INTRODUCTION

HDACs have emerged as typical effective therapeutic targets for anticancer agents. To date, five HDAC inhibitors have been approved for cancer therapy and many more in different phases of clinical trials for diverse indications (Chen et al., 2018; Rahman et al., 2016; Zang et al., 2018). The hydroxamic acid-based compounds are among those HDAC inhibitors, which received the widely studies (Mottamal, Zheng, Huang, & Wang, 2015). Among them, Pracinostat is a promising compound, which has been granted breakthrough therapy designation by FDA for the treatment of patients with newly diagnosed acute myelocytic leukemia (AML) who are ≥75 years of age or unfit for intensive chemotherapy (Abaza et al., 2017; Garcia-Manero et al., 2017). Currently, Pracinostat is undergoing phase III clinical trials (Abaza et al., 2017; Ganai, 2016; Garcia-Manero et al., 2017; Kang et al., 2017; Novotny-Diermayr et al., 2012).

In preclinical animal models, Pracinostat is a potent pan-HDAC inhibitor with favorable pharmacokinetic properties (Ning et al., 2013; Razak et al., 2011). As one of the inventors, the corresponding author of this article has witnessed the broad and thoroughly structure–activity relationships based on the scaffold of Pracinostat (Wang et al., 2009, 2011). However, no examples were reported in the literature since about the synthesis and bioevaluation of Pracinostat derivatives with $N$-hydroxyacrylamide attached to the 6-position of benzimidazole ring, from our hands or from any other groups (Figure 1). Thus, we put ourselves to design, synthesize, and compare their differences in biological activities in vitro.

EXPERIMENTS AND METHODS

The aim of this study is to first access whether similar compounds with only structural position changes could induce any differences in their enzymatic and cell-based inhibitory activities. Secondly, we would investigate their corresponding signaling pathways (Figure 2). To our knowledge, a typical HDAC inhibitor should be able to increase the expression of Ac-H3, Ac-H4, and Ac-α-tubulin, in addition to their corresponding enzymatic and cell-based inhibitory activities.
2.1 | General procedure for the synthesis of target compounds 2a–2p

The synthesis of target compounds 2 are shown in Scheme 2. 3-Chloro-4-nitrobenzoic acid was selectively reduced by borane-THF under room temperature and 4 thus obtained in high yield. Compound 6 could be obtained from 4 by managed oxidation and Horner-Wadsworth-Emmons reaction (Ando & Yamada, 2011). Amination of ethyl (E)-3-(3-chloro-4-nitrophenyl) acrylate 6 with amine \( R^1\text{NH}_2 \) under basic conditions gave the intermediates 7a–7h. Heating a mixture of the 7, \( R^2\text{CHO} \) and \( \text{SnCl}_2\cdot2\text{H}_2\text{O} \) in \( \text{AcOH}/\text{MeOH} \) (1:9) at 45°C overnight led to the desired compounds 8a–8p. Finally, treatment of the intermediate 8 with excessive hydroxylamine hydrochloride in sodium methoxide/methanol gave the objective hydroxamates 2 (Scheme 1).

The synthetic route for the Pracinostat is shown in Scheme 2, which was prepared from ready available 4-chloro-3-nitrocinnamic acid, 9.

2.2 | Molecular docking

The crystal structure of human HDAC homolog (PDB code: 1C3R) was retrieved from the Protein Data Bank. All target compounds were docked in the active sites by MOE. Both HDAC homolog and ligand were structurally optimized prior to the actual docking simulation. Prior to the docking, the receptor was pretreated according to the standard procedure.

**SCHEME 1** | Reagents and conditions: (a) THF-BH3 (1 equiv), THF, rt, yield 98%; (b) Dess-Martin periodinane (1.1 equiv), DCM, 0°C, yield 98%; (c) triethyl phosphonoacetate (1.5 equiv), DBU (0.1 equiv), K2CO3 (2 equiv), H2O (8 equiv), THF, rt, yield 90%–96%; (d) K2CO3 (3 equiv), dioxane, 85°C, yield 65%–70%; (e) SnCl2·2H2O (5 equiv), AcOH/MeOH (1:9), 45°C, yield 47%–55%; (f) NH2OH·HCl (10 equiv)/NaOMe (20 equiv)/MeOH, 0°C to rt, yield 77%–83%
provided by MOE and energy minimized using the Amber-99 force field. The standard protocol implemented in MOE was performed in docking calculations. The binding modes were analyzed by 2D and 3D tool as well as SiteView of the MOE software.

