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# Identification of new quinic acid derivatives as histone deacetylase inhibitors by fluorescence-based cellular assay

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ABSTRACT

A fluorescence-based cellular assay system was established to identify potential epigenetic modulator ligands. This assay method is to detect the de-repression of an EGFP reporter in cancer cells by the treatment of HDAC (histone deacetylase) or DNMT (DNA methyltransferase) inhibitor. Using this system, we conducted a preliminary screening of in-house natural product library containing extracts and pure compounds, and identified several active compounds. Among them, novel quinic acid derivatives were recognized as excellent HDAC inhibitors by both enzymatic and cell-based HDAC assays.



Epigenetic modulation comes from interaction between various enzymes, such as HDAC (histone deacetylase), HAT (histone acetyltransferase), DNMT (DNA methyltransferase), HMT (histone methyltransferase), and etc, and these interactions depend on the balance of the presence of the enzymes.[1] Particularly, the acetylation of histone plays an important role in the epigenetic regulation of the cell. The acetylation of histone will be increased or decreased, depending on the interactivity between histone acetyl-transferases (HATs) and HDACs. Although these enzymes are best of description for regulation of chromatin architecture, in the recent days, general control mechanisms involving various protein complexes are being reported.[2,3] A synthetic hydroxamate HDAC inhibitors (SAHA,[4] belinostat,[5] and panobinostat[6]), and the natural product cyclic depsipeptide romidepsin,[7] have already been approved for the treatment of cancer. Such successful cases lead many scientists to be interested in developing drugs targeting epigenetic pathways.

In our study, to identify potential HDAC or DNMT inhibitors by an efficient fluorescence imaging-based screening method, we selected the cell line in which EGFP expression is repressed by DNMT and/or HDAC (Fig. 1).[8,9] This method is established based on the hypothesis that methylated DNA is specifically recognized by methyl CpG-binding domain (MBD) proteins which can recruit transcriptional co-repressors, including HDAC.[10] When these cells are treated with HDAC or DNMT inhibitors, the EGFP expression is derepressed.[9] This method is useful to preliminarily screen out candidate epigenetic modulators in cells.[12] The hit compounds identified from this assay will be further tested by enzymatic HDAC or DNMT assay to confirm the activity.

In-house 200 natural product compounds and 103 extracts were tested against EGFP-repressed c127LT cells,[13] and among them, 13 compounds and 18 extracts reactivated the EGFP expression. The fluorescence-based assay result for RLE12 (3,5-*O*-*trans*-dicaffeoylquinic acid methyl ester), one of the representative hit compound in Figure 2, is demonstrated in Table 1, in comparison with TSA[14] and MS275.[15] Then, enzymatic[16] and cell-based HDAC assays[17] were conducted to evaluate the HDAC inhibition activities of 13 hit compounds, and only 5 compounds showed micro molar HDAC inhibitory activities. Figure 3a demonstrates the results of enzymatic HDAC assay for the most active hit RLE12,[18–20] and Figure 3b illustrates cell-based HDAC assay. To confirm the binding of RLE12 in the substrate site for HDAC, an enzyme kinetic assay was conducted in comparison with TSA, and the Lineweaver–Burk plot revealed that RLE12 engages in competitive inhibition against acetylated lysine substrate (Fig. S2 in Supplementary material). As a result, RLE12 is identified as HDAC inhibitor with $IC_{50}$ value of 4.99 μM, which is comparable to well-known nonhydroxamate HDAC inhibitor MS275 ($IC_{50}$ = 6.73 μM in enzymatic assay).[15] RLE12 is also nonhydroxamate HDAC inhibitor, a methyl quinate derivative having both 3- and 5-hydroxy group connected with caffeic acid. The structurally related natural product compounds were reported to have mild HDAC inhibition activity, such as caffeic acid ($IC_{50}$ = 2.54 mM) and chlorogenic acid ($IC_{50}$ = 375 μM), and DNMT inhibition activity.[11,21,22] Next, we also tested other quinic acid derivatives (chlorogenic acid, RLE30, and RLE13)[18,20] by

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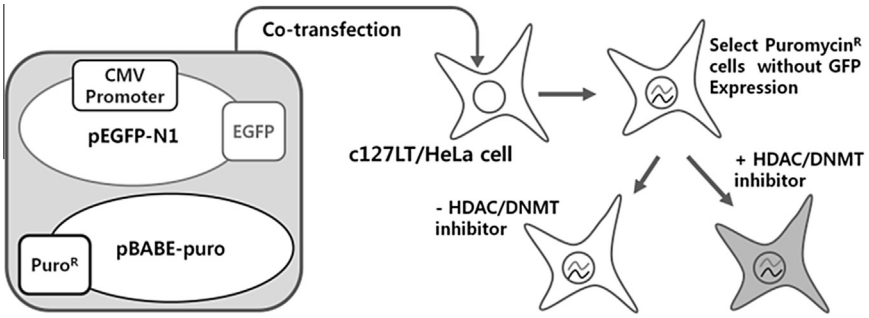



