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Short Note

Methyl 2-Amino-4-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1,3-selenazole-5-carboxylate

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Abstract: Methyl 2-amino-4-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1,3-selenazole-5-carboxylate as a newly functionalized heterocyclic amino acid was obtained via [3+2] cycloaddition. The structure of the novel 1,3-selenazole was unequivocally confirmed by detailed ^{1}H, ^{13}C, ^{15}N, and ^{77}Se NMR spectroscopic experiments, HRMS and elemental analysis.

Keywords: azetidine; 1,3-selenazole; heterocyclic amino acids; Hantzsch synthesis

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1. Introduction

Selenium is an essential bio-trace element that plays an important role in antioxidant selenoproteins for protection against oxidative stress in humans and other animal species [1]. Functionalized organoselenium compounds possess a wide range of biological activities and are present in many pharmacologically important substances [2]. Well-known representative bioactive molecules that contain selenium in their structure are potent the antiviral agent selenazofurin [3], histamine H2-agonist known as amselamine [4], as well as ebselen and its analogues exhibiting anti-inflammatory, antioxidant, and cytoprotective properties [5,6]. Moreover, the synthesis of new Se-containing β-lactams such as selenapenam, selenacepham and selenazepine as a potential antibacterial agents has been reported [7] (Figure 1).

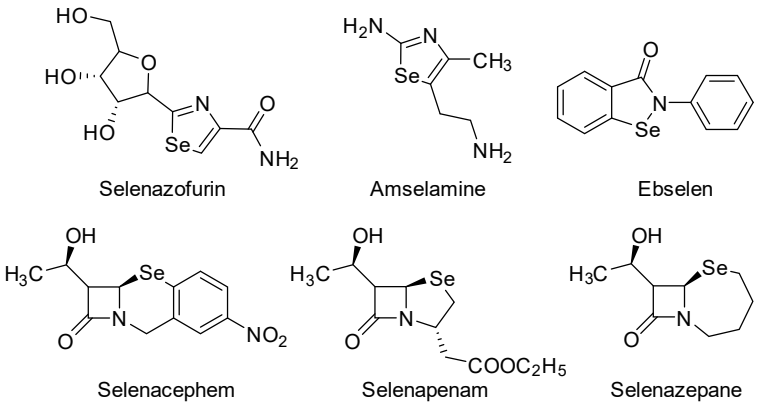


Figure 1. Biologically active organoselenium compounds.

On the other hand, the azetidine ring has been identified as a conformationally restricted component of important pharmacological molecules [8], including synthetic analogues of natural amino acids, such as γ-aminobutyric acid (GABA) [9]. The azetidine

ring is also present in the molecular structure of the well-known antihypertensive drug azelnidipine (Figure 2) [10].

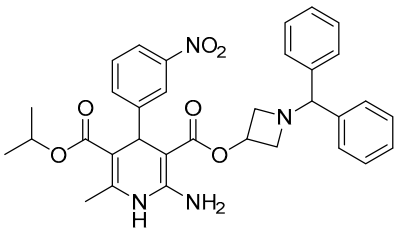


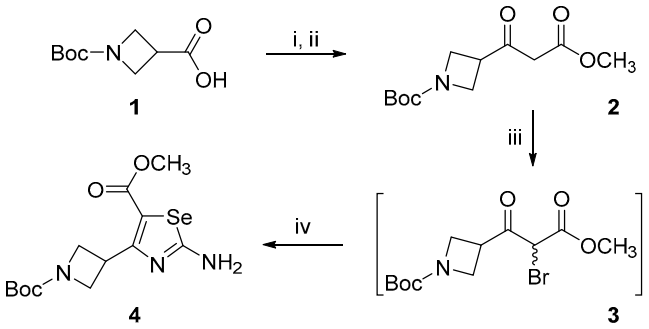
Figure 2. Structure of azelnidipine.

The aim of the present study was to extend our previous work on heterocyclic amino acids containing selenazole and azetidine cores [11–14], as it is still a new and potentially relevant field. In this work, a molecular system containing both selenazole and azetidine scaffolds was synthesized through Hantzsch cyclization of *β*-ketoester.

2. Results and Discussion

Selenazoles can be obtained using analogous synthetic strategies similar to the ones used for thiazoles. Among a variety of ring construction reactions, the most widely used approach is Hantzsch synthesis and its variations [15–17]. This methodology reliably leads to the formation of diverse heterocyclic rings in good yields.