2.3 | Biology

2.3.1 | HDAC inhibitory assay

HDAC inhibition was detected using the Amplite™ Fluorimetric HDAC Activity Assay Kit (Green Fluorescence, AAT Bioquest®, Inc), Hela nuclear extract (BioVision), and HDAC6 (BPS Bioscience) following the manufacturer’s protocol. The assay was performed in a volume of 25 μl at 37°C in 384-well white plates. The final components of the assay ingredients were 10 μl enzyme solution, 2.5 μl test compounds, and 12.5 μl HDAC Green™ Substrate. The compounds were dissolved in dimethyl sulphoxide (DMSO) at a concentration of 10 mM and diluted with medium at different concentrations before use. All compounds were dissolved in DMSO to make a stock solution at 10 mM and diluted with medium at 5% carbon dioxide. Cells plated in 96-well plates were treated with Pracinostat or Pracinostat derivatives for 72 hr at 37°C and then incubated with MTT for 4 hr. After MTT removal, 150 μl DMSO was added into each well and the absorption values were measured at 490 nm using a Cytation 5 imaging reader (Bio Tek Instruments. Inc. USA). The IC₅₀ values were obtained by Prism GraphPad Prism v.5 software.

2.3.2 | Cell proliferation assay

The antiproliferative activity of synthesized compounds 2a–2p was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. Four human cancer cell lines (HCT-116, A549, MCF-7, and SW-1990) were treated for 72 hr with various concentration of the isolated compounds. All cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were kept at 37°C in a humidified incubator with 5% carbon dioxide. All compounds were dissolved in DMSO to make a stock solution at 10 mM and diluted with medium at 4°C. The supernatants were collected, and protein concentration was quantified by a BCA Protein Assay Kit (Solarbio). Twenty-five micrograms of proteins were fractionated by SDS-PAGE (8%–14% gradient gels), electrophoresed, and transferred on to a PVDF membrane and blocked with 5% non-fat milk in TBS for at least 1 hr. The membranes were incubated with the primary and secondary antibodies and detected using the ChemiDoc XRS+ Gel Imaging System (Bio-Rad). Primary antibodies against human acetyl-histone H3 and acetyl-histone H4 were obtained from Cell Signaling Technology. Acetyl α-tubulin antibodies were purchased from Abbkine, Inc.
3 | RESULTS AND DISCUSSION

All the structures of 2a–2p and key intermediates were characterized by $^1$H NMR, $^{13}$C NMR, and ESI-MS and in full agreement with the proposed structures. All above spectra are available in Supplemental Material.

Docking compound 2a into the histone deacetylase-like protein (HDL, homology of HDAC. PDB code: 1C3R) by MOE software (Figure 3), which revealed that the structural moiety of N-hydroxyacylamide of the target compound 2a interacted with the residues in the active sites of HDAC homology model in a similar way as that of Pracinostat, while the rest of the substituents at position-1 and position-2 are quite different. As illustrated in Figure 2a, the N-hydroxyacylamide group of the Pracinostat and 2a has three H-bond interactions with the residues Tyr297, Gly140, and His132 in the ligand-binding pocket, respectively. The length of the hydrogen bonds formed between the N-hydroxyacylamide group of the Pracinostat with the residues Tyr297, Gly140, and His132 are 2.46, 2.68, and 2.55 Å, respectively, and the corresponding hydrogen bond energies are −1.4, −1.1, and −0.9 kcal/mol. Similarly, the length of the hydrogen bonds formed between the N-hydroxyacylamide group of 2a with the residues Tyr297, Gly140, and His132 are 2.81Å, 2.59Å, and

| Table 1 | HDAC inhibitory activities of prepared compounds |
|---|---|---|---|
| Compound | $R^1$ | $R^2$ | Inhibition of HDAC |
| | | | pan-HDAC | at 20 μM (%) | HDAC6 IC_{50} (μM) |
| Pracinostat, 1 | — | — | 98.87 | 0.14 ± 0.03 | 0.10 ± 0.03 |
| 2a | | | 60.21 | 2.94 ± 0.58 | 1.67 ± 0.08 |
| 2b | | | 42.84 | >20 | 1.89 ± 0.11 |
| 2c | | | 63.45 | 7.96 ± 0.83 | 2.18 ± 0.05 |
| 2d | | | 27.98 | >20 | 2.06 ± 0.01 |
| 2e | | | 65.85 | 9.62 ± 1.46 | 1.03 ± 0.04 |
| 2f | | | 36.19 | >20 | 1.43 ± 0.10 |
| 2g | | | 81.28 | 2.32 ± 0.29 | 1.31 ± 0.01 |
| 2h | | | 76.29 | 4.42 ± 0.55 | 0.97 ± 0.01 |
| 2i | | | 59.09 | 7.53 ± 0.95 | 1.11 ± 0.02 |
| 2j | | | 53.97 | 5.92 ± 0.82 | 1.58 ± 0.02 |
| 2k | | | 60.33 | 15.72 ± 0.06 | 1.30 ± 0.11 |
| 2l | | | 55.19 | 14.83 ± 3.75 | 1.24 ± 0.08 |
| 2m | | | 62.78 | 10.16 ± 1.05 | 1.28 ± 0.07 |
| 2n | | | 54.77 | 12.99 ± 1.10 | 1.46 ± 0.10 |
| 2o | | | 79.95 | 2.57 ± 0.41 | 1.27 ± 0.01 |
| 2p | | | 72.67 | 3.15 ± 1.84 | 0.99 ± 0.001 |

