Synthesis and Reactions of New Chiral Linear Dipeptide Candidates Using Nalidixic Acid as Starting Material

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A series of dipeptide heterocyclic derivatives **4–15** were synthesized using methyl 2-{[(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)carbonyl]amino}-3-ethylbutanoate (**3**) as starting material. Treatment of **3** with L-phenylalanine methyl ester hydrochloride afforded the corresponding dipeptide methyl ester derivative **4**, which was treated with hydrazine hydrate to afford the dipeptide acid hydrazide **5**. Compound **5** was coupled with aldehyde and acetophenone derivatives to afford the corresponding Schiff bases **6a**–**f**. The hydrazide derivative **5** was reacted with ethyl acetoacetate or acetone to give compounds **7** and **8**, respectively. Reaction of **5** with carbon disulfide at different conditions afforded compounds **9** and **10**, which were treated with hydrazine hydrate to give the 1-amino-2-dipeptido-1,3,4-triazole derivative **12**, which was cyclized with sodium hydroxide to the dipeptido 1-phenyl-1,3,4-triazole derivative **13**. Finally, treatment of **13** with methyl iodide afforded the *S*-methyl derivative **14**, which was reacted with hydrazine hydrate to give the hydrazine **15**.

Key words: Nalidixic Acid, Amino Acids, Chiral Dipeptide Candidates

Introduction

In previous work, Koskin and Merchant reported that certain substituted heterocyclic systems were synthesized *via* α , β -diketoesters using 2,3-pyrrolidinedione- α -acetic acid ethyl esters as starting materials [1, 2]. Peptides rarely function well as drugs due to their low bioavailability and rapid degradation within cells [3]. The conversion of these active peptides into peptidomimetics has been a successful approach for making new biologically active compounds [4]. Interestingly, some specific amino acids, exemplified by valine, leucine, isoleucine, glutamine, and phenylalanine, were reported early on to have anti-inflammatory properties [5–8]. Additionally, the specific inhibition of the inflammatory enzyme cyclooxygenase-2 (COX-2) by the natriuretic

peptide has also been reported [9]. These types of heterocyclic molecules have been shown to have various important biological activities such as antimicrobial [10], antileukemic [11], antihelminthic, anticonvulsant [12], antibacterial [13], antifungal [14], antitubercular [15], and anticancer [16] activity. In continuation of our previous work, we reported the synthesis of some heterocyclic candidates from dipicolinic acid with amino acids and he results of their biological activity screening [17-23]. Recently we also reported the synthesis of some linear and macrocyclic peptide candidates [24, 25] as PVC membrane [26] and miniaturized potentiometric sensors [27]. In view of these observations and as a continuation of our previous work in peptide-heterocyclic chemistry, we have synthesized some new dipeptide candidates that are bonded to a nalidixic acid moiety.

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Scheme 1. Synthetic route to compounds 2–6.

Results and Discussion

Chemistry

In the present study, we describe the synthesis and characterization of chiral dipeptides containing a nalidixic acid moiety and chiral amino acids. Synthesis of the acid **3** as starting material from coupling of **1** (nalidixic acid) with L-isoleucine methyl ester gave the corresponding peptide methyl ester **2**, which was hydrolyzed with methanolic sodium hydroxide according to the reported procedure [24]. Treatment of carboxamide acid **3** with L-phenylalanine methyl ester hydrochloride in the presence of ethyl chloroformate in dichloromethane afforded the corresponding dipeptide methyl ester derivative **4**, which was treated with methanolic hydrazine hydrate to afford the corresponding dipeptide acid hydrazide **5**. Compound **5** was condensed with appropriate ketonic derivatives to afford the corresponding Schiff bases **6a–f** (Scheme 1).

The hydrazide derivative **5** was reacted with refluxing ethyl acetoacetate or acetone to give the corresponding dipeptide pyrazole and dimethyl hydrazone derivatives **7** and **8**, respectively. Reaction of hydrazide **5** with carbon disulfide at room temperature afforded the corresponding potassium salt **9**, which was cyclized in the presence of potassium hydroxide to the oxadiazole derivative **10**. The latter compound can be



Scheme 2. Synthetic route to compounds 7–11.

obtained directly from compound **5** by heating with carbon disulfide. Treatment of compounds **9** and **10** with hydrazine hydrate gave the corresponding 1-amino 2-dipeptido-1,3,4-triazole derivative **11** (Scheme 2).

The dipeptide hydrazide derivative **5** was reacted with phenyl isothiocyanate to give the corresponding thiosemicarbazide derivative **12**, which was cyclized with sodium hydroxide to the corresponding dipeptido 1-phenyl-1,3,4-triazole derivative **13**. Finally, treatment of **13** with methyl iodide in DMF in the presence of anhydrous potassium carbonate afforded the corresponding *S*-methyl derivative **14**, which was reacted with hydrazine hydrate to give the hydrazine derivative **15**. Compound **15** can be obtained directly from compound **13** by heating with hydrazine hydrate (Scheme 3).



Scheme 3. Synthetic route to compounds 12-15.

