

Research Article

Stereochemical Investigations of Diastereomeric N-[2-(Aryl)-5methyl-4-oxo-1,3-thiazolidine-3-yl]-pyridine-3-carboxamides by Nuclear Magnetic Resonance Spectroscopy (1D and 2D)

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Some new *N*-[2-(aryl)-5-methyl-4-oxo-1,3-thiazolidine-3-yl]-pyridine-3-carboxamides were synthesized and their structures were investigated by IR, NMR (¹H, ¹³C, and 2D), and mass spectra. The presence of C-2 and C-5 stereogenic centers on the thiazolidinone ring resulted in diastereoisomeric pairs. The configurations of two stereogenic centers were assigned based upon ¹H NMR analysis of coupling constants and 2D nuclear overhauser enhancement spectroscopy (NOESY) experiment. Resolution of the diastereoisomers was performed by high performance liquid chromatography (HPLC) using a chiral stationary phase.

1. Introduction

Pyridine-3-carboxamide (nicotinamide), known as vitamin PP (pellagra protective), is part of the vitamin B group and plays an important role in biological oxidative chemistry. Pyridine-3-carboxamide derivatives have gained attention because of their diverse pharmacological activities, such as cytoprotective [1], antiviral [2], antitumor [3], and anxiolytic [4] activities.

Thiazolidin-4-one derivatives possess versatile biological activities [5], including antifungal [6], antibacterial [7, 8], anticancer [9, 10], anti-inflammatory [11–13], analgesic [14], anticonvulsant [15, 16], antiviral [17, 18], and antidiabetic activities [19, 20].

Currently, nearly 50% of the drugs are in use as racemates. But stereochemical factors generally have important influence on biological activity of the drug molecules. The two enantiomers present in a racemic mixture can possess different biological activities; that is, one enantiomer has therapeutic value; the other enantiomer may be less effective, inactive, or highly toxic [21–27]. Therefore, the identification and separation of stereoisomers are considered to be important. Chiral compounds bearing thiazolidin-4-one ring have also been studied for their stereochemistry. Several studies have been done on these compounds regarding enantiodifferentiation of stereoisomers in the presence of chiral auxiliary [28], separation of enantiomers by chiral HPLC [29, 30], and determination of absolute conformations [31, 32].

It is well known that combinations of two or more heterocyclic scaffolds in one molecule can provide a series of compounds with a broad spectrum of biological activity. Here, we combine thiazolidin-4-one and pyridine-3-carboxamide scaffolds together as part of an ongoing project directed towards the design and synthesis of biologically active nitrogen and sulfur containing heterocyclic compounds [33]. Our research focused on stereochemical investigations on diastereomeric N-[2-(aryl)-5-methyl-4-oxo-1,3-thiazolidine-3-yl]-pyridine-3-carboxamides (**2a**-**f**) (Figure 1) by one- and two-dimensional NMR techniques. In addition, the analytical chromatographic separation of some derivatives by chiral HPLC has been examined using a chiral column.