**Figure 1.** Fluorescence-based cellular screening system.

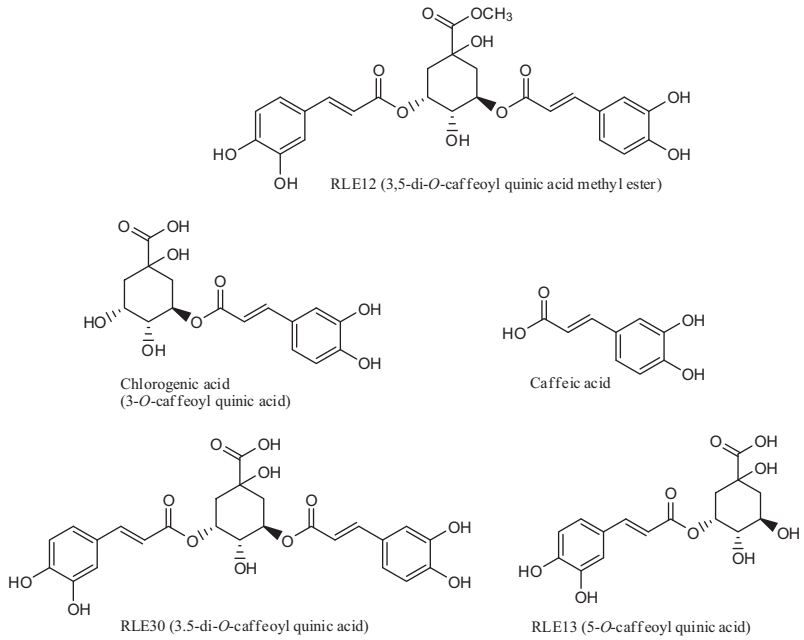


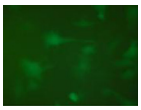
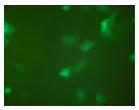

**Figure 2.** Structures of quinic acid derivatives and caffeic acid.

fluorescence imaging-based assay and HDAC assay. With the exception of RLE12 (Table 2), all other quinic acid derivatives induced EGFP expression very slightly, and in parallel, they showed HDAC inhibition activities noticeably lower than RLE12 (Table 3). These results suggest that intermolecular interactions between drug and HDAC active site might be affected by the methyl quinate group, and the chirality and number of caffeoyl ester group.

Docking study[14,23] was carried out using the Surflex-Dock[24] to examine the differences in binding poses of RLE12 derivatives, in correlation with HDAC inhibitory activities. To compare the Surflex-Dock score output (Table 3), RLE12 obtained the highest binding score ($-\log K_d = 9.03$) out of all other quinic acid derivatives, in agreement with its highest rank of HDAC inhibition activity. The binding score for carboxylate derivative RLE30 is 7.73, indicating that predicted $K_d$ value of RLE30 is about 20 times higher than that of RLE12. Considering that the $IC_{50}$ value of RLE30 is also 29 times higher than that of RLE12, the difference in HDAC inhibition activity of these compounds should be due to the differences in intrinsic interaction energy upon binding to the active site of HDAC.

The binding pose of RLE12 is the active site of human HDAC2[14] is shown in Figure 4a, in comparison with the X-ray pose of SAHA. RLE12 fits into the SAHA-binding pocket, with one caffeoyl group inserted into the zinc-containing narrow pocket and the other capped the entrance of the active site.

**Table 1**
Activation of EGFP expression by RLE12 and known HDAC inhibitors in EGFP-repressed c127LT cells

| Control | TSA(200nM) | MS275(1μM) | RLE12 (10μM) |
|---|---|---|---|
|  |  |  |  |

Inside the pocket, the catechol OH groups not only binds to zinc, but also forms a bidentate hydrogen bond with the nitrogen atom of the imidazole His145 and His146. The other catechol laying over the surface of hHDAC2 forms a hydrogen bond network with Gln31 (Fig. 4b). The T-shaped pose of RLE12 makes the drug cover a wide range of area at the capping group binding site of HDAC. The channel of binding site is composed of the hydrophobic side chains, such as Phe155, Phe210, and Tyr308 and interacts with aliphatic linker unit of RLE12 by forming CH–π interaction.[25] Figure 4c demonstrates the hydrophobic interaction formed between hydrophobic region (dark brown) and drug molecules at the entrance of the active site. Particularly, RLE12 only has interaction