Experimental

Melting points were determined in open glass capillary tubes with an Electro Thermal Digital melting point apparatus (model: IA9100) and are uncorrected. Elemental microanalysis data for carbon, hydrogen and nitrogen (Microanalytical Unit, NRC) were found within the acceptable limits of the calculated values. Infrared spectra (KBr) were recorded on a Nexus 670 FTIR Nicolet, Fourier Transform infrared spectrometer. Proton nuclear magnetic resonance (¹H NMR) spectra were run in ([D₆]DMSO) on Jeol 500 MHz instruments. Mass spectra were run on a MAT Finnigan SSQ 7000 spectrometer, using the electron impact technique (EI). Analytical thin layer chromatography (TLC) was performed on silica gel aluminum sheets, 60 F_{254} (E. Merck). Specific optical rotations were measured with an A. Krauss, Optronic, P8000 polarimeter, in a 1 dm length observation tube, at the indicated conditions, and according to the equation: $[\alpha]_D^T = 100\alpha(cl)^{-1}$, where: $\alpha =$ observed rotation angle, D = sodium line ($\lambda = 589$ nm), c = concentration (g per 100 mL), l = path length in dm, and T = experimental temperature (°C).

Synthesis of ethyl 2-[2-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)carboxamido-3-methylpentanoyl]amino-3-phenylpropanoate (**4**)

To a cold and stirred dry dichloromethane solution (25 mL, $-20 \degree C$) of the acid **3** (1 mmol), ethyl chloroformate (1 mmol) and triethylamine (1 mmol) were successively added. 10 min later, a cold methylene chloride solution (10 mL, $-20 \degree C$) of isoleucine methyl ester (1 mmol) was added. Stirring of the cold reaction mixture ($-20 \degree C$) was continued for 3 h, and at

r. t. for overnight. The solution was then washed with water, 1 N hydrochloric acid, 1 N sodium bicarbonate, and finally with water (250 mL). The dried solution (anhydrous CaCl₂) was evaporated, and the obtained oily residue was solidified by dry ether trituration, filtered off, dried under vacuum, and crystallized from methanol to afford the ester 4 in 57 % yield, m. p. $148 - 150 \,^{\circ}\text{C}$. $- [\alpha]_{\text{D}}^{25} = -102$ (c = 0.5, MeOH). - IR(KBr): v = 3375, 3219 (2NH), 1738 – 1668 (4 C=O) cm⁻¹. -¹H NMR (500 MHz, [D₆]DMSO): $\delta = 0.95$ (t, 3H, CH₃), 0.98 (t, 3H, CH₃), 1.09 (d, 3H, CH₃), 1.40 (m, 2H, CH₂), 2.35 (s, 3H, CH₃), 2.61 (m, 1H, CH), 3.02 (q, 2H, CH₂), 3.25 (d, 2H, CH₂), 3.81 (s, 3H, OCH₃), 4.56 (d, 1H, CH), 5.12 (t, 1H, CH), 6.85-8.11 (m, 8H, Ar-H), 8.96, 9.43 ppm (2s, 2H, 2NH, exchangeable with D_2O). – ¹³C NMR (125 MHz, $[D_6]DMSO$): $\delta = 11.53$, 13.27, 15.21, 25.01, 25.62, 36.87, 37.41, 49.46, 52.17, 54.22, 56.73, 112.65, 114.34, 119.02, 126.11, 127.86, 128.67, 137.96, 139.58, 148.87, 155.74, 159.84, 162.68, 170.98, 172.01, 178.33 ppm. - MS (EI, 70 eV): m/z (%) = 506 (22) [M]⁺. - C₂₈H₃₄N₄O₅ (506.59): calcd. C 66.38, H 6.76, N 11.06; found C 66.30, H 6.70, N 11.00.

Synthesis of [(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8naphthyridine)-2-carboxamide]-2-[(3-methylpentanoyl)amino]-3-phenylpropionic acid hydrazide (5)

A mixture of 4 (1 mmol) and hydrazine hydrate (16 mmol) in methanol (10 mL) was refluxed for 6 h. The solvent was evaporated under reduced pressure, the obtained residue was triturated with ether, filtered off, dried, and crystallized from methanol to afford acid hydrazide 5 in 54% yield, m.p. $183 - 185 \circ \text{C.} - [\alpha]_{\text{D}}^{25} = -32 \ (c = 0.5, \text{ MeOH}). - \text{IR (KBr):}$ v = 3413 - 3286 (3NH, NH₂), 1725 - 1660 (4 C=O) cm⁻¹. -¹H NMR (500 MHz, [D₆]DMSO): $\delta = 0.95$ (t, 3H, CH₃), 1.02 (t, 3H, CH₃), 1.10 (d, 3H, CH₃), 1.42 (qm, 2H, CH₂), 2.31 (s, 3H, CH₃), 2.64 (m, 1H, CH), 3.12 (q, 2H, CH₂), 3.21 (d, 2H, CH₂), 4.29 (s, 2H, NH₂, exchangeable with D₂O), 4.58 (d, 1H, CH), 5.10 (t, 1H, CH), 6.91-8.13 (m, 8H, Ar-H), 8.83, 9.12, 11.56 ppm (3s, 3H, 3NH, exchangeable with D_2O). – ¹³C NMR (125 MHz, [D₆]DMSO): δ = 11.37, 13.25, 14.74, 24.86, 25.19, 36.97, 38.01, 49.22, 53.81, 56.89, 112.47, 114.38, 118.90, 126.14, 127.85, 128.71, 138.17, 139.27, 149.01, 155.69, 159.88, 163.00, 171.24, 172.11, 179.02 ppm. – MS (EI, 70 eV): m/z (%) = 506 (12) [M]⁺. - C₂₇H₃₄N₆O₄ (506.60): calcd. C 64.01, H 6.76, N 16.59; found C 63.89, H 6.70, N 16.52.