The synthetic strategy for obtaining novel selenazole-azetidine building blocks is outlined in Scheme 1. The target compound was synthesized in four steps using commercially available *N*-Boc-protected azetidine-3-carboxylic acid **1**. The synthetic sequence was started with preparation of *β*-ketoester **2** by its adduct methanolysis with Meldrum's acid. In the following step, bromination of ester **2** was carried out in the presence of NBS in acetonitrile. The reaction afforded an *α*-bromocarbonyl compound **3**, which was immediately used in the final step. The condensation of **3** with selenourea afforded the desired methyl 2-amino-4-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1,3-selenazole-5-carboxylate **4**.



Scheme 1. Synthesis of methyl 2-amino-4-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1,3-selenazole-5-carboxylate (**4**). Reagents and conditions: (i) Meldrum's acid, DMAP, EDC, DCM, r.t., 18 h; (ii) MeOH, 60 °C, 18 h; (iii) NBS, ACN, r.t., 2 h; (iv) selenourea, MeOH, r.t., 2 h.

The final structure of compound **4** was easily deduced after a detailed analysis of spectral data (Figures S1–S8). The ^{1}H-^{15}N HSQC experiment revealed that the most downfield protons at 7.25 ppm belong to the NH_2 group, which resonates at −294.6 ppm. The multiplicity-edited ^{1}H-^{13}C HSQC spectrum revealed a cross-peak correlating a methine proton at 4.67 ppm with the ^{13}C signal at 28.1 ppm from the azetidine ring (Figure 3).

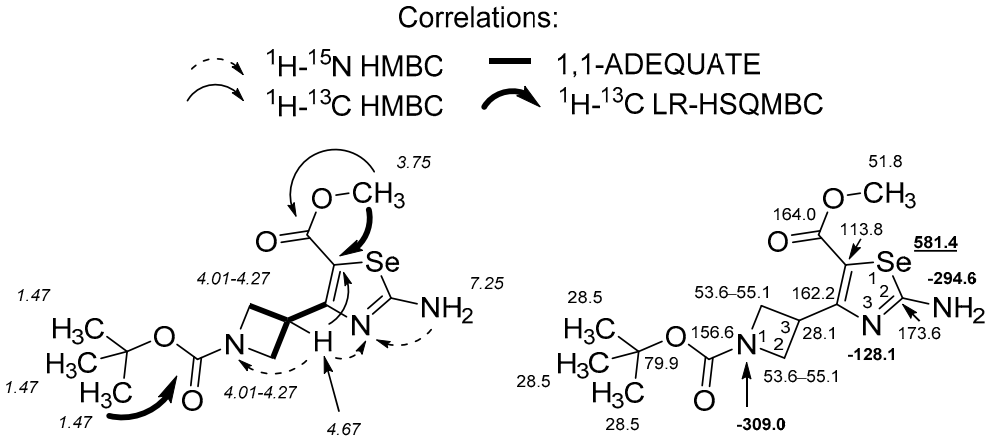


Figure 3. Relevant 1,1-ADEQUATE, ^{1}H-^{13}C HMBC, ^{1}H-^{15}N HMBC, ^{1}H-^{13}C LR-HSQMBC correlations and ^{1}H NMR (in italics), ^{15}N NMR (in bold), ^{77}Se (in bold, underlined) and ^{13}C-NMR chemical shifts of compound **4**.

The connectivity of the 2-amino-1,3-selenazole-5-carboxylate moiety and the *N*-Boc-protected azetidine fragment could be confirmed based on long-range ^{1}H-^{13}C and ^{1}H-^{15}N correlations, obtained from gs-HMBC spectra of the aforementioned protons. The ^{1}H-^{15}N HMBC experiment revealed a strong three-bond correlation between the 1,3-selenazole N-3 nitrogen, which resonated at −128.1 ppm, and the protons from the 2-amino functional group. In the case of 3-H from the azetidine ring system, it showed a strong correlation with the selenazole N-3, and additionally revealed data for azetidine N-1 at −309.0 ppm. The data from ^{1}H–^{13}C HMBC and LR-HSQMBC experiments allowed an unambiguous assignment of the 1,3-selenazole ring system, as we were able to easily distinguish the carbonyl carbons from the *N*-Boc and carboxylate moieties. Lastly, the protonated azetidine carbon C-3 at 21.8 ppm showed a correlation with an adjacent selenazole quaternary carbon C-4 at 162.2 ppm in the 1,1-ADEQUATE spectrum. By a process of elimination, this allowed the assignment of the last selenazole C-2 signal, which resonated at 173.6 ppm. The ^{77}Se NMR spectra contained a singlet at 581.4 ppm. The observed ^{15}N and ^{77}Se chemical shifts are consistent with data reported in the literature for 1,3-selenazoles possessing similar structures [18].