2. Experimental

2.1. General. 1D ¹H and ¹³C NMR spectra of all compounds were recorded on a Varian-Unity Inova 500 spectrometer

Entry	C-6 methyl	C-5 methine	C-2 methine	CO-NH
2a	1.55 (d, J = 7.0 Hz)	4.13 (qd, $J = 7.0$ Hz, 0.97 Hz) 4.23 (qd, $J = 7.0, 1.47$ Hz) ^b	5.92 (s)	10.94 (s)
2b	1.54 (d, J = 7.0) 1.55 (d, I = 6.8 Hz)	4.12 (q, $J = 6.8 \text{ Hz})^{\text{b}}$ 4.22 (q, $I = 6.8 \text{ Hz})^{\text{c}}$	5.90 (s)	10.94 (s) 10.95 (s)
2c	$\frac{1.55 \text{ (d, } J = 7.3 \text{ Hz})}{1.57 \text{ (d, } J = 7.3 \text{ Hz})}$	$4.15 (q, J = 6.8 Hz)^{b}$ 4.25 (qd, J = 6.8, 1.47 Hz)	6.01 (d, J = 1.47 Hz) 6.02 (s)	10.99 (s) 11.01 (s)
2d	1.53 (d, <i>J</i> = 7.3 Hz) 1.55 (d, <i>J</i> = 7.3 Hz)	4.10 (q, $J = 7.3$ Hz) 4.16 (qd, $J = 7.3, 1.47$ Hz) ^b	5.87 (s) 5.88 (d, J = 1.47 Hz)	10.90 (s)
2e	1.54 (d, <i>J</i> = 7.3) 1.55 (d, <i>J</i> = 6.8 Hz)	4.11 (q, $J = 6.8 \text{ Hz})^{\text{b}}$ 4.18 (qd, $J = 6.8, 1.46 \text{ Hz})$	5.89 (s) 5.90 (s)	10.97 (s)
2f	1.48 (d, $J = 7.3$) 1.52 (d, $J = 6.8$ Hz)	4.10 (q, $J = 7.0$ Hz) ^b 4.21 (qd, $J = 7.0, 1.96$ Hz)	6.22 (d, <i>J</i> = 1.96 Hz) 6.26 (s)	11.04 (s) 11.05 (s)

TABLE 1: ¹H NMR (500 MHz) data of compounds 2a-2f in DMSO- d_6 .^a

^aFor ¹H NMR data of the other protons, see Section 2.

^bThe signals corresponding to major diastereomer.

^cCoupling with C-2 methine was observed as a shoulder.



FIGURE 1: The synthesized compounds, 2a-f.

operating at 499.7 MHz for ¹H and 124.9 MHz for ¹³C, using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) were reported in parts per million (ppm). Spectral widths of 14 and 230 ppm were used in ¹H and ¹³C NMR, respectively. The splitting patterns of ¹H NMR were designed as follows: s: singlet, d: doublet, q: quartet, qd: quartet of doublets, dd: doublet of doublets, and m: multiplet. NOESY experiment was performed on a Varian-Mercury VX-400-BB (spectrometer frequency: 399.98 MHz, temperature: 24°C, relaxation delay: 2.0 sec, acquisition time: 0.15 sec, number of increments: the number of points in tl: 200, number of points in each FID (t2): 1920, and spectral width: ¹H channel; 14 ppm). HMBC experiment was performed on a Varian-Unity Inova 500 spectrometer (spectrometer frequency: 499.7 MHz, temperature: 30°C, relaxation delay: 1.0 sec, acquisition time: 0.128 sec, 400 increments, and spectral width: ¹H channel; 14 ppm, ¹³C channel: 230 ppm). IR analyses were performed on a Shimadzu IR Affinity-I FTIR using KBr discs; peaks are reported in cm⁻¹. UV analyses were performed on Shimadzu UV-1601; wavelengths are reported in nm. Liquid chromatography analyses were performed on Shimadzu SCL-10AVP with a diode array detector and using Chiralpak AD column (particle size: $5 \mu m$, column size: 250 × 4.6 mm). Eluent was n-hexane: 2-propanol (85:15) (v:v) with a flow rate of 0.9 mL min⁻¹. Reactions

were followed by TLC using silica gel 60- F_{254} . Elemental analyses were performed on Thermo Finnigan Flash EA 1112 CHNS-932 analyzer. Melting points were recorded using Buchi B-540 melting point apparatus. The mass spectra were obtained using Finnigan LCQ Advantage Max Waters 2695 Alliance Micromass ZQ.

