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Short Note

Methyl 9-(2-Iminothiazol-3(2H)-yl)-9-oxononanoate

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Abstract: Methyl 9-(2-iminothiazol-3(2H)-yl)-9-oxononanoate was synthesized through Schotten–Baumann type reaction between 2–aminothiazole and methyl 9-chloro-9-oxononanoate. The structure of the newly synthesized compound was elucidated based on ^{1}H-NMR, ^{13}C-NMR, DEPT, NOE, ESI-MS, FT-IR and UV-Vis spectroscopy.

Keywords: 2-aminothiazole; azelaic acid; Schotten–Baumann; cancer

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1. Introduction

2-Aminothiazole derivatives represent very important scaffolds in many fields of applied chemistry, such as dyes [1], pharmaceuticals [2], and medicinal chemistry [3–10]. For a long time, our interests have lain in thiazole chemistry both from the synthetic and mechanistic point of view and in the study of biological effects of 9-hydroxystearic acid (9-HSA, Figure 1a) and its derivatives. 9-HSA is an endogenous cellular product with antiproliferative activity against different human cancer cell lines, and, interestingly, it is not toxic for normal cells. It acts as a histone deacetylase inhibitor (HDACi) [11,12]. Looking at the structure of Vorinostat, also known as SAHA (suberoylanilide hydroxamic acid or *N*-Hydroxy-*N*′-phenyloctane diamide, Figure 1b), and 9-HSA, and considering that both act as inhibitors of HDAC by interacting, respectively, through hydroxamate- or carboxylate-zinc coordination modes, we realized that a structural modification of 9-HSA on the C-9, preserving the methylene chain as a spacer between the carboxylic group and an amide functionality, might produce promising candidates as inhibitors of HDAC. Thus, we incorporated a long aliphatic chain with ester or the carboxylic acid ending group to a series of amino aza-heterocycles via an amide connection (Figure 1c,d), and the compounds obtained showed antiproliferative ability against a series of human cancer cell lines [13,14].

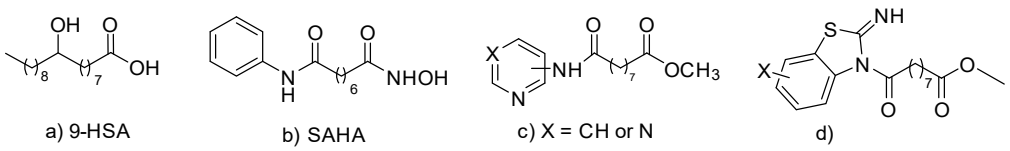


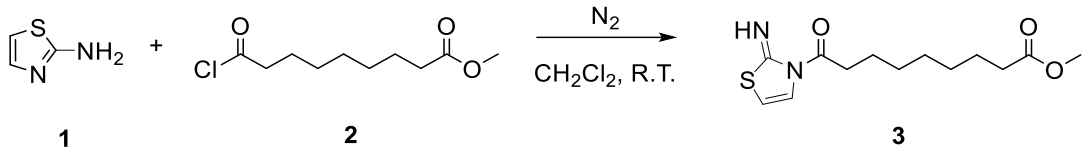
Figure 1. Structure of SAHA (**a**), 9-has and (**b**) Aza heterocycles bound to azelayl moiety (**c**,**d**).

Based on the above and considering that the 2-aminothiazolyl moiety is present in a lot of hybrid compounds of interest, such as anticancer drugs [15], we planned, and herein report, together with the preliminary results on its biological activity, the synthesis of a novel hybrid bearing both the 2-aminothiazolyl and azelayl moieties.

2. Results

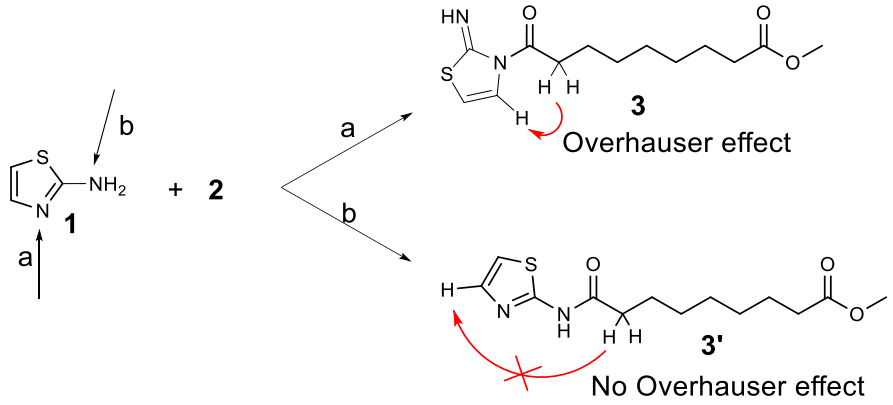
The synthesis of methyl 9-(2-iminothiazol-3(2*H*)-yl)-9-oxononanoate (**3**) (Scheme 1) was performed through a Schotten–Baumann-type reaction from 2-aminothiazole (**1**) and

methyl 9-chloro-9-oxononanoate (**2**) in dichloromethane at room temperature. At the end of the reaction, product **3** was purified by column chromatography on silica gel using a mixture of light petroleum and diethyl ether, 2/8 *v*/*v* ratio, as eluent (yield 43%); no presence of other isomers was detected. The newly synthesized compound was characterized by ^{1}H-NMR, ^{13}C-NMR, ESI-MS, FT-IR, and UV-Vis spectroscopy.



