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## Full Paper

# Preparation and *in-vitro* Evaluation of 4-Benzylsulfanylpiperidine-2-carbohydrazides as Potential Antituberculosis Agents

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A set of 4-benzylsulfanylpiperidine-2-carbohydrazides was synthesized and evaluated for *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis*, non-tuberculous mycobacteria, and multidrug-resistant *M. tuberculosis*. The activities expressed as the minimum inhibitory concentration (MIC) fall into a range of 2 to 125  $\mu\text{mol/L}$ , most often 4 to 32  $\mu\text{mol/L}$ . The results revealed that the substituents on the benzyl moiety do not influence the antimycobacterial efficacy. The substances exhibited similar activities against sensitive and resistant strains of *M. tuberculosis*. Furthermore, compounds show low antiproliferative effect and cytotoxicity.

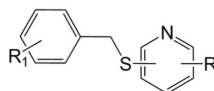
**Keywords:** 4-Benzylsulfanyl derivatives / Multidrug-resistant *M. tuberculosis* / *Mycobacterium tuberculosis* / Non-tuberculous mycobacteria / Piperidine-2-carbohydrazides

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## Introduction

Drugs for treating tuberculosis (TB) have been available for more than half a century and yet, the incidence of the disease worldwide continues to rise. TB annual incidence rates have peaked globally in the years 2003 / 2004, however, they are falling very slowly in all WHO regions and are stagnating in Eastern Europe [1]. Thus, multidrug-resistant strains (MDR-TB) are becoming a serious threat to TB control worldwide. In 2008, WHO recorded the highest rates of MDR-TB. In several MDR-TB “hot spot” areas (China (Henan province), Uzbekistan, Kazakhstan), rates of MDR-TB have reached levels of up to 14% among



R = CN, CSNH<sub>2</sub>; R<sub>1</sub> = H, Cl, Br, F, CH<sub>3</sub>, OCH<sub>3</sub>, CF<sub>3</sub>, CN, NO<sub>2</sub>.

**Figure 1.** Chemical structure of model compounds.

patients never treated and up to 40% in previously treated patients [2]. Even more serious is the emergence of extensively drug-resistant TB (XDR-TB) that has been confirmed in more than 45 countries. This increase of MDR-TB and the emergence of XDR-TB provide the rationale to search for new antimycobacterial drugs. The number of TB research publications with a drug-discovery focus increased in the last decade [3–5]. Numerous new molecules have been disclosed as potential leads for TB drug discovery and some of new compounds have advanced into clinical trials and development [6].

In our previous papers, we have reported the antimycobacterial activity of benzylsulfanyl derivatives of piperidine which were substituted by carbonitrile or carbo-

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**Abbreviations:** density functional theory (DFT); isoniazid (INH); multidrug-resistant tuberculosis strains (MDR-TB); extensively drug-resistant TB (XDR-TB)

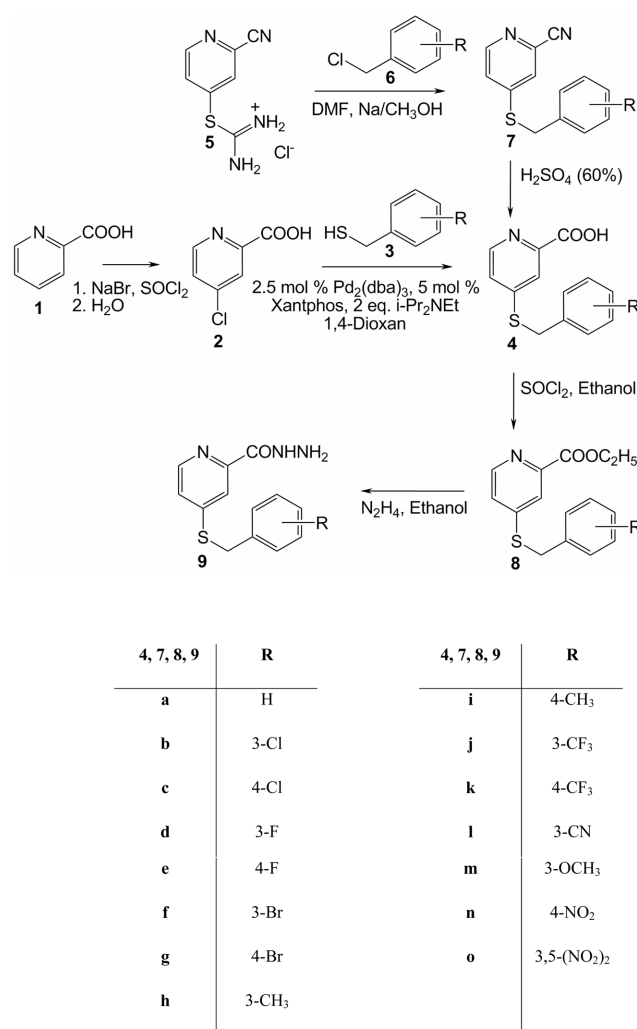
thioamide group on the pyridine moiety (Fig. 1) [7, 8]. The compounds exhibited significant *in-vitro* activity against obligate strain *Mycobacterium tuberculosis* and also against opportunistic non-tuberculous mycobacteria (*M. avium*, *M. kansasii*). We postulated that antimycobacterial activity of these compounds is connected with a benzylsulfanyl group [9]. Alkylsulfanyl group as a pharmacophore for antituberculosic activity was confirmed by further studies [10, 11], many compounds bearing an alkylsulfanyl group on the heterocyclic core exhibited antimycobacterial activity [12–14]. In the case of pyridine carbothioamides, it is very likely that the thioamide group contributed to the activity such as in the antituberculosic ethionamide. Ethionamide (2-ethylpyridine-4-carbothioamide) is a prodrug that is oxidized by EthA (a flavoprotein monooxygenase) to the sulfinic acid, and its mechanism of action is on the level of mycolic acid synthesis and then causes the inhibition of cell wall biosynthesis [4]. Encouraged by the significant activity shown by several 4-benzylsulfanylpyridine-2-carbothioamides, we decided to continue our synthetic work. We prepared the set of 4-benzylsulfanylpyridine-2-carbohydrazides with respect to the carbohydrazide group that is responsible for antimycobacterial activity of the most important antituberculosis drugs isoniazid (pyridine-4-carbohydrazide – INH). The mechanism of INH action is linked to inhibiting the antimycobacterial cell wall, the biological activity requires first the activation by KatG (an endogenous catalase-peroxidase) [4]. We assume that our new compounds could also act on the enzymatic pathways of the cell-wall biosynthesis.

In this paper, we report the synthesis of 4-benzylsulfanylpyridine-2-carbohydrazides and the results of the antimycobacterial-activity evaluation of these compounds. They were evaluated against *Mycobacterium tuberculosis*, non-tuberculous mycobacteria strains (*M. avium*, *M. kansasii*), and against three multidrug-resistant strains of *M. tuberculosis*. Selected compounds were tested for antiproliferative and cytotoxic effects.