2.1.1. General Procedure for the Preparation of N-[2-(Aryl)-5methyl-4-oxo-1,3-thiazolidine-3-yl]-pyridine-3-carboxamides. To a suspension of 0.01 mol of aryl N'-(substituted benzylidene)pyridine-3-carbohydrazide (**1a-f**) in 30 mL dry ben zene was added 2.5 mL (0.028 mol) of 2-sulfanylpropanoic acid. The mixture was refluxed for 6-18 hours using a Dean-Stark trap. Excess benzene was evaporated in vacuo. The resulting residue was triturated with NaHCO₃ solution until CO_2 evolution ceased and was allowed to stand refrigerated until solidification. The solid thus obtained was washed with water, dried, and recrystallized from ethanol.

Some spectral and X-ray crystallographic data of compounds **2a**, **2b**, and **2f** were reported regardless of stereochemistry in our previously published articles [34–36].

2.1.2. *N*-[2-(4-Chlorophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3yl]-pyridine-3-carboxamide (**2a**). Diastereomer ratio % (major/ minor): 54 : 46. ¹H-NMR (500 MHz, DMSO- d_6) &: 7.43–7.47 (2H, m, phenyl-H); 7.48–7.50 (1H, m, pyridine-H); 7.52-7.53 (2H, m, phenyl-H); 8.04–8.09 (1H, m, pyridine-H); 8.73 (1H, dd, *J* = 6.3 Hz, 1.4 Hz, pyridine-H); 8.84, 8.85 (1H, 2d, *J* = 2.9 Hz, 1.4 Hz, pyridine-H) [35] (for ¹H NMR data of other protons see Table 1). ¹³C-NMR (125 MHz) (DMSO- d_6) δ (ppm): 20.6 (C-6, CH₃); 39.8, 39.9 (C-5, CH); 60.4, 60.6 (C-2, CH); 124.3, 124.4 (C13, CH); 127.9, 128.0 (C9, C); 129.3, 129.4 (C16, 20, CH); 130.4, 130.7 (C17, 19, CH); 134.2, 134.4 (C18, C); 135.9, 136.0 (C14, CH); 137.2, 138.1 (C15, C); 149.1, 149.2 (C10, CH); 153.5, 153.6 (C12, CH); 164.5, 164.6 (C-8, C=O); 172.5, 172.6 (C-4, C=O) (for designations of carbons see Figure 3).

2.1.3. N-[2-(4-Bromophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3yl]-pyridine-3-carboxamide (**2b**). Diastereomer ratio % (major/ minor): 60: 40. ¹H-NMR (500 MHz, DMSO- d_6) $\delta:$ 7.44–7.46 (2H, m, phenyl-H); 7.49-7.56 (1H, m, pyridine-H); 7.57-7.60 (2H, m, phenyl-H); 8.05-8.09 (1H, m, pyridine-H); 8.72-8.73 (1H, m, pyridine-H); 8.86, 8.87 (1H, 2d, J = 1 Hz, pyridine-H) [36] (for ¹H NMR data of other protons see Table 1). ¹³C-NMR (125 MHz) (DMSO- d_6) δ (ppm): 20.2 (CH₃, C-6; 60%), 20.6 (CH₃, C-6; 40%); 39.7 (CH, C-5; 40%), 39.8 (CH, C-5; 60%); 60.4 (CH, C-2; 40%), 60.7 (CH, C-2; 60%); 122.8 (C, C18; 40%), 123.1 (C, C18; 60%); 124.3 (CH, C13; 40%), 124.4 (CH, C13; 60%); 127.8 (C, C9; 60%), 127.9 (C, C9; 40%); 130.7 (CH, C16, 20; 40%), 130.9 (CH, C16, 20; 60%); 132.2 (CH, C17, 19; 40%), 132.3 (CH, C17, 19; 60%); 135.9 (CH, C14; 60%), 136.0 (CH, C14; 40%); 137.7 (C, C15; 60%), 138.6 (C, C15; 40%); 149.1 (CH, C10; 60%), 149.2 (CH, C10; 40%); 153.6 (CH, C12; 40%), 153.7 (CH, C12; 60%); 164.5 (C=O, C-8; 40%), 164.6 (C=O, C-8; 60%); 172.6 (C=O, C-4; 40%), 172.7 (C=O, C-4; 60%) (for designations of carbons see Figure 3).