Scheme 1. Methyl 9-(2-iminothiazol-3(2H)-yl)-9-oxononanoate (**3**).

In principle, 2-aminothiazole has three nucleophilic sites, namely the C-5, the *endo*–cyclic nitrogen atom, and the *exo*–cyclic nitrogen atom. The ^{1}H NMR spectrum of the unique product that was recovered from the current reaction shows, in addition to the aliphatic chain and methoxy signals, two doublets at δ = 7.42 and 7.00 ppm due to C-4 and C-5 hydrogen atoms belonged to the thiazole ring. This information, however, does not permit discrimination between the two possible structures **3** and **3′** derived from the attack to the *endo*- or the *exo*-cyclic nitrogen atom, respectively (Scheme 2).



Scheme 2. Possible products derived from the attack of **2** to **1**.

Thus, a NOESY-1D experiment was carried out, and the result provided an unequivocal elucidation of the structure. In particular, the irradiation of the signal at 2.55 ppm (CH_2 in α position with respect to the amide group) produced a Nuclear Overhauser Effect on the signal at 7.42 ppm belonging to the C-4 hydrogen atom (Figure S2 in Supplementary Materials), indicating that the group irradiated was near to the H-4 and this was possible when the chain was bound to the endocyclic nitrogen atom of the thiazole ring.

This observation that resulted was crucial for assigning the structure of the reaction product to **3**, as derived from the attack on the *endo*–cyclic nitrogen atom.

We chose to test the effects of compound **3** on two cell lines since they came from the neoplastic transformation of two different human tissues: HT29 was isolated from a primary tumor obtained from a patient with colorectal adenocarcinoma, while U2OS was derived from a moderately differentiated sarcoma of an osteosarcoma patient.

The effects on cell viability were evaluated using the MTT assay, and the results are shown in Table 1. Interestingly, compound **3** exhibits inhibitory activity only on U2OS, indicating a cell-specific activity.

Table 1. IC_{50} values of compound **3**.

Cell Line	IC_{50}
HT29	≥100 μM
U2OS	50 μM

HT-29 cells have a mutation in the p53 gene at position 273, while U2OS are wt for this protein. This difference is important as it explains the different biological responses to therapeutic agents. Indeed, it has been demonstrated that the administration of 9-HSA to HT29 inhibits HDAC1-inducing histone hyperacetylation, which is associated with a cytostatic effect [11]. On the contrary, the treatment of U2OS produces a hyperacetylation of p53 with consequent cytotoxic effects [16]. Moreover, 9-HSA is a selective inhibitor of class I HDACs [12], while other agents, in particular, SAHA (non-selective for HDACi), affect the hyperacetylation of all histones and cause massive cell death [17].

3. Materials and Methods

The ^{1}H, ^{13}C, and DEPT (Distortionless Enhancement by Polarization Transfer), NOE (Nuclear Overhauser Effect) spectra were recorded on an Inova 600 (Varian, Palo Alto, CA, USA) spectrometer operating at 600 MHz (for ^{1}H NMR) and at 150 MHz (for ^{13}C NMR). Chemical shifts were referenced to the solvent for ^{1}H and ^{13}C NMR (δ = 7.26 ppm and δ = 77.0 ppm, respectively, for $CDCl_3$). Signal multiplicities were established by DEPT experiments. Chemical shifts are measured in δ (ppm). *J* values are given in Hertz. Electron spray ionization mass spectra (ESI-MS) were recorded with a WATERS 2Q 4000 instrument (Waters Corporation, Milford, MA, USA). IR spectra were recorded on a Perkin Elmer FT-IR Mod. 1600 spectrophotometer (Perkin Elmer, Waltham, MA, USA). UV/Vis spectra were recorded on a PerkinElmer Lambda 12 spectrophotometer (Perkin Elmer, Waltham, MA, USA) at 20 °C in $CHCl_3$ and in quartz cells (path length cell: 1 cm). Melting points (m.p.) were measured on a Büchi 535 apparatus (Büchi, Flawil, Switzerland). Chromatographic purifications (FC) were carried out on glass columns packed with silica gel (Merck grade 9385, 230−400 mesh particle size, 60 Å pore size) at medium pressure (Merck & Co. Readington, NJ, USA). Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} coated aluminum foils (Fluka, Buchs, Switzerland). Methyl 9-chloro-9-oxononanoate was prepared by us as previously reported [13]; 2-aminothiazole was purchased by Sigma-Aldrich (Milan, Italy), as well as all the solvents used.

