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# Design, synthesis, and preliminary bioactivity studies of substituted purine hydroxamic acid derivatives as novel histone deacetylase (HDAC) inhibitors†

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Histone deacetylase (HDAC) is a clinically validated target for anti-tumor therapy. In order to increase HDAC inhibition and efficiency, we developed a series of novel substituted purine hydroxamic acids as potent HDAC inhibitors. The biological evaluation suggests that compound **5r** ($IC_{50}$ = 0.075 μmol $L^{-1}$) exhibits better HDAC1 and 2 inhibitory activity compared to the approved drug SAHA ($IC_{50}$ = 0.14 μmol $L^{-1}$). Further biological evaluation indicated that compounds **5r**, **5w**, and **5x** have potent anti-proliferative activities against eight tumor cells, including MDA-MB231, KG1, PC3, U937 and so on.

## Introduction

It is well known that cancer is related to genetic and epigenetic events which lead to neoplastic transformation. Scientists have found that multiple stages of tumorigenesis can be influenced by epigenetic alterations. In cancer cells, an imbalance of histone acetylation often exists due to the over-expression of histone deacetylase (HDAC), which leads to silencing of the expression of tumor suppressor genes (such as p21/p53) through hypoacetylation. HDAC inhibitors (HDACi) can reverse the situation and induce cell cycle arrest, differentiation, and apoptosis. HDACi have shown significant clinical benefits for the treatment of cancers. Continued efforts have been devoted to the discovery of competitive HDAC inhibitors for chemotherapeutic intervention.[1–3]

To date, more than 20 HDAC inhibitors are under preclinical and clinical investigation as single agents or in combination therapies against different cancers. Two HDAC inhibitors, Zolinza (vorinostat or SAHA) and Istodax (romidepsin or FK228), have been launched for the treatment of cutaneous T-cell lymphoma. The latter was also approved for peripheral T-cell lymphoma (Fig. 1).[4]

HDAC inhibitors generally conform to a three-motif pharmacophoric model consisting of a surface recognition cap group, a hydrophobic linker, and a zinc-binding group (ZBG) (Fig. 2). The cap group permits extraordinary variability. The linker group can pass through the tunnel of the active site while the zinc binding group (ZBG) chelates the active site zinc ion ($Zn^{2+}$). The hydroxamate moiety is by far the most common ZBG, which is also found in naturally occurring HDAC inhibitors.[2,5,6]

Our group has made great efforts to develop novel HDAC inhibitors by introducing different heterocycles as the cap group to improve their efficacy against HDACs. We have prepared tetrahydroisoquinoline,[7–9] *N*-hydroxy benzamide,[10,11] tyrosine-based hydroxamic acid,[12] and 1,3,4-thiadiazole[13] as potent HDAC inhibitors. Recently, we found that compounds with 1,2-dihydrobenzo[*d*]isothiazol-3-one-1,1-dioxide (saccharin) as the cap group also showed significant inhibition to HDAC.[14] Purine derivatives[15,16] or purine isomers[17] have been incorporated into HDAC inhibitors in a few cases. Systematic investigations have not been conducted on the SAR of HDACi with different substituted purine derivatives as the cap group. Recently, our group reported the anti-tumor activities of some

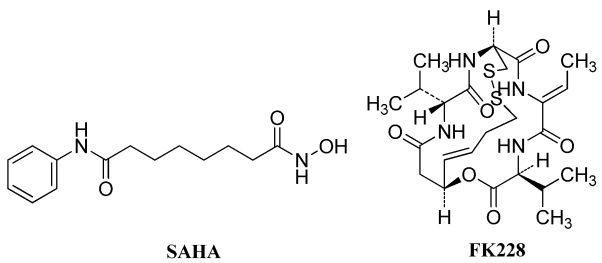


Fig. 1 Approved HDAC inhibitors by the FDA.

