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SelSA, Selenium Analogs of SAHA As Potent Histone Deacetylase Inhibitors

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Several etiological pathways like genetic, epigenetic, and cytogenetic process play a role in the transformation of normal cells to cancer cells. Regulation of transcription factors, as well as modification of chromatin structures (also known as epigenetic regulation) are critical regulatory steps in the cellular response to trauma and inflammation.^{1, 2} The reversible acetylation of the side chain of specific histone lysine residues by histone deacetylase (HDACs) and histone acetyl transferase (HATs) is one of the most widely studied chromatin modifications.³ The HDACs can be divided into two families, (1) the Zn²⁺-dependent HDAC family composed of Class I, Class IIa/b, and Class IV and (2) Zn²⁺-independent NAD-dependent class III enzymes. Class I comprises HDAC1, 2, 3, and 8 which are located in the nuclei of the cells. Class IIa/b contains HDAC4, 5, 6, 7, 9, and 10 primarily localized to the cytoplasm. HDAC11 has a conserved domain in the catalytic region of both Class I and Class II enzymes and it has been grouped to Class IV. Class III HDACs are NAD⁺-dependent deacetylase with non-histone protein as substrate and have been linked to regulation of caloric utilization of cells.^{4, 5}

HDAC catalyzes deacetylation of ϵ -amino group in lysines located near the N-terminal of core histone proteins.^{6, 7} Specific HDAC activity results in hypoacetylation that is associated with subsequent gene silencing, whereas histone hyperacetylation is associated with unwinding of the DNA and transcriptional activation.^{8, 9} Studies have shown that inhibition of HDAC elicits anticancer effects in several tumor cells by inhibition of cell growth, and induction of terminal differentiation in tumor cells. This has led to the development of HDAC inhibitors for anti-cancer chemotherapy¹⁰ mainly directed at Zn²⁺-dependent Class I and II HDACs. Structural-activity relationships (SAR) and reviews of different HDAC inhibitors and analogs have been previously published.^{2, 11–20} Most of these HDAC inhibitors were designed to have a hydrophobic cap that blocks the entrance to the active site, a polar site, and a hydroxamic acid type zinc-binding active site.¹⁵

Hydroxamic acids are the broadest class of inhibitors with high affinity for HDAC that has been shown to inhibit both Class I and Class II HDACs. Trichostatin A (TSA) belonging to hydroximates is one of the first natural product possess HDAC inhibitory activity and it is widely used as reference compound.^{21–23} TSA blocks proliferation, inhibits cell growth, decreases differentiation in ovarian cancer cells, and suppresses growth of pancreatic adenocarcinoma cells at nanomolar concentrations.^{24, 25} A second generation HDAC inhibitor, Suberoylanilide hydroxamic acid (SAHA) inhibits secretion of TNF- α , IL-1 β , IL-6, and IFN- γ in LPS-induced PBMC cells, inhibits their *in vivo* production as shown in an LPS induced animal model, as well as prevents formation of tumors in mice and rats.^{26–28} SAHA (Vorinostat) is under clinical trials in both hematological and nonhematological malignancies