## Results and discussion

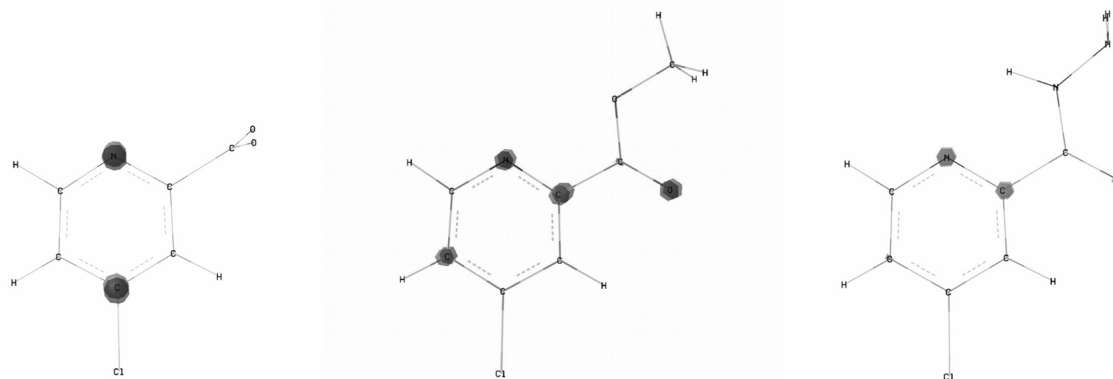
### Chemistry

The target compounds were synthesized following the steps depicted in Scheme 1. Since it was not possible to prepare the desired sulfides **9** directly either by the reaction of 4-mercaptopyridine-2-carbohydrazides with benzyl halides or 4-chloropyridine-2-carbohydrazides with benzylthiols, we synthesized sulfides **9** via the key intermediates **4**. These 4-benzylsulfanylpyridine-2-carboxylic acids **4** were formed via two routes. The first method



**Scheme 1.** Synthesis of the 4-benzylsulfanyl derivatives **4**, **7**, **8**, and **9**.

used for the preparation of **4** was based on Pd-catalyzed cross coupling of 4-chloropyridine-2-carboxylic acid **2** with benzylthiols **3** [15]. Acid **2**, which serves as a convenient starting material, was prepared according to a method described in the literature [16] from pyridine-2-carboxylic acid **1**. Thiols **3** were prepared by heating appropriate benzyl halides with thiourea in ethanol, and the resulting S-alkylisothiuronium salts were further hydrolyzed by an aqueous solution of sodium hydroxide. The treatment of **2** with various benzylthiols **3** was carried out in dioxane and *i*-Pr<sub>2</sub>NEt in the presence of catalyst Pd<sub>2</sub>(dba)<sub>3</sub> and Xantphos at reflux. The process required 9 to 12 h depending on the alkylating agent and furnished products **4** in 32 to 68% yields. This procedure failed for the derivatives with the nitro group on the benzyl moiety. For this reason, we used the formerly prepared sulfide-nitrile **7** [8] which was converted to the key inter-



**Figure 2.** The dislocation of LUMO orbitals in the 4-chloropyridine-2-carboxylate anion, methyl 4-chloropyridine-2-carboxylate, and 4-chloropyridine-2-carbohydrazide (RB3LYP/6-311+G(d,p) level, Isovalue = 0.17).

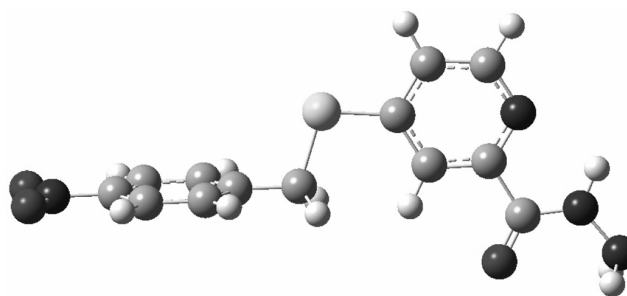
mediates **4** by hydrolysis in 60% sulfuric acid in good yields. Sulfides **7** were obtained by reacting isothiouonium salt **5** with the appropriate benzyl halides **6** in *N,N*-dimethylformamide in the presence of sodium methoxide, *i.e.*, the second method for the formation of a C-S bond. The reaction of **4** with  $\text{SOCl}_2$  in ethanol led to the formation of esters **8** which gave the target hydrazides **9** by the reaction with hydrazine.

The structures of the compounds were fully characterized by  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR, and IR spectral data, and their purity by elemental analysis. For the  $^1\text{H}$ -NMR spectra of benzylsulfanyl derivatives **4**, **8**, and **9**, the singlet of the  $\text{S-CH}_2$  group positioned in the region 4.40 to 4.76 ppm and the signal of the  $\text{O-CH}_2$  esters in the region 4.30 to 4.31 ppm are typical. The methyl-group signal of the ethyl esters can be found at 1.30 to 1.31 ppm. Multiplets of the benzene ring occur in the region between 6.81 to 8.19 ppm; for 3,5-dinitrosubstituted benzenes, the signal is located between 8.70 and 8.78 ppm. Signals of pyridine hydrogens are in the region 7.43 to 8.90 ppm.

The  $^{13}\text{C}$ -NMR signal of the  $\text{S-CH}_2$  group can be observed at 32.6 to 34.5 ppm, the signal of the  $\text{O-CH}_2$  esters can be found at 61.5 to 61.6 ppm, the signal of the  $\text{CH}_3$  group of ethyl esters lies at 14.2 to 14.3 ppm. The carbon signal of the  $\text{C=O}$  group can be found in the region 164.8 to 166.0 ppm for acids, 164.5 to 164.7 ppm for esters, and at 162.2 to 162.4 ppm for hydrazides. In infrared spectra, compounds **4**, **8**, and **9** show characteristic bands of the  $\text{C=O}$  group – acids at 1701 to 1722  $\text{cm}^{-1}$ , esters at 1714 to 1735  $\text{cm}^{-1}$ , and hydrazides at 1660 to 1699  $\text{cm}^{-1}$ ; bending vibration of the  $\text{NH}_2$  group of hydrazides will be seen in the region 1617 to 1629  $\text{cm}^{-1}$ .

### Molecular modeling

Using the procedure to synthesize the title compounds, at first, we tried to prepare sulfides from 4-chloropyri-



**Figure 3.** 3D Model of compound **9n**.

dine-2-carbohydrazide, yet without success. Therefore, we computed 3D models (RB3LYP/6-311+G(d,p) level, software Gaussian03W, v. 6.1; Gaussian, Inc., Wallingford, CT, USA) of the following derivatives of 4-chloropyridine-2-carboxylic acid, which could most likely be used as starting compound for the synthesis: carboxylate anion, methyl ester, and hydrazide. We searched for the dislocation of the LUMO orbital which shows the possible position of the nucleophilic attack on the pyridine ring. Only the carboxylate anion shows a higher location of this orbital on position 4 of the ring (Fig. 2, Isovalue = 0.17, software GaussView, v. 4.1.2; Gaussian, Inc.), and therefore, we used 4-chloropyridine-2-carboxylic acid in alkaline conditions to synthesize the sulfides in this work; this reaction gave good yields.