The human colon cancer cell line HT29 and the human bone osteosarcoma U2OS cell line were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in an RPMI 1640 medium (Labtek Eurobio, Milan, Italy), supplemented with 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) or fetal calf serum (Euroclone, Milan, Italy) 100 U/mL penicillin, 100 g/mL streptomycin (GE Healthcare, Chicago, IL, USA) and 2mM L-glutamine (Sigma-Aldrich, Milan, Italy), at 37 °C in a 5% CO_2 atmosphere. Compound **3** was dissolved in DMSO (Sigma-Aldrich, Milan, Italy) in a stock solution.

3.1. Methyl 9-(2-Iminothiazol-3(2H)-yl)-9-oxononanoate (3)

A solution of 2-aminothiazole (**1**) (150 mg, 1 mmol, in 1 mL of anhydrous CH_2Cl_2) was added to a solution of 9-chloro-9-oxononanoate (**2**) (110 mg, 0.5 mmol), prepared from azelaic acid mono-methyl ester and oxalyl chloride, in 5 mL of anhydrous CH_2Cl_2, under a nitrogen atmosphere, as reported in ref. [13]. The mixture was magnetically stirred for 3 h. The reaction course was monitored by TLC (eluent diethyl ether/petroleum ether 9/1). The product was purified by chromatography on silica gel (eluent diethyl ether/petroleum ether 8/2). The product yield was 61 mg, 43%.

Pale yellow solid, m.p.: 105.5–105.8 °C; ^{1}H-NMR (600 MHz, $CDCl_3$, 25 °C) δ, ppm: 12.25 (s, 1H, NH), 7.42 (d, *J* = 3.6 Hz, 1H, C<u>H</u>=), 7.00 (d, *J* = 3.6 Hz, 1H, C<u>H</u>=), 3.66 (s, 3H, COOC<u>H</u>$_3$), 2.54 (t, *J* = 7.5 Hz, 2H, C<u>H</u>$_2$CON), 2.29 (t, *J* = 7.5 Hz, 2H, C<u>H</u>$_2$COOCH$_3$),

1.80–1.74 (m, 2H, CH_2CH_2CON), 1.64–1.58 (m, 2H, $CH_2CH_2COOCH_3$), 1.43–1.30 (m, 6H, aliphatic CH_2); ^{13}C NMR: (150 MHz, $CDCl_3$, 25 °C) δ ppm: 174.2 (C), 171.2 (C), 160.1 (C), 136.1 (CH), 113.5 (CH), 51.4 (CH_3), 36.1 (CH_2), 34.0 (CH_2), 29.0 (CH_2), 28.95 (CH_2), 28.87 (CH_2), 25.0 (CH_2), 24.8 (CH_2); FT-IR ($CHCl_3$), ν (cm^{-1}): 3019, 2930, 2860, 2397, 1731, 1691, 1552, 1532, 1217; UV-Vis λmax = 266 nm; ESI-MS (*m*/*z*): 285 $[M+H]^+$, 307 $[M + Na]^+$, 323 $[M + K]^+$; Elemental Analysis for $C_{13}H_{20}N_2O_3S$, Calculated C, 54.91; H, 7.09; N, 9.85; found C, 54.97; H, 7.12; N, 9.81.

3.2. Cell Viability Assays

To evaluate the compound activity, the cells were treated for 24 h with a vehicle (DMSO, as control) or with the test sample at concentrations between 0.01 μM and 250 μM. Cell growth was assessed by the colorimetric 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide assay (MTT, Sigma-Aldrich, Milan, Italy). As for MTT assays, the culture medium was removed, and cells were incubated with 0.1 mL of MTT dissolved in PBS at a concentration of 0.2 mg/mL following Micheletti et.al. [18]. The absorbance at 570 nm was measured using a multiwell plate reader (Tecan, Männedorf, Switzerland). The IC_{50} was determined from the dose–response curve by using Prism Graph Pad Software v.6.0 (GraphPad Software, San Diego, CA, USA).

Supplementary Materials: The following are available online, Figure S1. ^{1}H-NMR spectrum in $CDCl_3$ of compound **3**; Figure S2. NOE spectrum in $CDCl_3$ of compound **3**; Figure S3. ^{13}C-NMR spectrum in $CDCl_3$ of compound **3**; Figure S4. DEPT spectrum in $CDCl_3$ of compound **3**; Figure S5. ESI-MS spectrum of compound **3**.

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