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and is approved for treatment of cutaneous T-cell lymphoma.^{29, 30} Another class of HDAC inhibitors includes a group of synthetic benzamide derivatives such as MS-275 and CI-994 that are effective inhibitors of solid tumors in a murine model, but did not inhibit HDAC directly.³¹ This class of compounds inhibits both histone deacetylation and cellular proliferation at the G1-S phase.³² MS-275 and CCI-994 are undergoing clinical trials.^{33, 34} Another class, a cyclic peptide natural product include Trapoxin, having epoxide group may act by chemically modifying an active site nucleophile with the epoxide group and forming hydrogen bonds through the ketone.³⁵ Trapoxin is supposed to trap HDACs through the reaction of the epoxide moiety with the zinc cation or an amino acid in the binding pocket.^{36 – 38} FK228 (also referred as depsipeptide) is a natural product derived from *Chromobacterium violaceum*, inhibit HDACs at nanomolar concentrations, and exhibits potent antitumor activity.³⁹ The mechanism of action of FK228 is unknown; however, according to one hypothesis, a disulfide bond is reduced inside the cell or organism and the mercaptobutyryl residue then fits inside the HDAC catalytic pocket.³⁵ FK228 is currently undergoing evaluation in clinical trials.^{40 – 42} Thus HDACs have been suggested to be a potential targets for anticancer drug development and many other non-malignant diseases such as rheumatoid arthritis and osteoporosis.⁴³ Therefore, demand for new HDAC inhibitors having strong inhibitory action is increasing. In this communication, we report the syntheses of two newly developed selenium-based HDAC inhibitors (namely, SelSA-1 and SelSA-2) and evaluation of their HDAC activity compare to SAHA and TSA, a known HDAC inhibitors.

Several structurally diverse HDAC inhibitors have been reported and many of them belong to the family of hydroxamic acid derivatives.⁴⁴ Metabolic instability and pharmacokinetic problems such as glucuronide and sulfate conjugates that could result in short half life of the drug in biological systems. Therefore, several new non-hydroxamic HDAC inhibitors have been reported in the literature.⁹ However, they have a reduced potency compared to hydroxamate inhibitors. A cyclic peptide HDAC inhibitor FK228 is a potent HDAC inhibitor having a disulfide bond in the molecule that is reduced in the cellular environment releasing the free thiol analog as the active species.⁴⁵ Therefore, in a similar manner we hypothesize that in the cellular environment, the selenium dimer (SelSA-1) and selenocyanide (SelSA-2) will be reduced and free SeH will be release as the active species that will bind to the acetate group and cause the potent HDAC inhibitory activities. Based on our hypothesis, we have synthesized two selenium compounds. Synthesis of SelSA-1 was accomplished as illustrated in Scheme 1.

The amino group of aniline was acetylated with the appropriate acid chloride to give the amide **1** in quantitative yield.^{46, 47} Amide **1** was treated with selenium powder under basic condition in a biphasic system using phase transfer catalyst to give the desired dimer SelSA-1 in 60% yield⁴⁸.

Synthesis of SelSA-2 was accomplished by reacting of amide **1** with KSeCN in CH₃CN as shown in Scheme 2.⁴⁹ Acetonitrile was the solvent of choice for the reaction to avoid side products as reported earlier with other solvent.⁵⁰

HeLa cell nuclear extract was used as the source of the HDAC activity with HDAC1 and HDAC2 being the major contributors.^{51, 52} We found that SelSA-1 and SelSA-2 inhibited HDAC activity approximately by 81% and 95% at 50nM, respectively. The inhibitory activity of SelSA-2 was statistically significant higher than TSA (90%) at the same concentrations (50nM).⁵³

Both SelSA-2 and TSA showed higher inhibitory activity than SAHA (77%) at 50nM which was not different than SelSA-1 at 50nM. Based on these findings, we assessed the IC₅₀ of

SelSA-2, TSA and SAHA. The IC₅₀ concentrations of SelSA-2, TSA and SAHA were 8.9, 28.9 and 196nM, respectively.

In summary, we have developed novel selenium based HDAC inhibitors and evaluated their inhibitory effect on HDACs. Both selenium compounds are superior in their inhibitory effect (more than 20 fold) on HDAC than the known inhibitor, SAHA. Indeed, SAHA is currently in clinical use for lymphoma and under active evaluation for other indications.⁵ However, these selenium- based small molecules may play an important role in the fight against cancer. Currently, we are pursuing the structural activity relationship (SAR) studies with analogs of SelSA as HDAC inhibitors.