We also calculated the 3D model (RB3LYP/6-311+G(d,p) level, software Gaussian03W, v. 6.1; Gaussian, Inc.) of compound **9n** (Fig. 3) and, based on this structure, calculated the vibrational spectrum of this compound for easier, exact allocation of measured bands of the real spectrum. First, we did the vibrational analysis, and then the calculated bands were scaled using the factor 0.9688 [17], which corrects the systematic error of the used DFT (density functional theory) method. The hybrid functional we

**Table 1.** Calculated and measured vibrations in the infrared spectrum of the compound **9n** (in  $\text{cm}^{-1}$ ).

Calculated	Scaled	Spectrum	Type of vibration
1119	1084	1118	$\delta(\text{C-H})_{\text{benzene}}$
1285	1244	1256	$\delta(\text{C-N-H}) + \nu(\text{C-N})$
1357	1314	1301	$\nu(\text{C-C})_{\text{benzene}}$
1370	1327	1349	$\nu_s(\text{NO}_2)$
1527	1479	1467	$\delta(\text{C-N-H})$
1580	1531	1514	$\nu_{\text{as}}(\text{NO}_2)$
1613	1562	1536	$\nu(\text{C-C})_{\text{pyridine}}$
1640	1588	1582	$\nu(\text{C-C})_{\text{benzene}}$
1646	1594	1605	$\nu(\text{C-C})_{\text{benzene}} + \nu_{\text{as}}(\text{NO}_2)$
1702	1649	1628	$\delta(\text{NH}_2)$
1730	1676	1676	$\nu(\text{C=O})$
3063	2967	2990	$\nu_{\text{as}}(\text{CH}_2)$

used should give the most exact results after scaling from the common DFT methods. The comparison of the calculated and measured main bands is given in Table 1.

### Biological activity

Benzylsulfanyl derivatives of pyridinecarbohydrazides **9** were evaluated *in vitro* against *M. tuberculosis* and non-tuberculous mycobacteria – *M. kansasii* and *M. avium*. The values of minimum inhibitory concentration (MIC) expressed in  $\mu\text{mol/L}$  are summarized in Table 2. In several cases (denoted >), the MIC value could not be determined due to the limited solubility of the compounds in

the test medium. For the sake of comparison, the MIC values for the standard isoniazid (INH) were also included.

As seen from the data of Table 2, all evaluated compounds **9** exhibited *in-vitro* activity against all tested mycobacterial strains. The compounds are of comparable activity against *M. tuberculosis* My 331/88 and *M. kansasii* My 235/80 with the MICs values within the range of 2 to 32  $\mu\text{mol/L}$ , most often between 8 to 16  $\mu\text{mol/L}$ . The biological activity is slightly different against *M. avium* My 330/88 and clinical isolate of *M. kansasii* 6 509/96. MICs values are within the range of 8 to 250  $\mu\text{mol/L}$ , most often between 16 to 32  $\mu\text{mol/L}$ . Careful examination of the biological activity data reveals the different activity profiles of the newly prepared compounds and INH. Whereas *M. avium* and *M. kansasii* My 235/80 are resistant to INH, our compounds display a significant activity against these strains. This finding indicates that our compounds may employ a different mechanism of action compared to INH. It is important to point out that the activity is not affected by the electronic properties of the substituents on the benzyl moiety, practically all compounds exhibited similar activity. This is in contradiction to our model compounds (Fig. 1) where the electron-withdrawing substituents cause the increase of the activity [18]. Especially incorporating two nitro groups into the benzyl moiety led to the most active compounds [7, 8]. The same conclusions were obtained for the benzylsulfanyl derivatives of benzimidazole [19, 20], benzothiazole, and benzoxazole

**Table 2.** *In-vitro* antimycobacterial activity of 4-(benzylsulfanyl)pyridine-2-carbohydrazide expressed as MIC ( $\mu\text{mol/L}$ ).

Compound	Strains									
	<i>Mycobacterium tuberculosis</i> My 331/88		<i>Mycobacterium kansasii</i> My 235/80			<i>Mycobacterium kansasii</i> 6 509/96			<i>Mycobacterium avium</i> My 330/88	
	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d	14 d	21 d
<b>9a</b>	16	16	4	8	16	16	32	32	125	>125
<b>9b</b>	8	16	8	8	16	16	16	32	62	62
<b>9c</b>	8	8	4	8	8	16	32	32	62	62
<b>9d</b>	8	16	4	8	8	16	32	32	62	125
<b>9e</b>	16	16	8	8	16	16	16	32	125	250
<b>9f</b>	8	16	4	4	8	16	32	62	62	125
<b>9g</b>	4	8	2	4	8	8	16	32	32	62
<b>9h</b>	8	16	4	8	16	32	62	62	62	125
<b>9i</b>	8	16	4	8	8	8	16	32	32	62
<b>9j</b>	16	32	4	8	16	16	32	62	62	125
<b>9k</b>	8	16	4	8	16	8	16	16	32	62
<b>9l</b>	8	16	4	8	8	32	>62	>125	>62	>125
<b>9m</b>	8	16	4	8	8	32	62	125	32	62
<b>9n</b>	8	8	8	8	16	8	16	16	62	62
<b>9o</b>	>62	125	>62	>62	>62	16	>16	>32	>32	>32
<b>INH</b>	0.5	1	>250	>250	>250	2	2	4	>250	>250

**Table 3.** In vitro antituberculous activity against MDR *M. tuberculosis* (MIC expressed in  $\mu\text{mol/L}$ ).

Compound	Strains					
	<i>Mycobacterium tuberculosis</i> 7357/98 <sup>a)</sup>		<i>Mycobacterium tuberculosis</i> 9449/06 <sup>b)</sup>		<i>Mycobacterium tuberculosis</i> 2092/05 <sup>c)</sup>	
	14 d	21 d	14 d	21 d	14 d	21 d
<b>9c</b>	4	8	4	8	2	4
<b>9g</b>	4	4	4	4	4	8
<b>9m</b>	4	8	8	16	8	16
<b>9n</b>	8	8	8	8	4	8
<b>9o</b>	>16	>62	>32	>62	32	>125

<sup>a)</sup> Resistant to isoniazid, rifampicin, streptomycin, ethambutol, ofloxacin, ansamycin.

<sup>b)</sup> Resistant to isoniazid, rifampicin, streptomycin, ansamycin.

<sup>c)</sup> Resistant to isoniazid, rifampicin, streptomycin, ethambutol, ofloxacin, ansamycin.