*The IC_{50} values represent an average of triplicate experiments. *$^{11}$IC_{50} values ± SD.
and 2.69 Å, respectively, and the corresponding hydrogen bond energies are −1.0, −2.5, and −3.3 kcal/mol. Notably, the substituent in the 1-position of benzimidazole ring of Pracinostat forms a hydrogen bond with the residue Tyr91 (N–H separation = 2.62 Å, hydrogen bond energies = −0.7 kcal/mol), whereas the compound 2a does not establish this interaction.

The enzyme inhibition assay results showed that all the prepared compounds (2a–2p) exhibited significantly weaker Pan-HDAC inhibitory activities than Pracinostat (Table 1). Specifically, the IC_{50} values of the prepared compounds tested against HDAC6 varied from 0.97 to 2.18 μM, higher than that of Pracinostat (IC_{50} = 0.10 μM).

The compounds 2a–2p were evaluated for their cellular potency against HCT-116, MCF-7, A549, and SW1990 cell lines in comparison with Pracinostat. Consistent with the results of the HDAC enzyme activity, all the target compounds showed poorer antiproliferative activities than Pracinostat, as listed in Table 2. In all cell lines tested, Pracinostat showed potent antiproliferative activities with IC_{50} values ranging from 0.32 to 0.52 μM. Compounds 2a–2p showed weak inhibitory activity against SW1990 and MCF-7 while seemed almost lost activity against A549 and HCT-116 cells.

Compound 2a was chosen for further investigation in comparison with Pracinostat. The acetylation of histone 3 (Ac-H3), histone 4 (Ac-H4), and α-tubulin (Ac-α-tubulin) was measured in HCT-116. The experiments (Figure 4) demonstrated that compound 2a did not increase the acetylation level of histone H3, histone H4, as well as α-tubulin. The results corresponded to the enzymatic and cell-based assays.

4 | CONCLUSIONS

In summary, we have demonstrated that the attached position of N-hydroxyacrylamide of Pracinostat is vital important for the HDAC inhibitory activities. The N-hydroxyacrylamide
attached to the position-5 related compounds such as Pracinostat is a potent HDAC inhibitor with acetylation levels increased significantly for H3, H4, and α-tubulin. However, all these activities dramatically decreased even lost when N-hydroxyacrylamide was attached to the position-6 of the benzimidazole ring. These results were also consisted with the cell-based inhibitor activities. Docking results reveals that the hydrogen bond interaction between amino unit of the side chain at position 1 (Figure 1) and the hydroxyl group of the tyrosine (Tyr91) plays an important role in HDAC inhibition by Pracinostat. Thus, minor structural modifications might produce big changes in its biological responses and we hope this observation might be useful in the arena of drug hunting.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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REFERENCES

Abaza, Y. M., Kadia, T. M., Jabbour, E. J., Konopleva, M. Y., Borthakur, G., Ferrajoli, A., … Garcia-Manero, G. (2017). Phase 1 dose escalation multicenter trial of Pracinostat alone and in combination with azacitidine in patients with advanced hematologic malignancies. Cancer, 123, 4851–4859. https://doi.org/10.1002/cncr.30949

Ando, K., & Yamada, K. (2011). Highly E-selective solvent-free Horner–Wadsworth–Emmons reaction catalyzed by DBU. Green Chemistry, 13, 1143–1146. https://doi.org/10.1039/c1gc15134g

Chen, W., Dong, G. Q., Wu, Y., Zhang, W. N., Miao, C. Y., & Sheng, C. Q. (2018). Dual NAMPT/HDAC inhibitors as a new strategy for multitargeting antitumor drug discovery. ACS Medicinal Chemistry Letters, 9, 34–38. https://doi.org/10.1021/acsmedchemlett.7b00414