The optical properties of compound **4** were investigated by UV/vis spectroscopy. The electronic absorption spectra of compound **4** in tetrahydrofuran (THF) contained an intense absorption band at 314 nm. Fluorescence spectra displayed an emission maximum at 375 nm (Figure 4).

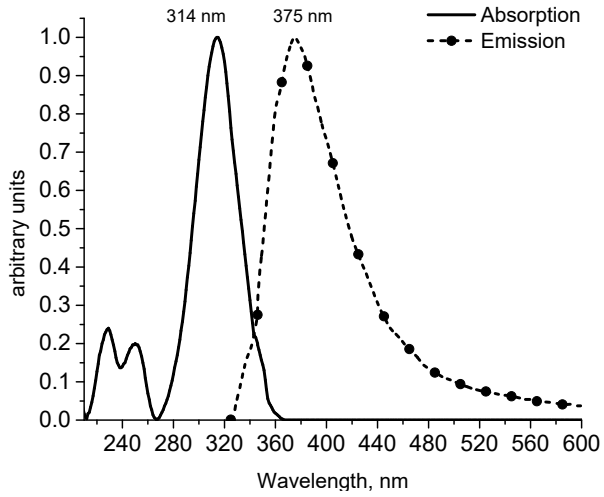


Figure 4. Absorption and emission (λex = 320 nm) spectrum of compound **4** in THF.

3. Materials and Methods

3.1. General

All chemicals and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification unless otherwise specified. Prior to use, dichloromethane (DCM) was stored over molecular sieves (4 Å). Reaction progress was monitored by thin-layer chromatography (TLC) analysis on Macherey–Nagel™ ALUGRAM® Xtra SIL G/UV_{254} plates (Macherey−Nagel™, Düren, Germany). TLC plates were visualized with ultraviolet (UV) light (wavelength 254 nm). Compounds were purified by flash chromatography in a glass column (stationary phase—silica gel, high-purity grade 9385, pore size 60 Å, particle size: 230–400 mesh, Sigma-Aldrich, St. Louis, MO, USA). The ^{1}H, ^{13}C and ^{15}N-NMR spectra were recorded in chloroform-D ($CDCl_3$) at 25 °C on a Bruker Avance III 700 (700 MHz for ^{1}H, 176 MHz for ^{13}C, 71 MHz for ^{15}N) spectrometer equipped with a 5 mm TCI ^{1}H-^{13}C/^{15}N/D z-gradient cryoprobe (Bruker BioSpin AG, Fallanden, Switzerland). ^{77}Se NMR spectra (76.31 MHz, absolute referencing via Ξ ratio) were obtained on a Bruker Avance III 400 (Bruker BioSpin AG, Fallanden, Switzerland) instrument with a 'directly' detecting broadband observe probe (BBO). The chemical shifts, expressed in ppm, were relative to tetramethylsilane (TMS). The ^{15}N NMR spectrum was referenced to neat, external nitromethane (coaxial capillary). The full and unambiguous assignments of the ^{1}H, ^{13}C, ^{15}N, and ^{77}Se-NMR resonances were achieved using standard Bruker software (TopSpin 3.5.6, Bruker BioSpin AG, Fallanden, Switzerland) and a combination of standard NMR spectroscopic techniques, such as distortionless enhancement by polarization transfer (DEPT), homonuclear correlation spectroscopy (COSY), gradient-selected heteronuclear single quantum coherence (gs-HSQC), gradient-selected heteronuclear multiple bond correlation (gs-HMBC), heteronuclear 2-bond correlation (H2BC), long-range heteronuclear single-quantum multiple-bond correlation (LR-HSQMBC) and 1,1-ADEQUATE experiments. The infrared (IR) spectra were recorded on a Bruker TENSOR 27 spectrometer (Bruker Optik GmbH, Ettlingen, Germany) using potassium bromide (KBr) pellets. The melting point was determined in open capillary tubes with a DigiMelt MPA160 apparatus (temperature gradient: 1 °C/min) and was uncorrected. The HRMS spectrum was obtained in electrospray ionization (ESI) mode on a Bruker MicrOTOF-Q III spectrometer (Bruker Daltonik GmbH, Bremen, Germany). Elemental analysis (C, H and N) was determined using a CE-440 Elemental Analyzer (Exeter Analytical Ltd, Coventry, UK), Model 440 CHN/O/S. The UV/vis spectrum was recorded using 10^{-4} M solution of the compound in THF on a Shimadzu 2600 UV/vis spectrometer (Shimadzu EUROPA GmbH, Duisburg, Germany). The fluorescence spectrum was recorded on a FL920 fluorescence spectrometer from Edinburgh Instruments (Edinburg Instruments Ltd, Livingston, UK).