2.1.4. N-[2-(4-Trifluoromethylphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-pyridine-3-carboxamide (2c). Diastereomer ratio % (major/minor): 53:47. White powder (2.78 g, 73%); mp 170.0–173.1°C; ¹H-NMR (500 MHz, DMSO- d_6) δ : 7.49–7.51 (1H, m, pyridine-H); 7.72-7.78 (4H, m, 2-phenyl-H); 8.05-8.10 (1H, m, pyridine-H); 8.72–8.73 (1H, m, pyridine-H); 8.85, 8.88 (1H, 2d, J = 2.0 Hz, pyridine-H) (for ¹H NMR data of other protons see Table 1); 13 C-NMR (100 MHz, DMSO- d_6) δ: 20.2, 20.3 (C-6, CH₃); 38.6, 39.2 (C-5, CH); 60.1, 60.4 (C-2, CH); 124.2, 124.3 (C13, CH); 124.6 (CF₃, q, J = 271 Hz); 126.1, 126.2 (C17, 19, CH); 127.6, 127.7 (C9, C); 129.0, 129.4 (C16, 20, CH); 129.8 and 130.0 (C18, C, q, J = 32 Hz); 135.8, 135.9 (C14, CH); 143.1, 144.0 (C15, C); 148.9, 149.0 (C10, CH); 153.5, 153.6 (C12, CH); 164.4, 164.5 (C-8, C=O); 172.5, 172.7 (C-4, C=O) (for designations of carbons see Figure 3); IR (KBr): $v_{\text{max}} = 3143, 3037, 1732, 1676, 1620, 1595, 1544; \text{UV}$ (EtOH): $\lambda_{\max}(\log \varepsilon) = 203.2$ (28135), 219.6 (23655), 262.8 (63.68); ESI MS: m/z = 380.08 ([M - H]⁻, 100); Anal. Calcd. for C₁₇H₁₄F₃N₃O₂S: C, 53.54; H, 3.70; N, 11.02%. Found: C, 53.75; H, 3.92; N, 10.96%.

2.1.5. N-[2-(4-Benzyloxyphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-pyridine-3-carboxamide (2d). Diastereomer ratio % (major/minor): 80:20. White powder; yield: 3.68 g (88%); mp 140.4–143.5°C; ¹H-NMR (500 MHz, DMSO- d_6) δ : 5.08 (2H, s, O-CH₂-C₆H₅); 6.99-7.02 (2H, m, 2-phenyl-H); 7.31-7.35 (1H, m, pyridine-H); 7.37-7.51 (7H, m, 2-phenyl and -O-CH₂-C₆H₅); 8.06-8.09 (1H, m, pyridine-H); 8.73 (1H, dd, J = 7.5 Hz, 2.0 Hz, pyridine-H); 8.84, 8.86 (1H, 2d, 1H); 8.84, 8.86 (1H); 8.84,J = 2.0 Hz, pyridine-H) (for ¹H NMR data of other protons see Table 1); 13 C-NMR (125 MHz, DMSO- d_6) δ : 20.0 (CH₃, C-6; 20%), 20.8 (CH₃, C-6; 80%); 39.8 (CH, C-5; 20%), 39.9 (CH, C-5; 80%); 60.8 (CH, C-2); 70.0 (CH₂, C21); 115.5 (C17, 19, CH); 124.3 (CH, C13; 80%), 124.4 (CH, C13; 20%); 128.0 (C9, C); 128.3 (CH, C24, 26; 80%), 128.4 (CH, C24, 26; 20%); 128.6 (CH, C25); 129.1 (CH, C23, 27); 130.0 (CH, C16, 20; 80%), 130.3 (CH, C16, 20; 20%); 130.6 (C15, C); 135.9 (CH, C14; 20%), 136.0 (CH, C14; 80%); 137.5 (C, C22; 20%), 137.6 (C, C22; 80%); 149.1 (CH, C10; 20%), 149.2 (CH, C10; 80%); 153.6 (C12, CH); 159.6 (C18, C); 164.5 (C-8, C=O); 172.6 (C-4, C=O) (for designations of carbons see Figure 5); IR (KBr): $\nu_{\rm max}=3473,\,3163,\,3066,\,1710,\,1672,\,1606,\,1591;\,1244.$ UV (EtOH): $\lambda_{\rm max}(\log\varepsilon)=204.8$ (59593), 233.2 (280.89), 266.8 (13560). ESI MS: m/z=418.10 ([M – H]⁻, 100); Anal. Calcd. for C₂₃H₂₁N₃O₃S: C, 64.47; H, 5.17; N, 9.81%. Found: C, 64.63; H, 5.06; N, 9.76%.

