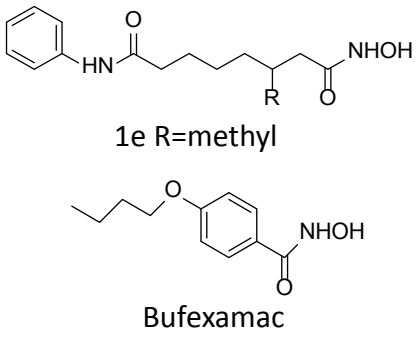


Figure 9. Structure of bufexamac and 1e.

4.3. *Sulfamides*

From the study of Jones *et al*. (*52,53*), we found a potent HDAC inhibitor with a methylketone ZBG. Based on their study, researchers tried to find if compounds with the sulfamide at the e-nitrogen are potent and selective for HDAC6 inhibition. Compounds **14a** and **14b** were synthesized with a lysine scaffold, while compounds **13e** and **13f** were synthesized by removal of the ZBG as linear long-chain-based analoges (Figure 11). Both of the two types of compounds were

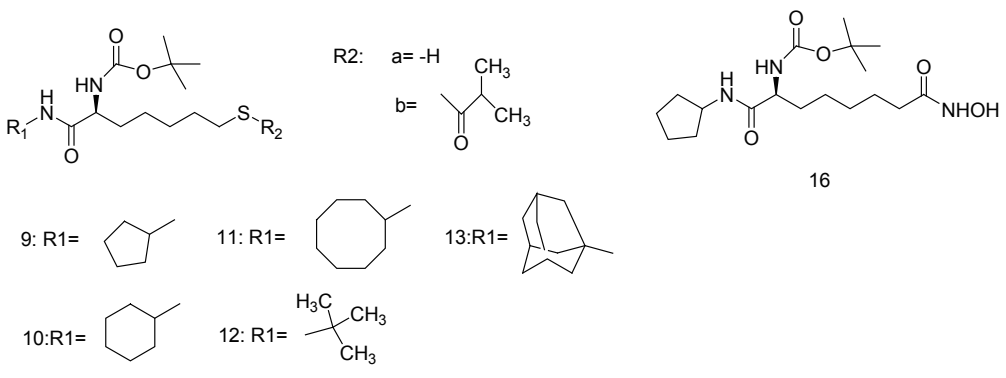


Figure 10. Structures of compounds with thiol.

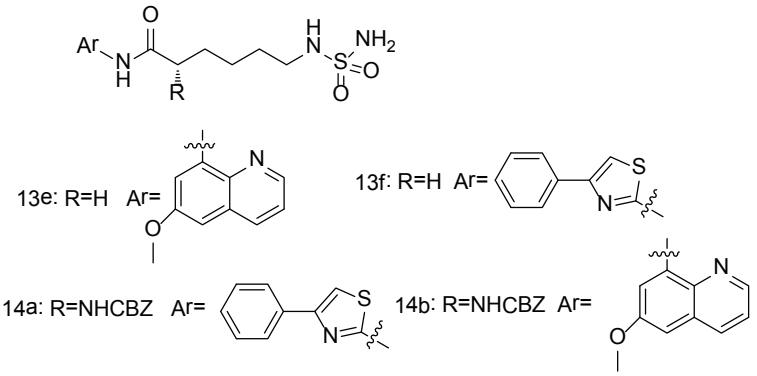
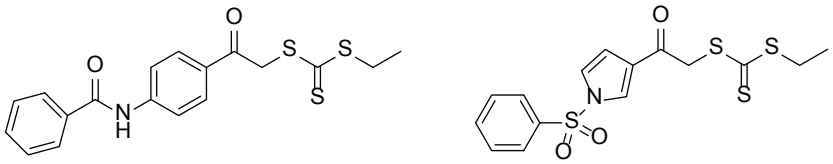


Figure 11. Structures of 13e, 13f, 14a, and 14b.



12ac 13

Figure 12. Structures of compounds 12ac and 13.

evaluated in *in vitro* enzyme assays with HDAC1 and HDAC6.

Compounds **13e** and **13f** showed higher potency for tubulin acetylation than histone 3 confirming enzyme assays for HDAC6 and HDAC1 (*54*). This suggested that compounds with linear long-chains showed obvious selectivity for HDAC6. The most potent compound **14b** exhibited an EC_{50} of 0.35 μM and 0.2 μM towards H3Ac and TubAc respectively (*54*), its activity is similar to that of SAHA.

The observed enzymatic potencies and cellular activities of compounds with a sulfamide moiety showed a new way for designing selective HDAC6 inhibitors which can be characterized with linear long-chains and sulfamide ZBG. Of course if you just want to increase the potency for HDAC6, synthesis of compounds with a lysine scaffold is a good choice.