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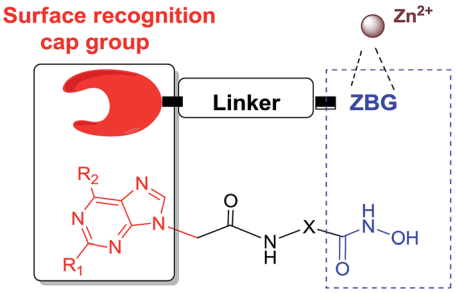
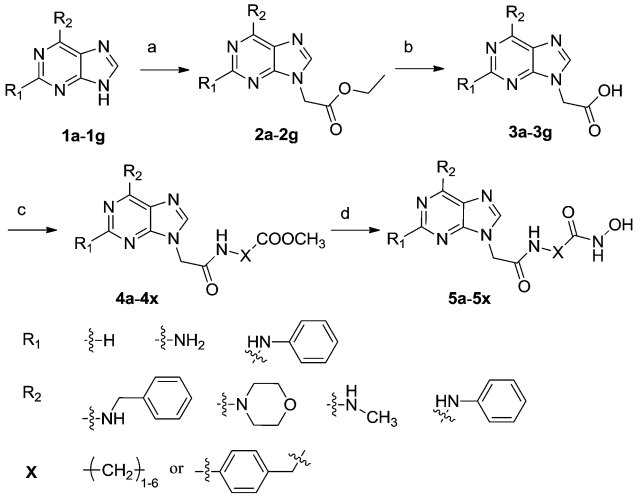



Fig. 2 HDACi pharmacophoric model and purine-based hydroxamic acid derivatives.

substituted purine derivatives.[18] To explore the possibility of synergistic effects, we prepared a series of novel HDACi, where the substituted purine derivatives were linked with the hydroxamic acid moiety, and evaluated the inhibitory activities against HDAC (Fig. 2).




Scheme 1 Reagents and reaction conditions: (a) ethyl chloroacetate, $K_2CO_3$, DMSO, rt; (b) (i) 2 mol $L^{-1}$ NaOH, THF/MeOH = 3 : 1, rt; (ii) 3 mol $L^{-1}$ HCl, $H_2O$, rt; (c) amino acid methyl ester hydrochloride; EDCI, HOBt, TEA, anhydrous DCM, 0 °C – rt; (d) $NH_2OK$, MeOH, rt.

## Chemistry

The synthesis route to our newly designed HDACi is outlined in Scheme 1. Compounds **1a–1g** (ref. 19 and 20) reacted with ethyl chloroacetate under basic conditions at room temperature to obtain **2a–2g**. Compounds **3a–3g** were then prepared after saponification and acidification. Carboxylic acids **3a–3g** were next coupled with various amino acid methyl esters to obtain intermediates **4a–4x**. Finally, hydroxamic acids **5a–5x** were synthesized by reaction with freshly prepared potassium hydroxylamine according to reported procedures.[21]

## Results and discussion

A Color de Lys™ assay (BML–AK501, Enzo® Life Sciences) was used to evaluate the inhibitory activities of purine hydroxamic acids against HDACs which mainly include HDAC1 and HDAC2. The inhibitory results are summarized in Fig. 3. The $IC_{50}$ values of more potent compounds are calculated and tabulated in Table 1.

We found that the compounds with one to three methylene units as the linker showed poor enzymatic inhibition even at 5 μmol $L^{-1}$, such as **5a–5c**, **5e**, **5f**, **5i**, and **5j**. When the linker was phenyl methyl, the compounds (**5h** and **5l**) did not display inhibition to HDAC either. Compounds bearing four to six methylene units as the linker (**5d**, **5g**, and **5k**) showed better inhibitory activities. On the other hand, the substitutions on purine $C_2$ and $C_6$-positions also played an important role in the inhibitory activities. According to the $IC_{50}$ values in Table 1, introducing an aromatic ring in the $C_2$-position of purine could enhance the potency significantly. For example, **5v–5x** ($R_1$ = –NHPh) were better than the corresponding compounds without $C_2$ substitution (**5s–5u**, $R_1$ = H). Compound **5r** ($IC_{50}$ = 0.075 μmol $L^{-1}$) behaved much better than **5k** ($IC_{50}$ = 4.53 μmol $L^{-1}$) and the inhibitory activity increased 60-fold. In addition, aromatic rings on the purine $C_6$-position also increased the inhibitory activities. For example, compounds **5t** and **5d** with a phenyl or benzyl substitution on the purine $N_6$-position showed much better HDAC inhibitory activities compared with