Synthesis of [(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8naphthyridine)-2-carboxamide]-2-[(3-methylpentanoyl)amino]-3-phenylpropionic acid hydrazone derivatives **6a–e**

A solution of hydrazide **5** (1 mmol) and an aromatic ketone, namely, 4-isopropylbenzaldehyde, 4methoxybenzaldehyde, 3-indolecarboxaldehyde, 4-methoxyacetophenone or 3-acetylindole (1 mmol) in absolute methanol (50 mL) was refluxed for 5 h with stirring. The reaction mixture was allowed to stand at r.t. overnight, and volatiles evaporated under reduced pressure. The obtained residue was triturated with ether, filtered off, dried, and crystallized from ethanol to afford the corresponding hydrazone derivatives 6a-e.

6a: Yield 69%, m. p. 143-145 °C. - $[\alpha]_D^{25} = -184$ (c = 0.5, MeOH). – IR (KBr): v = 3382 - 3217 (3 NH), 1720-1668 (4 C=O) cm⁻¹. - ¹H NMR (500 MHz, $[D_6]DMSO$: $\delta = 0.98$ (t, 3H, CH₃), 1.05 (t, 3H, CH₃), 1.12 (d, 3H, CH₃), 1.37 (d, 6H, 2CH₃), 1.43 (m, 2H, CH₂), 2.32 (s, 3H, CH₃), 2.67 (m, 1H, CH), 3.09 (q, 2H, CH₂), 3.27 (d, 2H, CH₂), 3.32 (m, 1H, CH), 4.55 (d, 1H, CH), 5.14 (t, 1H, CH), 6.94-8.12 (m, 13H, Ar-H+=CH), 8.52, 9.15, 11.87 ppm (3s 3H, 3NH, exchangeable with D_2O). – ¹³C NMR (125 MHz, [D₆]DMSO): $\delta = 11.31, 13.62, 14.53,$ 23.48, 24.89, 25.12, 36.35, 36.92, 37.81, 49.25, 54.99, 56.34, 113.04, 114.21, 118.87, 126.10, 126.37, 127.76, 128.73, 129.11, 132.01, 138.22, 139.42, 143.33, 148.90, 151.28, 155.74, 159.83, 162.84, 171.20, 173.54, 178.67 ppm. -MS (EI, 70 eV): m/z (%) = 637 (8) [M]⁺. - C₃₇H₄₄N₆O₄ (636.78): calcd. C 69.79, H 6.96, N 13.20; found C 69.70, H 6.90, N 13.06.

6b: Yield 62%, m. p. 201–203 °C. – $[\alpha]_D^{25} = -96$ (c = 0.5, MeOH). – IR (KBr): v = 3426 - 3194 (3 NH), 1727 - 1675 (4 C=O) cm⁻¹. - ¹H NMR (500 MHz, $[D_6]DMSO$: $\delta = 0.95$ (t, 3H, CH₃), 1.02 (t, 3H, CH₃), 1.14 (d, 3H, CH₃), 1.42 (m, 2H, CH₂), 2.30 (s, 3H, CH₃), 2.68 (m, 1H, CH), 3.11 (q, 2H, CH₂), 3.29 (d, 2H, CH₂), 3.81 (s, 3H, CH₃), 4.59 (d, 1H, CH), 5.10 (t, 1H, CH), 6.89-8.14 (m, 13H, Ar-H + =CH), 8.64, 9.32, 12.05 ppm (3s, 3H, 3NH, exchangeable with D_2O). – ¹³C NMR (125 MHz, [D_6]DMSO): $\delta = 11.28, 13.56, 14.62, 24.90, 25.09, 36.98, 38.12, 49.15,$ 55.67, 56.23, 56.43, 113.64, 114.28, 115.03, 118.83, 126.18, 126.35, 127.73, 128.77, 130.26, 138.17, 139.58, 143.29, 148.80, 155.68, 159.76, 162.57, 163.14, 171.36, 176.45, 179.42 ppm. – MS (EI, 70 eV): m/z (%) = 624 (16) [M]⁺. - C₃₅H₄₀N₆O₅ (624.73): calcd. C 67.29, H 6.45, N 13.45; found C 67.22, H 6.40, N 13.40.