2.1.6. N-[2-(3-Methoxyphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-piridine-3-carboxamide (2e). Diastereomer ratio % (major/minor): 73:27. White powder; yield: 2.24 g (65%); mp 101.0–105.0°C; ¹H-NMR (500 MHz, DMSO-*d*₆) δ: 3.74 (3h, s, OCH₃); 6.90-6.93 (1H, m, 2-phenyl-H); 7.02-7.04 (2H, m, 2-phenyl-H); 7.27-7.30 (1H, m, 2-phenyl-H); 7.50 (1H, dd, J = 5.3, 4.8 Hz, pyridine-H); 8.05-8.08 (1H, m, m)pyridine-H); 8.72 (1H, dd, *J* = 7.8, 2.0 Hz, pyridine-H); 8.85 (major diastereomer), 8.87 (minor diastereomer) (1H, 2d, J = 1.9 Hz, J = 1.9 Hz, pyridine-H) (for ¹H NMR data of other protons see Table 1). ¹³C-NMR (125 MHz, DMSO- d_6) δ: 20.2 (CH₃, C-6; 73%), 20.6 (CH₃, C-6; 27%); 39.8 (CH, C-5; 27%), 39.9 (CH, C-5; 73%); 55.8 (OCH₃); 60.9 (CH, C-2; 27%), 61.2 (CH, C-2; 73%); 113.5 (CH, C18; 27%), 113.8 (CH, C18; 73%); 115.3 (CH, C16; 27%), 115.4 (CH, C16; 73%); 120.4 (CH, C20; 27%), 120.8 (CH, C20; 73%); 124.3 (CH, C13; 27%), 124.4 (CH, C13; 73%); 127.9 (C, C9; 73%), 128.0 (C, C9; 27%); 130.4 (CH, C19; 27%), 130.5 (CH, C19; 73%); 136.0 (CH, C14; 73%), 136.1 (CH, C14; 27%); 139.8 (C, C15; 73%), 140.5 (C, C15; 27%); 149.1 (CH, C10; 73%), 149.2 (CH, C10; 27%); 153.6 (CH, C12); 160.1 (C, C17; 73%), 160.2 (C, C17; 27%); 164.6 (C-8, C=O); 172.8 (C=O, C-4; 27%), 172.9 (C=O, C-4; 73%) (for designations of carbons see Figure 3); IR (KBr): $v_{\rm max} = 3487, 3176, 3076, 1707, 1670, 1610, 1591, 1546; 1260; UV$ (EtOH): $\lambda_{\max}(\log \varepsilon) = 204.0$ (42863), 225.0 (17859), 263.8 (6101); ESI MS: m/z = 342.23 ([M – H]⁻, 100); Anal. Calcd. for $C_{17}H_{17}N_3O_3S$: C, 56.50, H, 5.30, N, 11.63%. Found: C, 56.47; H, 4.77; N, 11.50%.