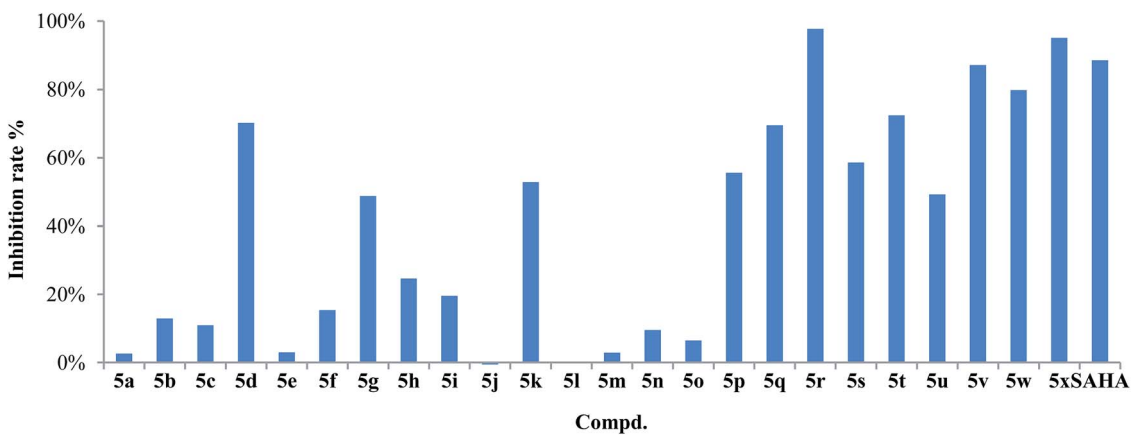



Fig. 3 Inhibition of all compounds against HDAC1 & 2 at 5 μmol $L^{-1}$.

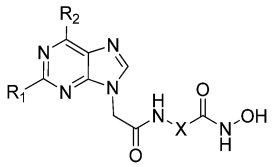
compound **5g** with the same linker ($R_1$ = H, $R_2$ = morpholinyl). The improved activity by the additional aromatic ring substitution on purine $N_2$ or $N_6$-positions may be due to the additional π–π interactions between the compounds and HDAC.

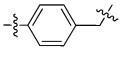
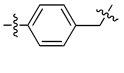
To study the interaction between the inhibitor and HDAC, compound **5r** and SAHA were docked into the active site of HDAC2 (PDB entry: 4LZX) using AutoDock4.2 (ref. 22–26) (Fig. 4). The results suggested that **5r** possessed a similar binding mode to SAHA in the active site of HDAC2. The hydroxamic acid moieties of **5r** and SAHA could both chelate to the $Zn^{2+}$ ion. Interestingly, the oxygen atom on the morpholine moiety could form two hydrogen bonds with Arg275. The amide attaching purine could form another hydrogen bond with Phe210, which further increased the interaction with HDAC. The above interactions could contribute to the higher potency of **5r** against HDAC compared to SAHA.

To further examine the activities of these HDAC inhibitors at the cellular level, the compounds which showed obvious inhibition in enzyme screening assays, were chosen to be evaluated for their anti-proliferative activities against the cancer cell lines. Three tumor cell lines, MDA-MB231, MCF7, and KG1, were tested using MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2*H*-tetrazoliumbromide) assay.

The results showed that most tested compounds displayed obvious anti-proliferative activities against the MDA-MB231 cell line except for **5s** (Table 2). At the same time, compounds **5d**, **5k**, **5s**, **5t**, and **5u** did not work on the KG1 and MCF7 cell lines. The compound **5v** showed weak inhibitory activities to KG1 and no inhibition to the MCF7 cell line. Among all tested compounds, compounds **5r**, **5w**, and **5x** possessed good anti-proliferative activities against all the three tumor cell lines. Therefore, these three compounds were chosen to be evaluated further for their anti-tumor activities against other cancer cell lines including PC3, A549, U937, HeLa cells, and ES-2 cell lines with MTT assay (Table 3). Our results indicate that these three compounds could significantly inhibit the growth of all of these tumor cell lines. Compound **5x** showed low $IC_{50}$ values (less than 10 μmol $L^{-1}$) against all the tumor cell lines. This compound also has similar anti-proliferative activities compared to the positive control. Further studies indicated that compounds **5r**, **5w**, and