**Table 4.** Antiproliferative and cytotoxic effects expressed in  $\mu\text{g/mL}$ .

Compound	Antiproliferative effect		Cytotoxicity
	Huvec GI <sub>50</sub>	K-562 GI <sub>50</sub>	HeLa CC <sub>50</sub>
<b>9a</b>	>50	50	35.7
<b>9c</b>	47.2	12.1	37.6
<b>9d</b>	>50	37.9	38.9
<b>9g</b>	>50	21.0	31.8
<b>9i</b>	>50	18.4	>50
<b>9j</b>	42.7	12.9	31.8
<b>9m</b>	>50	>50	48.4
<b>9n</b>	>50	>50	50.0
<b>9o</b>	>50	>50	>50

[21]. However, in our newly prepared series, compound **9o** having two nitro groups on the benzyl moiety, displays poor activity. The exact values of MICs could not be determined due to the insolubility of compound **9o** in the test medium. We assume that the low efficacy of this compound is connected with its low solubility.

The *in-vitro* activity of the selected compounds was determined against three multidrug-resistant (MDR) strains of *M. tuberculosis* isolated from TB patients. The results are summarized in the Table 3. MIC values are within the range of 2 to 16  $\mu\text{mol/L}$  except for the dinitro-derivative values. Due to insolubility, their MICs could not exactly be determined. In comparison to the sensitive strain of *M. tuberculosis*, compounds exhibited the same or better efficacies. As the tested MDR strains are resistant to INH, our results indicate a different mechanism of action for the newly prepared compounds compared to IHN. It is likely that the benzylsulfanyl group is responsible for the activity.

Several compounds were assayed against the cell lines K-562 and HUVEC for their antiproliferative effects (GI<sub>50</sub>: concentration which inhibits cell proliferation by 50% compared to control), and against HeLa cells for their

cytotoxic effects (CC<sub>50</sub>: concentration which is toxic for 50% of the cells compared to control; used particularly when referring to cell lysis). The cells were incubated with ten concentrations of the tested compounds. The compounds exhibited low antiproliferative and cytotoxic effects. The values expressed as GI<sub>50</sub> are ranging from 12 to >50  $\mu\text{g/mL}$  and values of CC<sub>50</sub> from 31 to 50  $\mu\text{g/mL}$  (Table 4).

## Conclusion

Here, we report a series of 4-benzylsulfanyl derivatives of pyridine-2-carbohydrazide that show antimycobacterial activity against *M. tuberculosis* as well as non-tuberculous mycobacteria – *M. kansasii* and *M. avium* and MDR strains of *M. tuberculosis*. The compounds exhibited low antiproliferative and cytotoxic effects.

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The authors have declared no conflict of interest.

## Experimental

### Chemistry

The melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried over P<sub>2</sub>O<sub>5</sub> at 78 °C or 25 °C and 2.4–2.6 kPa for 2 h. Elemental analyses were performed on CHNS-O CE instrument, FISON EA 1110 (Fisons, Milan,

Italy) and are within  $\pm 0.4\%$  of the theoretical values. IR spectra were obtained on a Nicolet Impact 400 spectrometer (Nicolet, Madison, WI, USA) in KBr pellets. NMR spectra were recorded with a Varian Mercury-Vx BB 300 spectrometer (operating at 300 MHz for  $^1\text{H}$  and 75 MHz for  $^{13}\text{C}$ ) in  $\text{DMSO}-d_6$ ; the solutions were at ambient temperature. Chemical shifts were recorded as  $\delta$  values in ppm and were indirectly referred to tetramethylsilane (TMS). Coupling constants ( $J$ ) are given in Hz. The reactions were monitored and the purity of the products was checked by TLC (TLC plates, silica gel 60  $\text{F}_{254}$ , aluminum back; Merck, Germany) in chloroform / methanol / triethylamine or butanol / formic acid / water (for acid derivatives). The spots were visualized using UV light.

#### General procedure for the synthesis of compounds 4

Method A: 4-Chloropyridine-2-carboxylic acid **2** (6 mmol),  $i$ - $\text{Pr}_2\text{NEt}$  (24 mmol), and dry 1,4-dioxane (50 mL) were placed in a round-bottom flask. The flask was evacuated and backfilled with argon (3 cycles). Catalyst  $\text{Pd}_2(\text{dba})_3$  (0.15 mmol), Xantphos (0.3 mmol), and the appropriate benzylthiol **3** (6 mmol) were added, then the mixture was degassed twice more times. The mixture was heated under reflux for 9–12 h. Monitoring by silica gel TLC plates (butanol / formic acid / water 14 : 3 : 2) confirmed the completion of the reaction. Then, the reaction mixture was allowed to reach ambient temperature, filtered, neutralized by diluted  $\text{H}_2\text{SO}_4$ , and concentrated *in vacuo*. The crude product was dissolved in a small amount of ethanol, then poured into 100 mL ice-water and left overnight at  $-20^\circ\text{C}$ . The solid was filtered off and recrystallized from ethanol.

Method B: 3 mmol of **7** were dissolved in 9 mL of 60% sulfuric acid and heated at  $150^\circ\text{C}$  for two hours. After cooling to ambient temperature, the reaction mixture was neutralized by an aqueous solution of  $\text{Na}_2\text{CO}_3$  to pH 6. Precipitated crystals were recrystallized from ethanol.

#### 4-(Benzylsulfanyl)pyridine-2-carboxylic acid 4a

Yield: 67%; m.p.:  $170\text{--}172^\circ\text{C}$ ; IR (KBr)  $\lambda_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3500–2400 (COOH), 1718 (C=O);  $^1\text{H-NMR}$  (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.46 (dd,  $J = 5.3$  Hz,  $J = 0.5$  Hz, 1H,  $\text{H}_6$ ), 7.88 (dd,  $J = 2.0$  Hz,  $J = 0.5$  Hz, 1H,  $\text{H}_3$ ), 7.53 (dd,  $J = 5.3$  Hz,  $J = 2.0$  Hz, 1H,  $\text{H}_5$ ), 7.46–7.43 (m, 2H, Ar-H), 7.36–7.26 (m, 3H, Ar-H), 4.44 (s, 2H,  $\text{CH}_2$ );  $^{13}\text{C-NMR}$  (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 166.0, 150.4, 149.0, 148.4, 136.2, 129.1, 128.8, 127.7, 123.5, 121.4, 34.3.

#### 4-(3-Chlorobenzylsulfanyl)pyridine-2-carboxylic acid 4b

Yield: 68%; m.p.:  $143\text{--}145^\circ\text{C}$ ; IR (KBr)  $\lambda_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3500–2400 (COOH), 1718 (C=O);  $^1\text{H-NMR}$  (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.47 (dd,  $J = 5.3$  Hz,  $J = 0.6$  Hz, 1H,  $\text{H}_6$ ), 7.88 (dd,  $J = 2.0$  Hz,  $J = 0.6$  Hz, 1H,  $\text{H}_3$ ), 7.54–7.52 (m, 2H,  $\text{H}_5$ , Ar-H), 7.43–7.33 (m, 3H, Ar-H), 4.46 (s, 2H,  $\text{CH}_2$ );  $^{13}\text{C-NMR}$  (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 166.0, 149.9, 149.2, 148.4, 139.0, 133.3, 130.7, 128.9, 127.8, 127.6, 123.6, 121.5, 33.5.