2.1.7. N-[2-(2-Nitrophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]piridine-3-carboxamide (2f). Diastereomer ratio % (major/ minor): 52:48. ¹H-NMR (500 MHz, DMSO- d_6) δ : 7.51 (1H, dd, J = 4.8 Hz, 4.4 Hz, pyridine-H); 7.61-7.65 (1H, m, m)2-phenyl-H); 7.85-7.90 (2H, m, 2-phenyl-H); 8.04-8.11 (2H, m, 2-phenyl-H and pyridine-H); 8.73 (1H, dd, J = 8.3 Hz, 2.0 Hz, pyridine-H); 8.71, 8.79 (1H, 2d, *J* = 2.4 Hz, *J* = 2.4 Hz, pyridine-H) [34] (for ¹H NMR data of other protons see Table 1). ¹³C-NMR (125 MHz) (DMSO- d_6) δ (ppm): 19.0, 21.9 (C-6, CH₃); 37.2, 39.2 (C-5, CH); 56.6, 56.7 (C-2, CH); 124.2, 124.3 (C13, CH); 125.6, 125.7 (C9, C); 127.9, 128.0 (C17, CH); 128.3, 128.5 (C20, CH); 130,3, 130.5 (C19, CH); 135.3, 135,4 (C18, CH); 135.5, 135.8 (C15, C); 136.1, 136.2 (C14, CH); 148.1, 148.6 (C16, C); 149.2, 149.3 (C10, CH); 153.5, 153.6 (C12, CH); 164.7, 164.8 (C-8, C=O); 172.8, 173.2 (C-4, C=O) (for designations of carbons see Figure 3).

3. Results and Discussion

3.1. Chemistry. Novel compounds 2a-f have been synthesized by the reaction of compounds 1a-f with racemic (±)-2-sulfanylpropanoic acid in dry benzene (Figure 2).

The structures of the compounds were determined by microanalysis, IR, ¹H-NMR, ¹³C-NMR, HMBC, and ESI



Ar: 4-chlorophenyl, 4-bromophenyl, 4-trifluoromethylphenyl, 4-benzyloxyphenyl, 3methoxyphenyl, 2-nitrophenyl

FIGURE 2: The preparation of compounds 2a-f.



FIGURE 3: Selected HMBC correlations for 2b.

mass spectrometry. IR spectra of **2a–f** showed common characteristic absorption bands at 3142–3176 cm⁻¹ (NH), 1707– 1732 cm⁻¹ (thiazolidinone C=O), and 1670–1681 cm⁻¹ (NH-C=O) which provided evidence for the ring closure reaction between **1a–f** and 2-sulfanylpropanoic acid. Disappearance of the peak at 8 ppm corresponding to N=CH proton of **1a– f** [37] and the observation of C-2 proton of **2a–f** at 5.88– 6.30 ppm in the ¹H-NMR spectra were also taken as the proof of the formation of thiazolidin-4-one ring.

The structure of **2b** was confirmed by the HMBC spectrum in which the correlations of C-8 ($\delta_{\rm C}$ 164.5, 164.6 ppm) with H-10 ($\delta_{\rm H}$ 8.86 ppm), H-14 ($\delta_{\rm H}$ 8.07 ppm), and N-H (H-7) ($\delta_{\rm H}$ 10.94, 10.95 ppm); C-4 ($\delta_{\rm C}$ 172.6, 172.7 ppm) with H-5 ($\delta_{\rm H}$ 4.12, 4.22 ppm) and H-6 ($\delta_{\rm H}$ 1.54, 1.55 ppm); and C-6 ($\delta_{\rm C}$ 20.6, 20.2 ppm) with H-2 ($\delta_{\rm H}$ 5.90 ppm), H-5 ($\delta_{\rm H}$ 4.12, 4.22 ppm), and H-6 ($\delta_{\rm H}$ 1.54, 1.55 ppm) enabled definite assignment of CONH (C-8) and thiazolidinone C=O (C-4) carbons (Figure 3).