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46. General synthesis information: Melting points were recorded on a Fisher-Johnson melting point apparatus and are uncorrected. Unless stated otherwise, proton NMR spectra were recorded in CDCl₃ using a Bruker 500 MHz instrument. The chemical shifts are reported in ppm downfield from TMS. MS were run on 4000 Q trap hybrid triple quadrupole/linear ion trap instrument (Applied Biosystems/MDS Sciex) at the proteomic facility of the Penn State Hershey Cancer Institute, College of Medicine, Hershey, PA. High-resolution MS were determined at the Instrument Center, University of Buffalo, Buffalo, NY. Thin-layer chromatography (TLC) was performed on aluminum-supported, pre-coated silica gel plates (EM Industries, Gibbstown, NJ). All starting materials and reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification.
47. *Preparation of 7-Bromoheptanoic acid phenylamide (1)*: A flame-dried round bottom flask was charged with aniline (1g, 10.7mmol) in dry methylene chloride (20ml) and triethylamine (2.95 mL, 21.48 mmol). The mixture was cooled on ice for 10 minutes. Through a dropping funnel, a solution

of 6-bromohexanoylchloride (2.3g, 10.74 mmol) in methylene chloride (10 mL) was added over a period of 10 minutes. The mixture was allowed to warm up to room temperature and stirring was continued for an additional 18 hours. The mixture was poured into water and the organic layer was separated, dried over MgSO_4 , filtered, and evaporated to give crude product. Crude product was purified over a silica gel column using methylene chloride as an eluant to give bromo amide 1 (2.43 g, 84%). ^1H NMR δ 1.35 (q, 2H, $J=6.7\text{Hz}$), 1.57 (q, 2H, $J=7.0\text{Hz}$), 1.80 (q, 2H, $J=7.5\text{Hz}$), 1.95 (q, 2H, $J=7.0\text{Hz}$), 2.41 (t, 2H, $J=7.5\text{Hz}$), 3.46 (t, 2H, $\text{CH}_2\text{-Br}$, $J=7.0\text{Hz}$), 7.11 (bs, 1H, NH), 7.14 (t, 1H, aromatic, $J=7.5\text{Hz}$), 7.35 (t, 2H, aromatic, $J=7.5\text{Hz}$), 7.54 (d, 2H, aromatic, $J=8.0\text{Hz}$).