Ganai, S. A. (2016). Histone deacetylase inhibitor pracinostat in doublet therapy: A unique strategy to improve therapeutic efficacy and to tackle herculean cancer chemoresistance. Pharmacological Biology, 54, 1926–1935. https://doi.org/10.3109/1380209.2015.1135966

Garcia-Manero, G., Montalban-Bravo, G., Berdeja, J. G., Abaza, Y., Jabbour, E., Essell, J., … Roboz, G. J. (2017). Phase 2, randomized, double-blind study of pracinostat in combination with azacitidine in patients with untreated, higher-risk myelodysplastic syndromes. Cancer, 123, 994–1002. https://doi.org/10.1002/cncr.30533

Kang, S. W., Lee, S. M., Kim, J. Y., Kim, S. Y., Kim, Y. H., Kim, T. H., … Seo, S. K. (2017). Therapeutic activity of the histone deacetylase inhibitor SB939 on renal fibrosis. International Immunopharmacology, 42, 25–31. https://doi.org/10.1016/j.intimp.2016.11.008

Mottamal, N., Zheng, S., Huang, T. L., & Wang, G. (2015). Histone deacetylase inhibitors in clinical studies as templates for new anti-cancer agents. Molecules, 20, 3898–3941. https://doi.org/10.3390/molecules2003898

Ning, C. Q., Bi, Y. J., He, Y. J., Huang, W. Y., Liu, L. F., Li, Y., … Yu, N. F. (2013). Design, synthesis and biological evaluation of disubstituted cinnamic hydroxamic acids bearing urea/thiourea unit as potent histone deacetylase inhibitors. Bioorganic & Medicinal Chemistry Letters, 23, 6432–6435. https://doi.org/10.1016/j.bmcl.2013.09.051

Novotny-Diermayr, V., Hart, S., Goh, K. C., Cheong, A., Ong, L.-C., Hentze, H., … Wood, J. M. (2012). The oral HDAC inhibitor pracinostat (SB939) is efficacious and synergistic with the JAK2 inhibitor pacritinib (SB1518) in preclinical models of AML. Blood Cancer Journal, 2, e69. https://doi.org/10.1038/bcj.2012.14

Rahman, M. S., Jamil, H. M., Akhtar, N., Islam, R., Awal, S. M. A., Rana, M. M., & Asaduzzaman, S. M. (2016). Cancer epigenetics and epigenetical therapy. Journal of Experimental and Integrative Medicine, 6, 143–150. https://doi.org/10.5455/jeim.270616.rw.016

Razak, A. R. A., Hotte, S. J., Siu, L. L., Chen, E. X., Hirte, H. W., Powers, J., … Eisenhauer, E. A. (2011). Phase I clinical, pharmacokinetic and pharmacodynamic study of SB939, an oral histone deacetylase (HDAC) inhibitor, in patients with advanced solid tumours. British Journal of Cancer, 104, 756–762. https://doi.org/10.1038/bjc.2011.13

Wang, H. S., Yu, N. F., Chen, D. Z., Lee, K. C. L., Lye, P. L., Ethirajulu, K., & Sun, E. T. (2011). Discovery of (2E)-3-{[2-Butyl-1-[(2-di-ethylamino)ethyl]-1Hbenzimidazol-5-yl]-N-hydroxyacrylamide (SB939), an Orally Active Histone Deacetylase Inhibitor with a Superior Preclinical Profile. Journal of Medicinal Chemistry, 54, 4694–4720. https://doi.org/10.1021/jm2003552

Wang, H. S., Yu, N. F., Song, H. Y., Chen, D. Z., Zou, Y., Deng, W. P., … Entzeroth, M. (2009). N-Hydroxy-1,2-disubstituted-1H-benzimidazol-5-yl acrylamides as novel histone deacetylase inhibitors: design, synthesis, SAR studies, and in vivo antitumor activity. Bioorganic & Medicinal Chemistry Letters, 19, 1403–1408. https://doi.org/10.1016/j.bmcl.2009.01.041

Zang, J., Liang, X. C., Huang, Y. G., Jia, Y. P., … Zhang, Y. J. (2018). Discovery of novel pazopanib-based HDAC and VEGFR dual inhibitors targeting cancer epigenetics and angiogenesis simultaneously. Journal of Medicinal Chemistry, 61, 5304–5322. https://doi.org/10.1021/acs.jmedchem.8b00384

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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