3.2. Stereochemical Investigations. Due to the formation of a new stereocenter at C-2, in principle four stereoisomers were expected to form the following: two enantiomeric (2S-5R/2R-5S, 2S-5S/2R-5R) and two diastereomeric pairs (2S-5R/2S-5S, 2R-5S/2R-5R) (Figure 4). In fact, compounds **2a**f were obtained as mixtures of unequal composition of two diastereomers which were differentiated by their ¹H NMR spectra (Figure 5). It has been observed that the ratios of the major and minor diastereomers calculated from the integration values of the C-5 methine proton signals were 54%: 46%, 40%: 60%, 47%: 53%, 80%: 20%, 27%: 73%, and 48%: 52% for compounds **2a**-f, respectively. ¹³C signals at C-2, C-4, C-5, and C-6 positions for compounds **2b** and **2e** also appeared as double peaks in the HMBC spectra due to the formation diastereoisomers (see Section 2). Chiral HPLC of compounds **2b** and **2c** on the Chiralpak AD-H column resulted in four peaks (Figure 6) which further proved the presence of four stereoisomers.

For all diastereomeric compounds (Figure 4), it was observed that C-5 methine proton on the thiazolidinone moiety was coupled with C-6 methyl protons and appeared as two quartets (Table 1, Figure 5). Similarly the signal of C-6 methyl protons was coupled with C-5 methine and observed as two doublets for compounds **2b–2f**. In all of the ¹H NMR spectra of compounds 2a-2f (except 2b) the higher frequency signals of C-5 methine appeared as a quartet of doublets due to the long-range coupling with the C-2 proton. The two diastereotopic C-2 hydrogens could be observed separately only for compounds 2c-2f. Aromatic protons of pyridyl and C-2 aryl rings gave signals between 6.9 and 9.0 ppm. In this region some of the aromatic peaks corresponding to two diastereomers could also be observed separately for all compounds (Figure 5). The N-H proton was observed at around 11 ppm as two singlets with unequal integral ratios for compounds 2b, 2c, and 2f and only one singlet for 2a, 2d, and 2e.

We have previously elucidated the stereostructures of some oxazolidine derivatives by NOESY experiment [38-40]. The configurations of the major and minor stereoisomers of thiazolidin-4-one derivatives (2a-2f) were determined by means of ¹H NMR and NOESY spectra of compound 2f. The ¹H NMR spectrum of 2f showed that the major diastereomer had its C-5 methine signal (quartet) at a lower frequency (4.10 ppm, $J_{\text{H-5,CH}_3-6} = 7.0 \text{ Hz}$) than the signal of the minor component (4.21 ppm, qd, $J_{\text{H-5,CH}_3-6} = 7.0 \text{ Hz}$ $J_{\text{H-5,H-2}}$: 1.96 Hz). The signal of C-2 proton of compound **2f** was observed as two separate signals ($\Delta\delta$: 0.04 ppm) corresponding to two diastereomers: a singlet at 6.26 ppm for the major diastereomer and a doublet at 6.22 ppm (${}^{4}J_{H-2,H-5} =$ 1.96 Hz) for the minor diastereomer. The observed longrange coupling constant $({}^{4}J)$ of the doublet, which is characteristic of trans protons [41], was consistent with that of the higher frequency quartet of minor diastereomer. Based on these results, the stereochemistry of the minor diastereomer was assigned as 2S, 5S or 2R, 5R, in which C-2 and C-5 methine protons are trans to each other (Figure 4).

NOESY spectrum for compound **2f** was taken in order to further prove that the stereochemistry of the minor and the major diastereomers was 2*S*, *5S*/2*R*, *5R* and 2*S*, *5R*/2*R*, *5S*, respectively (Figure 7). Observation of the cross peaks at



FIGURE 4: The stereoisomers of compounds 2a-f.



FIGURE 5: 500 MHz $^1\mathrm{H}$ NMR spectrum of compound $\mathbf{2d}$ in DMSO- $d_6.$ S: solvent.



FIGURE 6: (a) HPLC chromatogram of compound **2b**; (b) HPLC chromatogram of compound **2c**. Peaks marked with the same sign belong to enantiomers according to their % areas. Column: Chiralpak AD-H; eluent: n-hexane: 2-propanol (85:15) (v:v); diode array detector.