3.2. Synthesis

1-*Boc*-azetidine-3-carboxylic acid **1** (3.267 g, 16.2 mmol), Meldrum's acid (2.811 g, 19.5 mmol), DMAP (2.969 g, 24.3 mmol) and EDC (3.738 g, 19.5 mmol) were dissolved in DCM (70 mL). The resulting mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with an additional volume of DCM (50 mL) and washed with 10% $KHSO_4$ aqueous solution (3 × 50 mL) and brine (1 × 100 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was dissolved in MeOH (70 mL) and stirred at 60 °C for 18 h. The solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, gradient from 6:1 to 1:1 *v*/*v*). Obtained *β*-ketoester **2** (3.458 g, 13.4 mmol) was dissolved in ACN (100 mL) and NBS (3.588 g, 20.2 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. After the reaction, the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (50 mL) and filtered through a pad of silica. The filter pad was washed out with EtOAc (150 mL). The filtrate was concentrated in vacuo. The residue was dissolved in MeOH (40 mL) and selenourea (0.915 g, 7.4 mmol) was added. The reaction mixture was stirred at room temperature. After 2 h, the resulting mixture was added dropwise to 2% Na_2CO_3

aqueous solution (100 mL) while stirring. The precipitate was filtered and dissolved in DCM (100 mL). The solution was dried over anhydrous Na_2SO_4, filtered, and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, gradient from 4:1 to 1:1 *v*/*v*) to afford compound **4**. The obtained solid was recrystallized from hexane. Colorless crystals (683 mg) were obtained with an overall of yield 21%. R_f = 0.19 (*n*-hexane/ethyl acetate 2/1, *v*/*v*), m.p. 167−168 °C. IR (KBr) ν_{max}, cm^{-1}: 3388 (NH), 3293 (NH), 3175, 2975, 2955, 2890, 1680 (C=O), 1628 (C=O), 1505, 1415, 1284, 1139, 1067, 909, 772 and 757, 527. ^{1}H NMR (700 MHz, $CDCl_3$) δ, ppm: 1.47 (9H, s, 3 × CH_3), 3.75 (3H, s, OCH_3), 4.01 (2H, br s, 2 × C$\underline{H_a}H_b$), 4.20−4.27 (2H, m, 2 × $CH_a\underline{H_b}$), 4.67 (1H, tt, *J* = 8.7, 5.5 Hz, CH), 7.25 (2H, s, NH_2). ^{13}C NMR (176 MHz, $CDCl_3$) δ, ppm: 28.1 (CH), 28.5 (3 × CH_3), 51.8 (OCH_3), 53.6 (CH_2), 55.1 (CH_2), 79.9 ($\underline{C}(CH_3)_3$), 113.8 (Sel C-5), 156.6 (Boc C=O), 162.2 (Sel C-4), 164.0 $\underline{C}OOCH_3$), 173.6 (Sel C-2). ^{15}N NMR (71 MHz, $CDCl_3$) δ, ppm: −309.0 (Az N-1), −294.6 (NH_2), −128.1 (Sel N-3). ^{77}Se NMR (76 MHz, $CDCl_3$) δ, ppm: 581.4. HRMS (ESI) for $C_{13}H_{19}N_3NaO_4Se$ ($[M + Na]^+$): found *m*/*z* 384.0434, calculated *m*/*z* 384.0434. Elem. An. for $C_{13}H_{19}N_3O_4Se$ (%): found C, 43.87; H, 5.33; N, 11.22; calculated C, 43.34; H, 5.32; N, 11.66.

4. Conclusions

In this short note, we reported the synthesis and structure elucidation of methyl 2-amino-4-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1,3-selenazole-5-carboxylate **4**. This compound is a valuable building block for more complex molecular systems as well as for the development of DNA-encoded chemical libraries.

Supplementary Materials: The following are available online: NMR, HRMS, and IR spectra of compound **4**. Figure S1: 1H NMR spectrum of compound **4**. Figure S2: 13C NMR spectrum of compound **4**. Figure S3: 1H-15N HSQC NMR spectrum of compound **4**. Figure S4: The overlaid 1H-13C gs-HSQC and gs-HMBC NMR spectra of compound **4**. Figure S5: 1H-15N HMBC NMR spectrum of compound **4**. Figure S6: 1H-13C 2 Hz LR-HSQMBC NMR spectra of compound **4**. Figure S7: The overlaid ^{1}H-^{13}C gs-HSQC (red) and 60 Hz 1,1-ADEQUATE (black) NMR spectra of compound **4**. Figure S8: 77Se NMR spectrum of compound **4**. Figure S9: IR spectrum of compound **4**. Figure S10: HRMS spectrum of compound **4**.

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Conflicts of Interest: The authors declare no conflict of interest.

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