#### 4-(4-Chlorobenzylsulfanyl)pyridine-2-carboxylic acid 4c

Yield: 66%; m.p.:  $159\text{--}161^\circ\text{C}$ ; IR (KBr)  $\lambda_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3500–400 (COOH), 1701 (C=O);  $^1\text{H-NMR}$  (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.46 (dd,  $J = 5.3$  Hz,  $J = 0.5$  Hz, 1H,  $\text{H}_6$ ), 7.86 (dd,  $J = 2.0$  Hz,  $J = 0.5$  Hz, 1H,  $\text{H}_3$ ), 7.52 (dd,  $J = 5.3$  Hz,  $J = 2.0$  Hz, 1H,  $\text{H}_5$ ), 7.49–7.46 (m, 2H, Ar-H), 7.40–7.37 (m, 2H, Ar-H), 4.44 (s, 2H,  $\text{CH}_2$ );  $^{13}\text{C-NMR}$  (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 166.0, 150.0, 149.1, 148.5, 135.4, 132.3, 130.9, 128.8, 123.6, 121.5, 33.4.

#### 4-(3-Fluorobenzylsulfanyl)pyridine-2-carboxylic acid 4d

Yield: 68%; m.p.:  $160\text{--}162^\circ\text{C}$ ; IR (KBr)  $\lambda_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3500–2400 (COOH), 1719 (C=O);  $^1\text{H-NMR}$  (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.50 (dd,  $J = 5.3$  Hz,  $J = 0.6$  Hz, 1H,  $\text{H}_6$ ), 7.88 (dd,  $J = 2.0$  Hz,  $J = 0.6$  Hz, 1H,  $\text{H}_3$ ), 7.55 (dd,  $J = 5.3$  Hz,  $J = 2.0$  Hz, 1H,  $\text{H}_5$ ), 7.41–7.28 (m, 3H, Ar-H), 7.14–7.06 (m, 1H, Ar-H), 4.47 (s, 2H,  $\text{CH}_2$ );  $^{13}\text{C-NMR}$  (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 164.8, 162.3 (d,  $J = 244.1$  Hz), 149.6, 148.2, 147.4, 139.3 (d,  $J = 7.6$  Hz), 130.8 (d,  $J = 8.6$  Hz), 125.2 (d,  $J = 2.9$  Hz), 123.5, 121.5, 115.8 (d,  $J = 21.9$  Hz), 114.5 (d,  $J = 21.3$  Hz), 33.6.

#### 4-(4-Fluorobenzylsulfanyl)pyridine-2-carboxylic acid 4e

Yield: 56%; m.p.:  $172\text{--}174^\circ\text{C}$ ; IR (KBr)  $\lambda_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3500–2400 (COOH), 1722 (C=O);  $^1\text{H-NMR}$  (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.50 (dd,  $J = 5.5$  Hz,  $J = 0.6$  Hz, 1H,  $\text{H}_6$ ), 7.96 (dd,  $J = 1.8$  Hz,  $J = 0.6$  Hz, 1H,  $\text{H}_3$ ), 7.65 (dd,  $J = 5.5$  Hz,  $J = 1.8$  Hz, 1H,  $\text{H}_5$ ), 7.52–7.48 (m, 2H, Ar-H), 7.20–7.14 (m, 2H, Ar-H), 4.49 (s, 2H,  $\text{CH}_2$ );  $^{13}\text{C-NMR}$  (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 164.8, 161.7 (d,  $J = 244.0$  Hz), 153.3, 147.3, 146.3, 132.1 (d,  $J = 3.0$  Hz), 131.2 (d,  $J = 8.2$  Hz), 123.7, 121.7, 115.7 (d,  $J = 21.4$  Hz), 33.6.

#### 4-(3-Bromobenzylsulfanyl)pyridine-2-carboxylic acid 4f

Yield: 50%; m.p.:  $156\text{--}158^\circ\text{C}$ ; IR (KBr)  $\lambda_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3600–2500 (COOH), 1717 (C=O);  $^1\text{H-NMR}$  (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.47 (dd,  $J = 5.3$  Hz,  $J = 0.5$  Hz, 1H,  $\text{H}_6$ ), 7.87 (dd,  $J = 1.9$  Hz,  $J = 0.5$  Hz, 1H,  $\text{H}_3$ ), 7.67–7.66 (m, 1H, Ar-H), 7.52 (dd,  $J = 5.3$  Hz,  $J = 1.9$  Hz, 1H,  $\text{H}_5$ ), 7.46 (dd,  $J = 7.8$  Hz,  $J = 1.8$  Hz, 2H, Ar-H), 7.31–7.26 (m, 1H, Ar-H), 4.45 (s, 2H,  $\text{CH}_2$ );  $^{13}\text{C-NMR}$  (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 166.0, 149.9, 149.1, 148.4, 139.3, 131.8, 130.9, 130.6, 128.1, 123.6, 121.9, 121.5, 33.5.

#### 4-(4-Bromobenzylsulfanyl)pyridine-2-carboxylic acid 4g

Yield: 42%; m.p.:  $166\text{--}168^\circ\text{C}$ ; IR (KBr)  $\lambda_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3600–2500 (COOH), 1718 (C=O);  $^1\text{H-NMR}$  (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.46 (dd,  $J = 5.3$  Hz,  $J = 0.6$  Hz, 1H,  $\text{H}_6$ ), 7.86 (dd,  $J = 2.0$  Hz,  $J = 0.6$  Hz, 1H,  $\text{H}_3$ ), 7.54–7.51 (m, 3H,  $\text{H}_5$ , Ar-H), 7.42–7.39 (m, 2H, Ar-H), 4.43 (s, 2H,  $\text{CH}_2$ );  $^{13}\text{C-NMR}$  (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 166.0, 149.9, 149.1, 148.4, 135.9, 131.7, 131.3, 123.6, 121.5, 120.8, 33.5.

#### 4-(3-Methylbenzylsulfanyl)pyridine-2-carboxylic acid 4h

Yield: 60%; m.p.:  $148\text{--}150^\circ\text{C}$ ; IR (KBr)  $\lambda_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3600–2400 (COOH), 1717 (C=O);  $^1\text{H-NMR}$  (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.47 (dd,  $J = 5.3$  Hz,  $J = 0.7$  Hz, 1H,  $\text{H}_6$ ), 7.89 (dd,  $J = 2.0$  Hz,  $J = 0.7$  Hz, 1H,  $\text{H}_3$ ), 7.54 (dd,  $J = 5.3$  Hz,  $J = 2.0$  Hz, 1H,  $\text{H}_5$ ), 7.27–7.22 (m, 3H, Ar-H), 7.10–7.08 (m, 1H, Ar-H), 4.40 (s, 2H,  $\text{CH}_2$ ), 2.28 (s, 3H,  $\text{CH}_3$ );  $^{13}\text{C-NMR}$  (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 166.0, 150.7, 149.0, 148.2, 138.0, 136.0, 129.7, 128.7, 128.4, 126.2, 123.5, 121.3, 34.3, 21.1.