FIGURE 7: Selected 2D NOESY correlations for compound **2f** (solvent: DMSO-*d*₆, 400 MHz).

TABLE 2: The configurations of C-2 and C-5 centers of major and minor diastereomers.

	Diastereomer	Configurations of C-2 and C-5		
Compounds	ratio, %	Major stereoisomer	Minor stereoisomer	
2a	54:46	2S, 5S or 2R, 5R	2S, 5R or 2R, 5S	
2b	60:40	2S, 5R or 2R, 5S	2S, 5S or 2R, 5R	
2c	53:47	2S, 5R or 2R, 5S	2S, 5S or 2R, 5R	
2d	80:20	2S, 5S or 2R, 5R	2S, 5R or 2R, 5S	
2e	73:27	2S, 5R or 2R, 5S	2S, 5S or 2R, 5R	
2f	52:48	2S, 5R or 2R, 5S	2S, 5S or 2R, 5R	

6.26 ppm and 4.10 ppm in 2D NOESY spectrum indicated the spatial proximity of C-2 and C-5 methine hydrogens of the major diastereomer (Figure 7(a)). Cross peaks at 1.52 ppm and 7.86 ppm also revealed that C-6 methyl and the hydrogens of the aryl ring [42, 43] of the major diastereomer are in close proximity (Figure 7(b)). These observations were consistent with the 2R, 5S or 2S, 5R configurations. Similarly, for the minor diastereomer cross peaks between the signals of C-6 methyl and C-2 methine hydrogens were observed (Figure 7(c)). A NOESY correlation between C-5 methine and aromatic protons (Figure 7(d)) further confirmed that the configurations of C-2 and C-5 positions of the minor diastereomer were 2S, 5S or 2R, 5R. Since the spectra of 2a-2f have the feature in common, by analogy, it could be concluded that all the deshielded signals of C-5 methine belong to 2S, 5S or 2R, 5R stereoisomer (Table 1). Based on these results, the configurations of C-2 and C-5 centers of the major and minor diastereomers are given in Table 2.

The diastereomeric isomer ratios of compounds **2b** and **2c** obtained by the integration of the ¹H NMR signals have been found identical with those obtained by HPLC analysis. Therefore, with the knowledge of the configurations of the C-2 and C-5 centers of the major and minor diastereomers of **2b** and **2c**, the HPLC peaks (Figures 6(a) and 6(b)) marked by "♣" could be assigned to 2*S*, 5*R* or 2*R*, 5*S* (major) and the others to 2*S*, 5*S* or 2*R*, 5*R* (minor).

In order to determine the reason of the diastereoselectivity of the synthesis, samples of **2d** and **2e** were recrystallized once again from ethanol and the composition of crystals precipitated first was analyzed by NMR. We have found a different composition for **2d** and **2e**. Therefore, the different isomer ratios showed that the obvious diastereoselectivity upon recrystallization from ethanol was due to different solubilities of the diastereomeric isomers in ethanol which was observed previously [29, 30, 39, 40] and not related to any remarkable favorable attack during ring closure. Nevertheless fractional crystallization of the product from ethanol allowed for an easy access to diastereomerically enriched **2b**, **2d**, and **2e** (Table 2).

4. Conclusions

The reaction of aryl N'-(substituted benzylidene)pyridine-3-carbohydrazide with 2-mercaptopropanoic acid produced mixtures of unequal composition of two diastereomeric N-[2-(aryl)-5-methyl-4-oxo-1,3-thiazolidine-3-yl]-pyridine-3-carboxamide derivatives which were differentiated by ¹H NMR spectra. The configurations of C-2 and C-5 stereogenic centers of thiazolidin-4-one ring for the major and the minor diastereomers have been found via one- and two-dimensional NMR spectroscopy. Four stereoisomers of

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

compounds **2b** and **2c** were resolved by chiral HPLC.

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