6c: Yield 55%, m. p. 256–258 °C. – $[\alpha]_D^{25} = -11$ (*c* = 0.5, MeOH). – IR (KBr): *v* = 3480–3215 (4NH), 1723–1668 (4 C=O) cm⁻¹. – ¹H NMR (500 MHz, [D₆]DMSO): δ = 0.96 (t, 3H, CH₃), 1.05 (t, 3H, CH₃), 1.11 (d, 3H, CH₃), 1.40 (m, 2H, CH₂), 2.35 (s, 3H, CH₃), 2.60 (m, 1H, CH), 3.14 (q, 2H, CH₂), 3.24 (d, 2H, CH₂), 4.68 (d, 1H, CH), 5.17 (t, 1H, CH), 6.94–8.13 (m, 14H, Ar-H + =CH), 8.70, 9.46, 11.83, 12.65 ppm (4s, 4H, 4NH, exchangeable with D₂O). – ¹³C NMR (125 MHz, [D₆]DMSO): δ = 11.24, 13.61, 14.58, 24.67, 37.01, 38.10, 49.12, 55.71, 56.43, 56.43, 105.23, 111.08, 113.84, 114.21, 118.65, 119.32, 121.04, 123.19, 126.02, 126.30, 127.79, 128.69, 131.21, 136.15, 138.20, 139.52, 143.21, 148.82, 155.59, 159.66, 162.58, 171.34, 175.47, 179.12 ppm. – MS (EI, 70 eV): m/z (%) = 633 [M]⁺. – C₃₆H₃₉N₇O₄ (633.74): calcd. C 68.23, H 6.20, N 15.47; found C 68.15, H 6.14, N 15.40.

6d: Yield 69%, m.p. 218–220 °C. – $[\alpha]_D^{25} = -39$ (c = 0.5, MeOH). – IR (KBr): v = 3376 - 3210 (3 NH), 1721 - 1673 (4 C=O) cm⁻¹. - ¹H NMR (500 MHz, $[D_6]DMSO$: $\delta = 0.99$ (t, 3H, CH₃), 1.00 (t, 3H, CH₃), 1.12 (d, 3H, CH₃), 1.26 (s, 3H, CH₃), 1.41 (m, 2H, CH₂), 2.36 (s, 3H, CH₃), 2.63 (m, 1H, CH), 3.12 (q, 2H, CH₂), 3.21 (d, 2H, CH₂), 3.86 (s, 3H, CH₃), 4.56 (d, 1H, CH), 5.14 (t, 1H, CH), 6.85-8.10 (m, 12H, Ar-H), 8.82, 9.17, 10.73 ppm (3s, 3H, 3NH, exchangeable with D_2O). – ¹³C NMR (125 MHz, $[D_6]DMSO$): $\delta = 11.34$, 13.26, 14.52, 21.46, 24.71, 24.89, 36.92, 38.03, 49.21, 55.68, 55.97, 56.47, 113.63, 114.25, 114.56, 118.79, 126.12, 126.38, 127.74, 128.71, 130.23, 138.15, 139.55, 148.78, 155.64, 159.62, 162.53, 163.21, 169.05, 171.29, 177.12, 179.00 ppm. – MS (EI, 70 eV): m/z $(\%) = 638 (24) [M]^+$. - C₃₆H₄₂N₆O₅ (638.76): calcd. C 67.69, H 6.63, N 13.16; found C 67.62, H 6.56, N 13.10.

6e: Yield 58%, m. p. 229–231 °C. – $[\alpha]_D^{25} = -134$ (c = 0.5, MeOH). – IR (KBr): v = 3428 - 3186 (4NH), 1719-1665 (4 C=O) cm⁻¹. - ¹H NMR (500 MHz, $[D_6]DMSO$): $\delta = 0.98$ (t, 3H, CH₃), 1.00 (t, 3H, CH₃), 1.10 (d, 3H, CH₃), 1.23 (s, 3H, CH₃), 1.43 (m, 2H, CH₂), 2.33 (s, 3H, CH₃), 2.67 (m, 1H, CH), 3.12 (q, 2H, CH₂), 3.20 (d, 2H, CH₂), 4.71 (d, 1H, CH), 5.15 (t, 1H, CH), 6.89-8.11 (m, 13H, Ar-H), 8.59, 9.25, 10.86, 12.05 ppm (4s, 4H, 4NH, exchangeable with D_2O). – ¹³C NMR (125 MHz, $[D_6]DMSO$: $\delta = 11.20, 13.46, 14.54, 21.57, 24.65, 24.88,$ 36.95, 37.78, 49.15, 55.73, 56.48, 111.03, 112.64, 113.82, 114.24, 118.67, 119.20, 120.35, 122.58, 126.10, 126.36, 127.83, 128.78, 130.91, 135.65, 138.26, 139.51, 148.79, 155.61, 159.60, 162.5, 168.75, 171.32, 176.82, 179.23 ppm. -MS (EI, 70 eV): m/z (%) = 647 (12) $[M]^+$. $-C_{37}H_{41}N_7O_4$ (647.77): calcd. C 68.60, H 6.38, N 15.14; found C 68.42, H 6.32, N 15.10.

Synthesis of (1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8naphthyridine-2-carboxamide)-2-[(3-methyl-pentanoyl) amino]-3-phenylpropionic acid mannosyl hydrazone (**6**f)

A mixture of hydrazide **5** (10 mmol) and D-mannose (10 mmol) in ethanol (30 mL) containing a few drops of acetic acid was refluxed for 2 h. After cooling, the precipitate was filtered off, washed with ethanol, dried, and crystallized from methanol to afford compound **6f** in 71% yield, m. p. 147–149 °C. – $[\alpha]_D^{25} = -112$ (c = 0.5, MeOH). – IR (KBr): v = 3486 - 3194 (OH, NH), 1728–1663 (4 C=O) cm⁻¹. – ¹H NMR (500 MHz, [D₆]DMSO): $\delta = 0.95$ (t, 3H, CH₃), 1.01 (t, 3H, CH₃), 1.12 (d, 3H, CH₃), 1.40 (m, 2H, CH₂), 2.36 (s, 3H, CH₃), 2.63 (m, 1H, CH), 3.09 (q, 2H, CH₂), 3.19