Table 1 The structures and HDAC1 & 2 inhibitory activities of all compounds



| | $R_1$ | $R_2$ | X | Inhibition at 5 μmol $L^{-1}$ | $IC_{50}$[a] (μmol $L^{-1}$) | | $R_1$ | $R_2$ | X | Inhibition at 5 μmol $L^{-1}$ | $IC_{50}$[a] (μmol $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **5a** | –H | $-NHCH_2Ph$ | $-CH_2-$ | 3% | Nd | **5m** | –H | $-NHCH_3$ | $-CH_2-$ | 3% | Nd |
| **5b** | –H | $-NHCH_2Ph$ | $-(CH_2)_2-$ | 13% | Nd | **5n** | –H | $-NHCH_3$ | $-(CH_2)_2-$ | 10% | Nd |
| **5c** | –H | $-NHCH_2Ph$ | $-(CH_2)_3-$ | 11% | Nd | **5o** | –H | $-NHCH_3$ | $-(CH_2)_3-$ | 6% | Nd |
| **5d** | –H | $-NHCH_2Ph$ | $-(CH_2)_5-$ | 70% | 2.05 ± 0.03 | **5p** | –NHPh |  | $-(CH_2)_2-$ | 56% | >5 |
| **5e** | –H |  | $-(CH_2)_2-$ | 3% | Nd | **5q** | –NHPh |  | $-(CH_2)_3-$ | 69% | >5 |
| **5f** | –H |  | $-(CH_2)_3-$ | 15% | Nd | **5r** | –NHPh |  | $-(CH_2)_5-$ | 98% | 0.075 ± 0.014 |
| **5g** | –H |  | $-(CH_2)_5-$ | 49% | >5 | **5s** | –H | –NHPh | $-(CH_2)_4-$ | 59% | 2.51 ± 0.28 |
| **5h** | –H |  |  | 25% | Nd | **5t** | –H | –NHPh | $-(CH_2)_5-$ | 72% | 2.09 ± 0.24 |
| **5i** | $-NH_2$ |  | $-(CH_2)_2-$ | 20% | Nd | **5u** | –H | –NHPh | $-(CH_2)_6-$ | 49% | 2.94 ± 0.06 |
| **5j** | $-NH_2$ |  | $-(CH_2)_3-$ | −1% | Nd | **5v** | –NHPh | –NHPh | $-(CH_2)_4-$ | 87% | 0.59 ± 0.03 |
| **5k** | $-NH_2$ |  | $-(CH_2)_5-$ | 53% | 4.53 ± 0.33 | **5w** | –NHPh | –NHPh | $-(CH_2)_5-$ | 80% | 0.30 ± 0.06 |
| **5l** | $-NH_2$ |  |  | 0% | Nd | **5x** | –NHPh | –NHPh | $-(CH_2)_6-$ | 95% | 0.51 ± 0.10 |
| SAHA | | | | 88% | 0.14 ± 0.02 | | | | | | |

[a] All of the compounds were assayed three times and expressed with standard deviations; Nd: not detected.

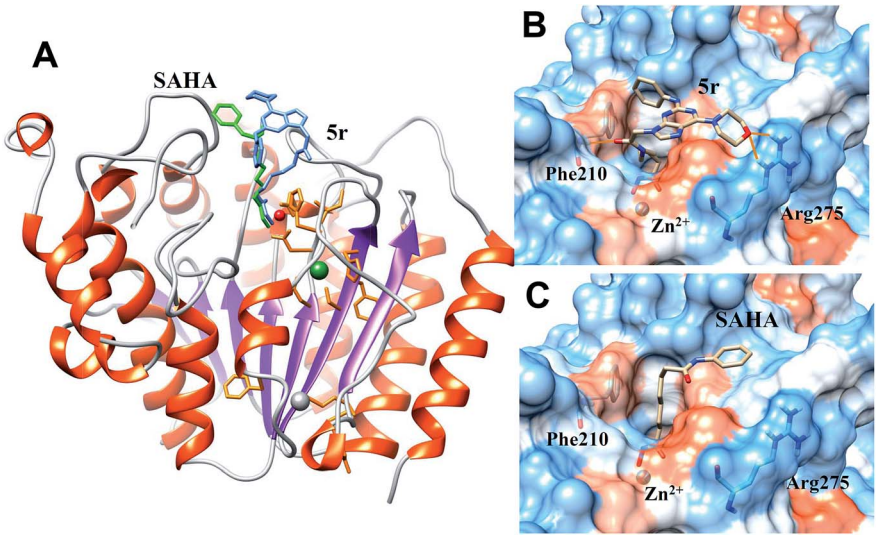



Fig. 4 (A) The docking modes of compound **5r** (depicted in blue) and SAHA (depicted in green) in the active site of HDAC2; (B) proposed binding preference of compound **5r**; (C) proposed binding preference of SAHA. Molecular graphics and analyses were performed with the UCSF Chimera package.[27]