#### 4-(4-Methylbenzylsulfanyl)pyridine-2-carboxylic acid 4i

Yield: 52%; m.p.:  $183\text{--}185^\circ\text{C}$ ; IR (KBr)  $\lambda_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3600–2500 (COOH), 1716 (C=O);  $^1\text{H-NMR}$  (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.47 (dd,  $J = 5.4$  Hz,  $J = 0.6$  Hz, 1H,  $\text{H}_6$ ), 7.91 (dd,  $J = 2.0$  Hz,  $J = 0.6$  Hz, 1H,  $\text{H}_3$ ), 7.57 (dd,  $J = 5.4$  Hz,  $J = 2.0$  Hz, 1H,  $\text{H}_5$ ), 7.34–7.31 (m, 2H, Ar-H), 7.15–7.12 (m, 2H, Ar-H), 4.40 (s, 2H,  $\text{CH}_2$ ), 2.25 (s, 3H,  $\text{CH}_3$ );  $^{13}\text{C-NMR}$  (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 165.5, 152.0, 148.2, 147.4, 137.0, 132.8, 129.4, 129.0, 123.6, 121.5, 34.1, 20.9.

#### 4-(3-Trifluoromethylbenzylsulfanyl)pyridine-2-carboxylic acid **4j**

Yield: 32%; m.p.: 150–152°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3600–2500 (COOH), 1717 (C=O), 1332 (CF<sub>3</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.48 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H<sub>6</sub>), 7.92 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H<sub>3</sub>), 7.84 (s, 1H, Ar-H), 7.77 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.65–7.57 (m, 3H, H<sub>5</sub>, Ar-H), 4.58 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 165.6, 150.6, 148.6, 147.9, 138.0, 133.2, 129.9, 129.4 (q, *J* = 31.8 Hz), 125.7 (q, *J* = 3.5 Hz), 124.5 (q, *J* = 3.5 Hz), 124.2 (q, *J* = 272.9 Hz), 123.8, 121.6, 33.5.

#### 4-(4-Trifluoromethylbenzylsulfanyl)pyridine-2-carboxylic acid **4k**

Yield: 45%; m.p.: 148–150°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3600–2500 (COOH), 1718 (C=O), 1326 (CF<sub>3</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.47 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H<sub>6</sub>), 7.88 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H<sub>3</sub>), 7.73–7.66 (m, 4H, Ar-H), 7.54 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 4.56 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 166.0, 149.7, 149.2, 148.4, 141.5, 129.8, 128.2 (q, *J* = 31.8 Hz), 125.7 (q, *J* = 3.9 Hz), 124.4 (q, *J* = 272.9 Hz), 123.6, 121.5, 33.6.

#### 4-(3-Cyanobenzylsulfanyl)pyridine-2-carboxylic acid **4l**

Yield: 44%; m.p.: 184–186°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3600–2500 (COOH), 2230 (CN), 1716 (C=O); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.48 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H<sub>6</sub>), 7.93 (s, 1H, Ar-H), 7.88 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H<sub>3</sub>), 7.80 (dd, *J* = 7.8 Hz, *J* = 1.0 Hz, 1H, Ar-H), 7.74 (dd, *J* = 7.8 Hz, *J* = 1.0 Hz, 1H, Ar-H), 7.57–7.52 (m, 2H, H<sub>5</sub>, Ar-H), 4.52 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 165.9, 149.8, 149.1, 148.3, 138.3, 134.0, 132.6, 131.5, 130.1, 123.6, 121.6, 118.7, 111.7, 33.3.

#### 4-(3-Methoxybenzylsulfanyl)pyridine-2-carboxylic acid **4m**

Yield: 37%; m.p.: 90–92°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3600–2500 (COOH), 1718 (C=O), 1270 (OCH<sub>3</sub>), 1040 (OCH<sub>3</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.50 (dd, *J* = 5.6 Hz, *J* = 0.6 Hz, 1H, H<sub>6</sub>), 8.01 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H<sub>3</sub>), 7.68 (dd, *J* = 5.6 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 7.25 (t, *J* = 7.8 Hz, 1H, Ar-H), 7.04–7.01 (m, 2H, Ar-H), 6.86–6.82 (m, 1H, Ar-H), 4.47 (s, 2H, CH<sub>2</sub>), 3.73 (s, 3H, OCH<sub>3</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 164.4, 159.6, 154.6, 146.7, 145.6, 137.3, 130.0, 123.8, 121.8, 121.3, 114.8, 113.3, 55.3, 34.5.

#### 4-(4-Nitrobenzylsulfanyl)pyridine-2-carboxylic acid **4n**

Yield: 81% (Method B); m.p.: 193–195°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3600–2500 (COOH), 1718 (C=O), 1519 (NO<sub>2</sub>), 1347 (NO<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.47 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H<sub>6</sub>), 8.20–8.17 (m, 2H, Ar-H), 7.88 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H<sub>3</sub>), 7.74–7.71 (m, 2H, Ar-H), 7.54 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 4.61 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 165.9, 149.3, 149.2, 148.5, 146.9, 144.7, 130.3, 123.9, 123.7, 121.6, 33.5.

#### 4-(3,5-Dinitrobenzylsulfanyl)pyridine-2-carboxylic acid **4o**

Yield: 49% (Method B); m.p.: 202–204°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3600–2500 (COOH), 1709 (C=O), 1544 (NO<sub>2</sub>), 1529 (NO<sub>2</sub>), 1342 (NO<sub>2</sub>), 1320 (NO<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.77 (d, *J* = 2.1 Hz, 2H, Ar-H), 8.70 (t, *J* = 2.1 Hz, 1H, Ar-H), 8.48 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H<sub>6</sub>), 7.92 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H<sub>3</sub>), 7.59 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 4.76 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR

(75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 166.0, 149.4, 148.7, 148.6, 148.3, 141.7, 129.4, 123.8, 121.8, 117.9, 32.6.

#### General procedure for the synthesis of compounds **7n, o**

These compounds were prepared according to the literature [8], by reacting isothiuronium salt **5** with nitrobenzyl halides **6**.

#### General procedure for the synthesis of compounds **8**

Thionyl chloride (2.2 mL, 30 mmol) was added in a few small portions – the temperature was watched not to exceed 0°C – to a solution of **4** (3 mmol) in 70 mL of absolute ethanol cooled to –2°C. After 10 min of stirring at room temperature, the reaction mixture was refluxed for 5–7 h. Monitoring by silica gel TLC plates (chloroform / methanol / triethylamine 9 : 1 : 0.25) confirmed the completion of the reaction. Thereafter, ethanol was partially evaporated, the remainder poured onto crushed ice, and alkalinized by the saturated aqueous solution of NaHCO<sub>3</sub> to pH 11. The mixture was left in the refrigerator overnight, and the precipitated product was filtered off and recrystallized from ethanol.