(d, 2H, CH₂), 3.26–3.34 (m, 2H, 6'-H, 6"-H), 3.45–3.52 (m, 3H, 5'-H, 4'-H, 6'-OH, exchangeable with D₂O), 4.15 (d, 1H, 5'-OH, exchangeable with D₂O), 4.38 (d, 1H, 4'-OH, exchangeable with D₂O), 4.49 (m, 3H, 2'-H, 3'-H, 3'-OH, exchangeable with D₂O), 4.65 (d, 1H, CH), 4.79 (d, 1H, 2'-OH, exchangeable with D₂O), 5.10 (t, 1H, CH), 6.91 (d, 1H, 1'-H), 6.95–8.12 (m, 9H, Ar-H + =CH), 8.72, 10.31, 11.57 ppm (3s, 3H, 3NH, exchangeable with D₂O). – ¹³C NMR (125 MHz, [D₆]DMSO): δ = 11.24, 13.54, 14.62, 24.65, 24.91, 37.12, 38.02, 49.10, 55.68, 56.41, 61.22, 64.75, 70.89, 72.16, 73.26, 113.82, 114.20, 118.61, 126.10, 127.74, 128.65, 138.29, 139.58, 148.88, 154.18, 155.61, 159.70, 162.56, 171.36, 178.01, 179.23 ppm. – MS (EI, 70 eV): m/z (%) = 669 (8) [M]⁺. – C₃₃H₄₄N₆O₉ (668.74): calcd. C 59.27, H 6.63, N 12.57; found C 59.22, H 6.56, N 12.50.

Synthesis of N-(1-(1-(3-methyl-5-oxopyrazol-2-yl)-2phenylethylcarbamoyl)-2-methylbutyl)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxamide (7)

To a mixture of acid hydrazide 5 (10 mmol) and ethyl acetoacetate (10 mmol) in ethanol (20 mL), a few drops of piperidine were added. The reaction mixture was refluxed for 8 h, the precipitated solid was filtered off, washed with water, dried, and recrystallized from ethanol to afford compound 7 in 51% yield; m. p. 260–262 °C. – $[\alpha]_D^{25} = -104$ (c = 0.5, MeOH). – IR (KBr): v = 3437, 3217 (2 NH), 1728 – 1675 (5 C=O) cm⁻¹. – ¹H NMR (500 MHz, [D₆]DMSO): δ = 0.96 (t, 3H, CH₃), 1.00 (t, 3H, CH₃), 1.12 (d, 3H, CH₃), 1.40 (m, 2H, CH₂), 1.84 (s, 3H, CH₃), 2.34 (s, 3H, CH₃), 2.68 (m, 1H, CH), 3.10 (q, 2H, CH₂), 3.26 (d, 2H, CH₂), 4.24 (s, 2H, CH₂), 4.55 (d, 1H, CH), 5.17 (t, 1H, CH), 6.93-8.14 (m, 8H, Ar-H), 8.76, 9.35 ppm (2s, 2H, 2NH, exchangeable with D₂O). $-{}^{13}$ C NMR (125 MHz, [D₆]DMSO): $\delta = 11.34$, 13.22, 14.71, 24.85, 25.10, 26.40, 36.93, 37.89, 49.16, 51.73, 53.62, 56.42, 112.54, 114.35, 118.96, 126.07, 127.84, 128.78, 138.21, 139.45, 148.76, 155.72, 159.49, 159.81, 162.50, 162.89, 171.21, 176.46, 179.11 ppm. - MS (EI, 70 eV): m/z (%) = 572 (6) [M]⁺. - C₃₁H₃₆N₆O₅ (572.65): calcd. C 65.02, H 6.34, N 14.68; found C 64.95, H 6.30, N 14.62.

Synthesis of N-(propan-2-ylidene)-4-(1H-pyrrol-1-yl)hydrazide (8)

A solution of acid hydrazide **5** (0.005 mol) in acetone (70 mL) was refluxed for 3 h. The solvent was evaporated under reduced pressure, and the obtained pale-yellow solid crystallized from ethanol to give compound **8** in 76% yield, m. p. 184–186 °C. – $[\alpha]_D^{25} = -136$ (c = 0.5, MeOH). – IR (KBr): v = 3476-3198 (3 NH), 1725–1660 (4 C=O) cm⁻¹. – ¹H NMR (500 MHz, [D₆]DMSO): $\delta = 0.97$ (t, 3H, CH₃), 1.02 (t, 3H, CH₃), 1.10 (d, 3H, CH₃), 1.46 (m, 2H, CH₂), 1.96 (s, 3H, CH₃), 2.01 (s, 3H, CH₃), 2.37 (s, 3H, CH₃), 2.71

(m, 1H, CH), 3.15 (q, 2H, CH₂), 3.20 (d, 2H, CH₂), 4.59 (d, 1H, CH), 5.18 (t, 1H, CH), 6.93 – 8.11 (m, 8H, Ar-H), 8.64, 9.48, 10.85 ppm (3s, 3H, 3NH, exchangeable with D₂O). – ¹³C NMR (125 MHz, [D₆]DMSO): δ = 11.26, 13.21, 14.78, 17.38, 24.80, 24.97, 25.12, 37.05, 38.03, 49.23, 55.64, 56.45, 113.87, 114.21, 118.73, 126.10, 127.89, 128.71, 138.27, 139.55, 148.77, 151.68, 155.83, 159.47, 162.58, 171.25, 177.34, 179.20 ppm. – MS (EI, 70 eV): m/z (%) = 547 (32) [M]⁺. – C₃₀H₃₈N₆O₄ (546.66): calcd. C 65.91, H 7.01, N 15.37; found C 65.84, H 6.90, N 15.30.