Table 2 Anti-proliferative activities of representative compounds to cancer cell lines: MDA-MB231, MCF7, and KG1

| | Anti-proliferative activities[a] (μmol $L^{-1}$) | | |
|---|---|---|---|
| Compd | MDA-MB231 | KG1 | MCF7 |
| **5d** | 34.18 ± 3.16 | >50 | >50 |
| **5k** | 41.32 ± 7.81 | Nd | >50 |
| **5r** | 1.56 ± 0.28 | 5.37 ± 0.25 | 18.63 ± 1.92 |
| **5s** | >50 | >50 | >50 |
| **5t** | 22.29 ± 6.13 | >50 | >50 |
| **5u** | 8.91 ± 0.99 | >50 | >50 |
| **5v** | 11.07 ± 0.35 | 34.74 ± 1.05 | >50 |
| **5w** | 1.61 ± 0.13 | 3.80 ± 0.25 | 9.58 ± 0.65 |
| **5x** | 1.55 ± 0.35 | 2.88 ± 0.71 | 9.04 ± 0.65 |
| SAHA | 1.11 ± 0.43 | 1.11 ± 0.16 | 3.26 ± 0.70 |

[a] Each value is reproduced in three independent assays and expressed with standard deviations; Nd: not detected.

**5x** showed similar inhibition to HDAC1 and HDAC8, but exhibited 8–18 fold enhanced activity against HDAC6 compared with SAHA (Table 4).

Table 4 *In vitro* inhibitory activities to HDAC subtypes of compounds **5r**, **5w**, and **5x**

| | $IC_{50}$ (μmol $L^{-1}$) | | | |
|---|---|---|---|---|
| Compd | HDAC1 & 2 | HDAC1[a] | HDAC6[a] | HDAC8[a] |
| **5r** | 0.075 | 0.035 | 0.0026 | 0.15 |
| **5w** | 0.30 | 0.028 | 0.0038 | 0.20 |
| **5x** | 0.51 | 0.063 | 0.0018 | 0.32 |
| SAHA | 0.14 | 0.051 | 0.032 | 0.23 |

[a] Values are the mean values of two determinations.

## Conclusions

Based on the classical pharmacophore structure model of HDAC inhibitors, a total of 24 new substituted purine hydroxamic acid derivatives were designed, synthesized, and evaluated as HDAC inhibitors. The preliminary enzyme assay showed that most compounds can effectively inhibit HDAC1 and 2. The potent compounds, such as **5r**, **5w**, and **5x**, showed

Table 3 Anti-proliferative activities of compounds **5r**, **5w**, and **5x**

| | Anti-proliferative activities[a] (μmol $L^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Compd | MDA-MB231 | KG1 | MCF7 | PC3 | A549 | U937 | HeLa | ES-2 |
| **5r** | 1.56 ± 0.28 | 5.37 ± 0.25 | 18.63 ± 1.92 | 6.44 ± 1.37 | 46.11 ± 3.76 | 6.50 ± 1.93 | 18.23 ± 2.63 | 28.02 ± 0.22 |
| **5w** | 1.61 ± 0.13 | 3.80 ± 0.25 | 9.58 ± 0.65 | 2.14 ± 0.52 | 18.00 ± 2.73 | 4.07 ± 0.97 | 9.08 ± 0.05 | 13.44 ± 2.17 |
| **5x** | 1.55 ± 0.35 | 2.88 ± 0.71 | 9.04 ± 0.65 | 4.26 ± 1.12 | 10.12 ± 1.87 | 3.91 ± 0.59 | 8.76 ± 1.16 | 8.95 ± 1.21 |
| SAHA | 1.11 ± 0.43 | 1.11 ± 0.16 | 3.26 ± 0.70 | 1.42 ± 0.17 | 4.97 ± 0.54 | 4.33 ± 2.06 | 6.07 ± 2.25 | 4.66 ± 0.61 |

[a] Each value was reproduced in three independent assays and expressed with standard deviations.

obvious anti-proliferative activities in eight cancer cell lines. In addition, the three compounds also had high affinity and good selectivity to HDAC6 compared with SAHA. The results suggested that 2,6-disubstituted purine hydroxamate derivatives could be used as lead compounds to develop novel anti-cancer agents.

## Acknowledgements

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