#### Ethyl 4-(benzylsulfanyl)pyridine-2-carboxylate **8a**

Yield: 53%; m.p.: 46–47°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 1720 (C=O); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.45 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H<sub>6</sub>), 7.88 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H<sub>3</sub>), 7.51 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 7.48–7.43 (m, 2H, Ar-H), 7.37–7.23 (m, 3H, Ar-H), 4.42 (s, 2H, CH<sub>2</sub>), 4.30 (q, *J* = 7.1 Hz, 2H, CH<sub>2</sub>), 1.30 (t, *J* = 7.1 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 164.7, 150.5, 149.4, 148.3, 136.2, 129.1, 128.8, 127.7, 123.8, 121.5, 61.5, 34.3, 14.3.

#### Ethyl 4-(3-chlorobenzylsulfanyl)pyridine-2-carboxylate **8b**

Yield: 58%; m.p.: 60–62°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 1735 (C=O); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.48 (dd, *J* = 5.2 Hz, *J* = 0.5 Hz, 1H, H<sub>6</sub>), 7.87 (dd, *J* = 1.9 Hz, *J* = 0.5 Hz, 1H, H<sub>3</sub>), 7.56–7.53 (m, 2H, H<sub>5</sub>, Ar-H), 7.44–7.31 (m, 3H, Ar-H), 4.46 (s, 2H, CH<sub>2</sub>), 4.31 (q, *J* = 7.1 Hz, 2H, CH<sub>2</sub>), 1.31 (t, *J* = 7.1 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 164.6, 149.7, 149.5, 147.8, 139.1, 133.3, 130.6, 128.9, 127.7, 127.6, 123.8, 121.6, 61.5, 33.5, 14.3.

#### Ethyl 4-(4-chlorobenzylsulfanyl)pyridine-2-carboxylate **8c**

Yield: 87%; m.p.: 101–103°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 1730 (C=O); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.47 (dd, *J* = 5.3 Hz, *J* = 0.7 Hz, 1H, H<sub>6</sub>), 7.86 (dd, *J* = 2.0 Hz, *J* = 0.7 Hz, 1H, H<sub>3</sub>), 7.54 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 7.49–7.46 (m, 2H, Ar-H), 7.40–7.37 (m, 2H, Ar-H), 4.45 (s, 2H, CH<sub>2</sub>), 4.31 (q, *J* = 7.1 Hz, 2H, CH<sub>2</sub>), 1.30 (t, *J* = 7.1 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 164.6, 149.9, 149.5, 147.7, 135.5, 132.3, 130.9, 128.8, 123.8, 121.6, 61.5, 33.5, 14.3.

#### Ethyl 4-(3-fluorobenzylsulfanyl)pyridine-2-carboxylate **8d**

Yield: 82%; m.p.: 75–77°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 1724 (C=O); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 8.48 (dd, *J* = 5.3 Hz, *J* = 0.7 Hz, 1H, H<sub>6</sub>), 7.87 (dd, *J* = 2.0 Hz, *J* = 0.7 Hz, 1H, H<sub>3</sub>), 7.55 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 7.41–7.28 (m, 3H, Ar-H), 7.13–7.06 (m, 1H, Ar-H), 4.47 (s, 2H, CH<sub>2</sub>), 4.31 (q, *J* = 7.1 Hz, 2H, CH<sub>2</sub>), 1.30 (t, *J* = 7.1 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 164.6, 162.3 (d, *J* = 244.1 Hz), 149.8, 149.5, 147.7, 139.3 (d, *J* = 7.6 Hz), 130.8 (d, *J* = 8.0 Hz), 125.2 (d, *J* = 2.8 Hz), 123.8, 121.6, 115.8 (d, *J* = 21.9 Hz), 114.5 (d, *J* = 20.7 Hz), 61.5, 33.6, 14.2.



**Ethyl 4-(4-fluorobenzylsulfanyl)pyridine-2-carboxylate 8e**

Yield: 61%; m.p.: 77–79°C; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1728 (C=O);  $^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.48 (dd,  $J = 5.2$  Hz,  $J = 1.1$  Hz, 1H, H<sub>6</sub>), 7.86 (dd,  $J = 1.7$  Hz,  $J = 1.1$  Hz, 1H, H<sub>3</sub>), 7.56–7.47 (m, 2H, H<sub>5</sub>, Ar-H), 7.36–7.32 (m, 1H, Ar-H), 7.20–7.13 (m, 2H, Ar-H), 4.44 (s, 2H, CH<sub>2</sub>), 4.31 (dq,  $J = 7.1$  Hz,  $J = 1.7$  Hz, 2H, CH<sub>2</sub>), 1.30 (dt,  $J = 7.1$  Hz,  $J = 1.7$  Hz, 3H, CH<sub>3</sub>);  $^{13}\text{C}$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 164.7, 161.6 (d,  $J = 243.0$  Hz), 150.0, 149.5, 147.7, 132.5 (d,  $J = 3.2$  Hz), 131.1 (d,  $J = 8.3$  Hz), 123.8, 121.6, 115.6 (d,  $J = 21.6$  Hz), 61.5, 33.4, 14.3.

**Ethyl 4-(3-bromobenzylsulfanyl)pyridine-2-carboxylate 8f**

Yield: 80%; m.p.: 66–68°C; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1733 (C=O);  $^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.47 (dd,  $J = 5.3$  Hz,  $J = 0.5$  Hz, 1H, H<sub>6</sub>), 7.86 (dd,  $J = 1.8$  Hz,  $J = 0.5$  Hz, 1H, H<sub>3</sub>), 7.67 (s, 1H, Ar-H), 7.54 (dd,  $J = 5.3$  Hz,  $J = 1.8$  Hz, 1H, H<sub>5</sub>), 7.46 (d,  $J = 7.8$  Hz, 2H, Ar-H), 7.29 (t,  $J = 7.8$  Hz, 1H, Ar-H), 4.45 (s, 2H, CH<sub>2</sub>), 4.31 (q,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>), 1.30 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>);  $^{13}\text{C}$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 164.6, 149.7, 149.5, 147.8, 139.3, 131.8, 130.9, 130.5, 128.1, 123.8, 121.9, 121.6, 61.5, 33.5, 14.3.

**Ethyl 4-(4-bromobenzylsulfanyl)pyridine-2-carboxylate 8g**

Yield: 83%; m.p.: 108–110°C; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1730 (C=O);  $^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.47 (dd,  $J = 5.3$  Hz,  $J = 0.5$  Hz, 1H, H<sub>6</sub>), 7.85 (dd,  $J = 1.9$  Hz,  $J = 0.5$  Hz, 1H, H<sub>3</sub>), 7.55–7.51 (m, 3H, H<sub>5</sub>, Ar-H), 7.43–7.40 (m, 2H, Ar-H), 4.43 (s, 2H, CH<sub>2</sub>), 4.31 (q,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>), 1.30 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>);  $^{13}\text{C}$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 164.6, 149.8, 149.5, 147.7, 135.9, 131.7, 131.2, 123.8, 121.6, 120.8, 61.5, 33.5, 14.3.