Synthesis of the potassium salt of the thiosemicarbazide derivative $\boldsymbol{9}$

To a cold stirred solution of acid hydrazide **5** (10 mmol) in absolute ethanol (100 mL) containing potassium hydroxide (15 mmol), carbon disulfide (15 mmol) was added gradually. The reaction mixture was stirred at room temperature for 8 h. A yellow precipitate of the corresponding potassium salt **9** separated. Then, dry ether (100 mL) was added to complete the precipitation of the formed salt which was filtered off and washed with dry ether (100 mL). The potassium salt was obtained in quantitative yield and used in the next step without further purification. Yield: 93 %; m. p. > 300 °C. – IR (KBr): v = 3480-3167 (4 NH), 1726–1664 (4 C=O) cm⁻¹. – MS (EI, 70 eV): m/z (%) = 621 (5) [M]⁺. – C₂₈H₃₃KN₆O₄S₂ (620.83): calcd. C 54.17, H 5.36, N 13.54, S 10.33; found C 54.10, H 5.30, N 13.50, S 10.26.

Synthesis of N-(1-(1-(5-mercapto-1,3,4-oxadiazol-2-yl)-2phenylethylcarbamoyl)-2-methylbutyl)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxamide (**10**)

Method A: Compound **5** (10 mmol) and CS₂ (10 mmol) were added to a solution of KOH (10 mmol) in a mixture of water/ethanol (100 mL, v/v 1 : 1). The reaction mixture was refluxed for 3 h, and then acidified with conc. HCl. The precipitate was filtered off, washed with H₂O, dried, and crystallized from ethanol to afford compound **10** in 78 % yield.

Method B: A solution of potassium hydroxide (15 mmol) and the potassium salt **9** (10 mmol) in absolute ethanol (100 mL) was refluxed for 4 h, till the evolution of H₂S ceased. The reaction mixture was diluted with water and acidified with HCl. The precipitated solid was filtered off, washed with water, dried, and finally crystallized with ethanol to give compound **10** in 81% yield; m. p. 243 – 245 °C. – $[\alpha]_D^{25} = -122$ (c = 0.5, MeOH). – IR (KBr): v = 3389, 3246 (2 NH), 1721 – 1667 (3 C=O) cm⁻¹. – ¹H NMR (500 MHz, [D₆]DMSO): $\delta = 0.95$ (t, 3H, CH₃), 1.03 (t, 3H, CH₃), 1.12 (d, 3H, CH₃), 1.43 (m, 2H, CH₂), 2.37 (s, 3H, CH₃), 2.71 (m, 1H, CH), 3.14 (q, 2H, CH₂), 3.18 (d, 2H, CH₂), 4.57 (d, 1H, CH), 5.19 (t, 1H, CH), 6.87–8.10 (m, 8H, Ar-H), 8.63, 9.16 (2s, 2H, 2NH, exchangeable with D₂O); 12.85 ppm (s, 1H, SH, exchangeable with D₂O). – ¹³C NMR (125 MHz, [D₆]DMSO): δ = 11.35, 13.22, 14.76, 24.82, 25.34, 36.91, 43.27, 49.12, 53.61, 56.49, 113.02, 114.32, 118.91, 126.04, 127.80, 128.76, 133.87, 138.25, 139.48, 148.73, 155.69, 159.52, 160.01, 162.59, 171.28, 178.16 ppm. – MS (EI, 70 eV): m/z (%) = 548 (18) [M]⁺. – C₂₈H₃₂N₆O₄S (548.66): calcd. C 61.30, H 5.88, N 15.32, S 5.84; found C 61.23, H 5.82, N 15.27, S 5.80.

Synthesis of N-(1-(1-(4-amino-5-mercapto-4H-1,2,4-triazol-2-yl)-2-phenylethylcarbamoyl)-2-methylbutyl)-1-ethyl-7methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (11)

Method A: The potassium salt **9** (10 mmol) was suspended in 80% hydrazine hydrate (5 mL) and the reaction mixture refluxed for 3 h. The formed solid was filtered off, washed with water, dried, and finally crystallized with DMF/ethanol to afford compound **11** in 65% yield.