48. *Synthesis of Bis(5-phenylcarbamoylpentyl) diselenide (SelSA-1) (2)*: To a stirring mixture of bromo compound 1 (50mg, 0.19 mmol) in methylene chloride (5 mL), selenium powder (25mg, 0.32mmol), and water (1 mL) was added. The mixture was stirred for 5 minutes and a drop of adogen and 40% KOH solution were added (1 mL). The mixture was stirred for an additional 5 minutes and hydrazine monohydrate (0.5 ml, 10mmol) was added and the mixture was stirred overnight at room temperature. The initial black color of the solution (due to selenium powder) turned yellow after overnight stirring. The organic layer was separated, dried over MgSO_4 , filtered, and evaporated to give a crude product. Column chromatography over silica gel using methylene chloride: EtOAc (98:2) as eluant gave SelSA-1 (30 mg, 60%); mp 128–130°C; ^1H NMR (d_6 -DMSO) δ 1.39 (p, 4H, CH_2 , $J=7.0\text{Hz}$), 1.61 (p, 4H, CH_2 , $J=7.5\text{Hz}$), 1.71 (p, 4H, CH_2 , $J=7.5\text{Hz}$), 2.30 (t, 4H, CH_2 , $J=7.5\text{Hz}$), 2.93 (t, 4H, CH_2 , $J=7.5\text{Hz}$), 7.02 (t, 2H, aromatic, $J=7.5\text{Hz}$), 7.28 (t, 4H, aromatic, $J=8.0\text{Hz}$), 7.59 (d, 4H, aromatic, $J=8.0\text{Hz}$), 9.85 (s, 2H, NH); MS, 563 ($\text{M}^+ + \text{Na}$, 100), 540 (M^+ , 10), 301 (40), 283 (45), 261 (50), 217 (45); HRMS 541.0867 (Calculated for $\text{C}_{24}\text{H}_{32}\text{N}_2\text{O}_2\text{Se}_2$, 541.0867).
49. *Synthesis of 5-phenylcarbamoylpentyl selenocyanide (SelSA 2) (3)*: The starting material bromo amide 1 (1g, 3.70 mmol) was dissolved in dry acetonitrile (15 mL) and the mixture was charged with KSeCN (0.64g, 4.44 mmol). The reaction mixture was stirred for 18h and poured into water. The aqueous layer was extracted with methylene chloride (3×25 ml). Combined organic layers were dried over MgSO_4 , filtered, and evaporated to yield a crude product that was purified by column chromatography using methylene chloride:EtOAc (99:1) as an eluent to give SelSA-2 (740mg, 64%), mp 87–88°C, ^1H NMR δ 1.60 (q, 2H, $J=7.0\text{Hz}$), 1.83 (q, 2H, $J=8.0\text{Hz}$), 2.00 (q, 2H, $J=7.5\text{Hz}$), 2.42 (t, 2H, CO-CH_2 , $J=7.5\text{Hz}$), 3.10 (t, 2H, $\text{CH}_2\text{-Se}$, $J=7.0\text{Hz}$), 7.1 (t, 1H, aromatic, $J=7.5\text{Hz}$), 7.17 (bs, 1H, NH), 7.35 (t, 2H, aromatic, $J=7.5\text{Hz}$), 7.54 (d, 2H, aromatic, $J=8.0\text{Hz}$); MS m/e 319 ($\text{M}^+ + \text{Na}$, 100), 297 (M^+ , 10), 261 (15), 245 (5), 217 (10), HRMS 297.0489 (Calculated For $\text{C}_{13}\text{H}_{16}\text{N}_2\text{OSe}$, 297.0501).
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51. *HDAC Assay*: HDAC Assay was performed using the colorimetric HDAC Colorimetric Assay/Drug Discovery Kit (Biomol; Plymouth Meeting, PA) according to manufacturer's instructions as reported by Huang *et al.*⁵² Briefly, HDAC inhibitors/candidates (10 μL ; 50nM TSA, 500nM SAHA, 50nM SelSA-1, and 50nM SelSA-2) were deposited in a 96-well plate. HeLa cell nuclear extract (5 μL) provided with the kit was added into each well. HDAC reaction was initiated by adding Color de Lys Substrate (25 μL) into each well and samples were incubated at RT for 15 min. Subsequently, the reaction was stopped by adding 50 μL of Color de Lys Developer for additional 30 min at 37°C. Colorimetric changes were measured at 405 nm using a plate reader. HDAC activity was expressed as percent inhibition based on the relative OD values of samples. Tests were conducted in triplicate sets. Negative and positive controls consisted of no HeLa nuclear extract or no inhibitor, respectively. IC_{50} of the inhibitor compounds was determined by performing HDAC assay with increasing concentrations (1, 3, 3, 10, 33, 100 and 333nM) of the inhibitor compounds (TSA, SAHA and SelSA-2) under the same conditions.
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53. *Statistical Analysis*: Data were combined from two separate HDAC assay experiments and analyzed. Statistical analysis was performed using a standard one-way ANOVA followed by Student Newman-Keuls post-hoc ANOVA test. Differences with $p < 0.05$ were considered significant.

Figure 1.
Structures of HDAC inhibitors

Figure 2.
% Inhibition of HDAC in HeLa Nuclear Extract

Figure 3.
IC₅₀ value of HDAC inhibitors

Scheme 1.

Synthesis of SelSA-1. Reagents and conditions: (a) TEA, RT, 18h, 84%. (b) KOH, Adogen, Se powder, NH_2NH_2 , RT, 60%.

Scheme 2.

Synthesis of SelSA-2. Reagents and conditions: (a) KSeCN, CH₃CN, RT, 18h, 64%.