4.4. *Trithiocarbonates*

As is mentioned above, thiol can be regarded as a ZBG in designing selective HDAC6 inhibitors. Because compounds displayed better activity *in vitro* rather than *in vivo*, scientists usually used thioesters which were analoges of relevant thiols to design more potent and selective HDAC6 inhibitors intracellularly. On that basis thioglycolamides, thiocarboxylates and thiol-substituted acetyls have been used as ZBGs for HDAC inhibitors. Through bioresearch and chemical study, trithiocarbonates are considered as a new ZBG for HDAC inhibitors.

Compound **12ac** (Figure 12) was described as a selective HDAC6 inhibitor of all compounds with trithiocarbonate, and compound **12ac** with a phenylacetyl moiety as the CAP group displayed better selectivity for HDAC6 (IC_{50} of 65 nm) over HDAC1(IC_{50} of 1.22 μM)in *in vitro* enzyme assays (*55*). The selective inhibition was also confirmed with the result that tubulin hyperacetylation (10 μM) was stronger than that of histone 3 (*55*). Compound **13** (Figure 13) structurally characterized with a pyrrole-N-sulfonamide displayed an IC_{50} of 9 nM for HDAC6 and an IC_{50} of 2.1 μM for HDAC1 (*55*).

Up to now such compounds were rarely studied, so compounds with trithiocarbonate as a new ZBG display a significant prospect for selective HDAC6 inhibitors.

4.5. *NQN naphthoquinone*

Recent research revealed an absolutely new selective HDAC inhibitor with a central naphthoquinone structure which didn't conform to the prototype of conventional HDAC inhibitors with CAP, linker and ZBG. The compound named NQN was screened by The Library of Pharmacologically Active Compounds (LOPAC, 1280 compounds) (*56*). As NQN is the central structure of vitamin K, analogues of vitamin K such as vitamin K3, NQN-1, NQN-2, and NQN-3 (Figure 13) were synthesized. In *in vitro* enzymeassays vitamin K3 was evaluated with an IC_{50} at low micromolar levels, NQN-1 was the most selective for HDAC6 with an IC_{50} of 5.54 μM, NQN-2 with one methylene extension of NQN-1 displayed an IC_{50} of 15.6 μM, NQN-3 with the carbonyl removed from NQN-1 was the least potent and selective for HDAC6 of this series of compounds with an IC_{50} > 180 μM (*56*). In AML MV4-11 cells, NQN-1 still induces hyperacetylation of tubulin rather than H3 and H4 (*56*). Interestingly NQN-1 can also induce

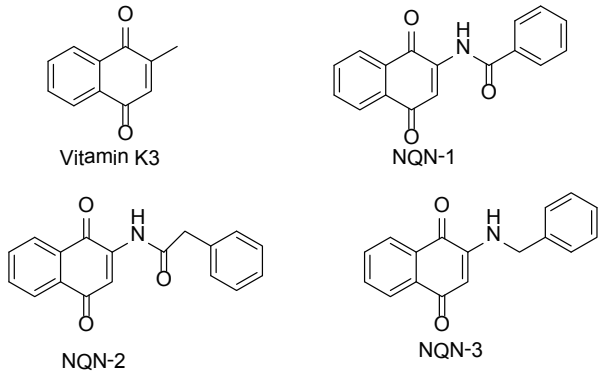


Figure 13. Structure of compounds vitamin K3, NQN-1, NQN-2, and NQN-3.

Hsp90 acetylation and FLT-3 and STAT5 depletions by comparison with SAHA, TSA, and tubastatin A (*56*).

It has been found that the carbonyl which links naphthoquinone and phenyl groups was necessary for HDAC6 inhibition through the inhibitory activity of vitamin K3 and NQN-3, and the larger phenyl group was not required for the NQN-2 result with a one carbon extension showing a less potent HDAC6 selectivity than NQN-1.

5. Summary and outlook

HDAC6 with specific structure and biological function plays a significant role in the carcinogenesis, progression and metastasis of tumors. HDACs inhibitors have been regarded as a new therapy for cancer and have also been used for neurological disorders. With the study of HDAC6 novel structurally diverse compounds were identified as HDAC6 inhibitors, and such new HDAC6 inhibitors didn't conform to the structure of typical HDAC inhibitors. The results show that we should break new ground in designing HDAC6 inhibitors, in the future more potent and selective HDAC6 inhibitors with absolutely new structures will be discovered. As the study of HDAC6 is in an initiation phase, HDAC6 will be a hot topic for cancer therapy.

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