Method B: A solution of oxadiazole 10 (10 mmol) in ethanol (20 mL) and 80% hydrazine hydrate (5 mL) was refluxed for 3 h, then allowed to cool, diluted with cold water, and acidified with HCl. The precipitated solid was filtered, washed with water, dried, and recrystallized with ethanol/DMF to give compound 11 in 59% yield; m.p. $187 - 189 \,^{\circ}\text{C}. - [\alpha]_{\text{D}}^{25} = +34 \ (c = 0.5, \text{ MeOH}). - \text{IR (KBr)}.$ v = 3478 - 3196 (2 NH, NH₂), 1726 - 1663 (3 C=O) cm⁻¹. -¹H NMR (500 MHz, [D₆]DMSO): $\delta = 0.96$ (t, 3H, CH₃), 0.99 (t, 3H, CH₃), 1.10 (d, 3H, CH₃), 1.41 (m, 2H, CH₂), 2.38 (s, 3H, CH₃), 2.65 (m, 1H, CH), 3.17 (q, 2H, CH₂), 3.22 (d, 2H, CH₂), 4.56 (d, 1H, CH), 5.15 (t, 1H, CH), 5.87 (s, 2H, NH₂, exchangeable with D₂O), 6.88-8.11 (m, 8H, Ar-H), 8.45, 9.39 (2s, 2H, 2NH, exchangeable with D₂O), 12.98 ppm (s, 1H, SH, exchangeable with D_2O). – ¹³C NMR (125 MHz, [D₆]DMSO): $\delta = 11.32$, 13.28, 14.70, 24.85, 25.11, 36.84, 43.15, 49.10, 53.66, 56.45, 113.23, 114.37, 118.92, 126.08, 127.84, 128.78, 138.29, 139.56, 148.67, 155.64, 159.47, 160.05, 162.61, 167.35, 171.23, 177.59 ppm. -MS (EI, 70 eV): m/z (%) = 562 (4) $[M]^+$. $-C_{28}H_{34}N_8O_3S$ (562.69): calcd. C 59.77, H 6.09, N 19.91, S, 5.70; found C 59.70, H 6.00, N 19.84, S, 5.64.

Synthesis of the hydrazinecarbothiamide derivative 12

A mixture of compound **5** (0.01 mol) and phenylisothiocyanate (0.01 mol) in ethanol (50 mL) was allowed to reflux for 3 h. After cooling, the obtained solid was filtered off, washed with cold ethanol, dried, and crystallized from ethanol to yield compound **12** in 87% yield; m. p. 146–148 °C. – $[\alpha]_D^{25} = +51$ (c = 0.5, MeOH). – IR (KBr): v = 3471 - 3168 (5 NH), 1722–1684 (3 C=O), 1305 (C=S) cm⁻¹. – ¹H NMR (500 MHz, [D₆]DMSO): $\delta = 0.98$ (t, 3H, CH₃), 1.05 (t, 3H, CH₃), 1.12 (d, 3H, CH₃), 1.40 (m, 2H, CH₂), 2.35 (s, 3H, CH₃), 2.67 (m, 1H, CH), 3.10 (q, 2H, CH₂), 3.24 (d, 2H, CH₂), 4.561 (d, 1H, CH), 5.13 (t, 1H, CH), 6.68–8.15 (m, 13H, Ar-H), 8.56, 9.70, 9.86, 10.84, 12.23 ppm (5s, 5H, 5NH, exchangeable with D₂O). – ¹³C NMR (125 MHz, [D₆]DMSO): $\delta = 11.29$, 13.25, 14.73, 24.88, 25.10, 36.95, 38.12, 49.16, 55.97, 56.42, 113.21, 114.35, 118.94, 124.87, 126.11, 126.49, 127.86, 128.75, 129.17, 137.23, 138.27, 139.55, 148.69, 155.64, 159.45, 162.68, 171.18, 171.75, 177.54, 181.24 ppm. – MS (EI, 70 eV): m/z (%) = 642 (6) [M]⁺. – C₃₄H₃₉N₇O₄S (641.78): calcd. C 63.63, H 6.13, N 15.28, S 5.00; found C 63.55, H 6.06, N 15.20, S 4.92.

Synthesis of N-(1-(1-(5-mercapto-4-phenyl-4H-1,2,4triazol-3-yl)-2-phenylethylcarbamoyl)-2-methylbutyl)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxamide (13)

A solution of hydrazine carbothiamide derivative 12 (0.01 mol) in sodium hydroxide (5 mL, 2 N) was refluxed for 3 h. The resulting solution was cooled to r.t. and acidified to pH 3-4 with 37% hydrochloric acid. The precipitate formed was filtered off, washed with distilled water, dried and crystallized from methanol to furnish the title compound **13** in 79 % yield, m. p. $127 - 129 \,^{\circ}\text{C}$. $- [\alpha]_{\text{D}}^{25} = -71 \ (c = 0.5,$ MeOH). - IR (KBr): v = 3341, 3256 (2 NH), 1720-1672 (3 C=O) cm⁻¹. – ¹H NMR (500 MHz, [D₆]DMSO): δ = 0.97 (t, 3H, CH₃), 1.00 (t, 3H, CH₃), 1.09 (d, 3H, CH₃), 1.44 (m, 2H, CH₂), 2.36 (s, 3H, CH₃), 2.61 (m, 1H, CH), 3.16 (q, 2H, CH₂), 3.25 (d, 2H, CH₂), 4.59 (d, 1H, CH), 5.22 (t, 1H, CH), 6.73-8.14 (m, 13H, Ar-H), 8.98, 11.34 (2s, 2H, 2NH, exchangeable with D₂O), 12.82 ppm (s, 1H, SH, exchangeable with D_2O). – ¹³C NMR (125 MHz, [D₆]DMSO): δ = 11.38, 13.26, 14.64, 24.81, 25.01, 36.89, 37.15, 49.12, 53.41, 55.62, 113.26, 113.77, 114.35, 117.46, 118.97, 126.02, 127.80, 128.74, 129.71, 138.25, 139.58, 144.58, 148.47, 148.69, 155.68, 159.44, 162.67, 169.21, 171.19, 177.51 ppm. - MS (EI, 70 eV): m/z (%) = 624 (15) [M]⁺. - C₃₄H₃₇N₇O₃S (623.77): calcd. C 65.47, H 5.98, N 15.72, S 5.14; found C 65.40, H 5.90, N 15.65, S 5.10.

Synthesis of N-(1-(1-(5-(methylthio)-4-phenyl-4H-1,2,4triazol-3-yl)-2-phenylethylcarbamoyl)-2-methyl-butyl)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxamide (14)

To a stirred solution of compound **13** (0.01 mol) in anhydrous DMF (10 mL), K_2CO_3 (0.01 mol) and methyl iodide (0.01 mol) were added. The stirring was continued at room temperature for 16 h. The reaction mixture was poured into cold water (150 mL), the resulting precipitate was collected by filtration and washed with small portions of water, methanol and ether, dried, and crystallized from DMF/H₂O to give compound 14 in 68% yield, m.p. 168-170 °C. – $[\alpha]_{\rm D}^{25} = -86 \ (c = 0.5, \text{ MeOH}). - \text{IR} \ (\text{KBr}): v = 3324, 3182$ (2 NH), 1719–1661 (3 C=O) cm⁻¹. – ¹H NMR (500 MHz, [D₆]DMSO): $\delta = 0.96$ (t, 3H, CH₃), 1.02 (t, 3H, CH₃), 1.12 (d, 3H, CH₃), 1.40 (m, 2H, CH₂), 2.31 (s, 3H, CH₃), 2.39 (s, 3H, SCH₃), 2.67 (m, 1H, CH), 3.11 (q, 2H, CH₂), 3.24 (d, 2H, CH₂), 4.54 (d, 1H, CH), 5.14 (t, 1H, CH), 6.68-8.12 (m, 13H, Ar-H), 8.61, 9.46 ppm (2s, 2H, 2NH, exchangeable with D_2O). – ¹³C NMR (125 MHz, [D₆]DMSO): $\delta = 11.35, 13.24, 14.66, 15.01, 24.79, 25.11, 36.92, 40.72,$ 49.15, 53.42, 56.02, 113.28, 114.30, 118.94, 126.05, 127.85, 128.00, 128.71, 128.76, 129.32, 130.04, 138.23, 139.54, 147.67, 148.65, 155.62, 159.58 162.75, 169.18, 171.25, 178.12 ppm. – MS (EI, 70 eV): m/z (%) = 638 (22) [M]⁺. - C35H39N7O3S (637.79): calcd. C 65.91, H 6.16, N 15.37, S 5.03; found C 65.84, H 6.10, N 15.30, S 4.97.

Synthesis of N-(1-(1-(5-hydrazino-4-phenyl-4H-1,2,4triazol-3-yl)-2-phenylethylcarbamoyl)-2-methyl-butyl)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxamide (15)

A mixture of 3-(5-(methylthio)-4-phenyl-4H-1,2,4triazol-3-yl) 13 (3 mmol) or the 3-(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)-derivative 14 (3 mmol) and hydrazine hydrate (80%, 5 mL) was refluxed for 6 h. The reaction mixture was evaporated under reduced pressure to remove excess hydrazine hydrate and was allowed to cool. The formed solid product was filtered off, washed with ethanol, and recrystallized from DMF/H2O to give compound **15** in 57% yield; m. p. 283-285 °C. - $[\alpha]_D^{25} = -152$ (c = 0.5, MeOH). – IR (KBr): v = 3476 - 3147 (3 NH, NH₂), 1723-1663 (3 C=O) cm⁻¹. - ¹H NMR (500 MHz, $[D_6]DMSO$: $\delta = 0.95$ (t, 3H, CH₃), 1.00 (t, 3H, CH₃), 1.10 (d, 3H, CH₃), 1.43 (m, 2H, CH₂), 2.33 (s, 3H, CH₃), 2.63 (m, 1H, CH), 3.17 (q, 2H, CH₂), 3.25 (d, 2H, CH₂), 4.57 (d, 1H, CH), 5.19 (t, 1H, CH), 5.79 (s, 2H, NH₂), 6.95-8.11 (m, 13H, Ar-H), 8.73, 9.43, 11.87 ppm (3s, 3H, 3NH, exchangeable with D_2O). – ¹³C NMR (125 MHz, $[D_6]DMSO$: $\delta = 11.31, 13.23, 14.67, 24.75, 25.09, 37.01,$ 41.55, 49.11, 53.49, 56.17, 113.26, 114.32, 118.92, 126.01, 127.80, 128.10, 128.65, 128.87, 129.38, 130.12, 138.28, 139.56, 147.98, 148.60, 155.61, 159.54 162.72, 169.17, 171.35, 177.73 ppm. – MS (EI, 70 eV): m/z (%) = 621 (34) $[M]^+$. – C₃₄H₃₉N₉O₃ (621.73): calcd. C 65.68, H 6.32, N 20.28; found C 65.60, H 6.24, N 20.20.

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