**Ethyl 4-(3-methylbenzylsulfanyl)pyridine-2-carboxylate 8h**

Yield: 63%; m.p.: 44–46°C; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1720 (C=O);  $^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.47 (dd,  $J = 5.3$  Hz,  $J = 0.6$  Hz, 1H, H<sub>6</sub>), 7.87 (dd,  $J = 2.0$  Hz,  $J = 0.6$  Hz, 1H, H<sub>3</sub>), 7.53 (dd,  $J = 5.3$  Hz,  $J = 2.0$  Hz, 1H, H<sub>5</sub>), 7.26–7.18 (m, 3H, Ar-H), 7.08–7.06 (m, 1H, Ar-H), 4.38 (s, 2H, CH<sub>2</sub>), 4.31 (q,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>), 2.27 (s, 3H, CH<sub>3</sub>), 1.31 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>);  $^{13}\text{C}$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 164.7, 150.3, 149.4, 147.7, 138.0, 136.0, 129.7, 128.7, 128.3, 126.2, 123.7, 121.5, 61.5, 34.3, 21.1, 14.3.

**Ethyl 4-(4-methylbenzylsulfanyl)pyridine-2-carboxylate 8i**

Yield: 60%; m.p.: 75–77°C; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1722 (C=O);  $^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.45 (dd,  $J = 5.3$  Hz,  $J = 0.5$  Hz, 1H, H<sub>6</sub>), 7.90 (dd,  $J = 2.0$  Hz,  $J = 0.5$  Hz, 1H, H<sub>3</sub>), 7.56 (dd,  $J = 5.3$  Hz,  $J = 2.0$  Hz, 1H, H<sub>5</sub>), 7.33 (d,  $J = 7.9$  Hz, 2H, Ar-H), 7.14 (d,  $J = 7.9$  Hz, 2H, Ar-H), 4.39 (s, 2H, CH<sub>2</sub>), 4.31 (q,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>), 2.25 (s, 3H, CH<sub>3</sub>), 1.30 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>);  $^{13}\text{C}$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 164.6, 150.4, 149.3, 147.8, 137.0, 132.9, 129.4, 129.0, 123.8, 121.6, 61.5, 34.0, 20.9, 14.3.

**Ethyl 4-(3-trifluoromethylbenzylsulfanyl)pyridine-2-carboxylate 8j**

Yield: 76%; m.p.: 45–47°C; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1716 (C=O), 1331 (CF<sub>3</sub>);  $^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.48 (dd,  $J = 5.3$  Hz,  $J = 0.5$  Hz, 1H, H<sub>6</sub>), 7.88 (dd,  $J = 1.9$  Hz,  $J = 0.5$  Hz, 1H, H<sub>3</sub>), 7.83 (s, 1H, Ar-H), 7.78–7.76 (m, 1H, Ar-H), 7.64–7.54 (m, 3H, H<sub>5</sub>, Ar-H), 4.56 (s, 2H, CH<sub>2</sub>), 4.31 (q,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>), 1.30 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>);  $^{13}\text{C}$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 164.6, 149.6, 149.5, 147.8, 138.1, 133.2, 129.9, 129.4 (q,  $J = 31.8$  Hz), 125.7 (q,  $J =$

3.9 Hz), 124.4 (q,  $J = 3.9$  Hz), 124.3, (q,  $J = 272.9$  Hz), 123.9, 121.7, 61.5, 33.5, 14.2.

**Ethyl 4-(4-trifluoromethylbenzylsulfanyl)pyridine-2-carboxylate 8k**

Yield: 68%; m.p.: 84–86°C; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1728 (C=O), 1327 (CF<sub>3</sub>);  $^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.48 (dd,  $J = 5.2$  Hz,  $J = 0.4$  Hz, 1H, H<sub>6</sub>), 7.87 (dd,  $J = 1.2$  Hz,  $J = 0.4$  Hz, 1H, H<sub>3</sub>), 7.70–7.65 (m, 4H, Ar-H), 7.56 (dd,  $J = 5.2$  Hz,  $J = 1.2$  Hz, 1H, H<sub>5</sub>), 4.56 (s, 2H, CH<sub>2</sub>), 4.31 (q,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>), 1.30 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>);  $^{13}\text{C}$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 164.6, 149.6, 149.5, 147.8, 141.5, 129.8, 128.2 (q,  $J = 31.8$  Hz), 125.7 (q,  $J = 3.9$  Hz), 124.3, (q,  $J = 272.9$  Hz), 123.8, 121.6, 61.5, 33.6, 14.2.

**Ethyl 4-(3-cyanobenzylsulfanyl)pyridine-2-carboxylate 8l**

Yield: 66%; m.p.: 104–106°C; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 2230 (CN), 1735 (C=O);  $^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.48 (dd,  $J = 5.3$  Hz,  $J = 0.5$  Hz, 1H, H<sub>6</sub>), 7.93 (s, 1H, Ar-H), 7.86 (dd,  $J = 1.9$  Hz,  $J = 0.5$  Hz, 1H, H<sub>3</sub>), 7.82–7.73 (m, 2H, Ar-H), 7.57–7.52 (m, 2H, H<sub>5</sub>, Ar-H), 4.51 (s, 2H, CH<sub>2</sub>), 4.31 (q,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>), 1.31 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>);  $^{13}\text{C}$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 164.6, 149.5, 149.4, 147.8, 138.4, 133.9, 132.6, 131.5, 130.1, 123.8, 121.7, 118.7, 111.7, 61.5, 33.3, 14.3.

**Ethyl 4-(3-methoxybenzylsulfanyl)pyridine-2-carboxylate 8m**

Yield: 66%; m.p.: 73–75°C; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1714 (C=O), 1266 (OCH<sub>3</sub>), 1044 (OCH<sub>3</sub>);  $^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.47 (dd,  $J = 5.3$  Hz,  $J = 0.5$  Hz, 1H, H<sub>6</sub>), 7.87 (dd,  $J = 2.0$  Hz,  $J = 0.5$  Hz, 1H, H<sub>3</sub>), 7.54 (dd,  $J = 5.3$  Hz,  $J = 2.0$  Hz, 1H, H<sub>5</sub>), 7.24 (t,  $J = 8.1$  Hz, 1H, Ar-H), 7.03–7.01 (m, 2H, Ar-H), 6.85–6.81 (m, 1H, Ar-H), 4.40 (s, 2H, CH<sub>2</sub>), 4.31 (q,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>), 3.72 (s, 3H, OCH<sub>3</sub>), 1.30 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>);  $^{13}\text{C}$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 164.7, 159.5, 150.3, 149.4, 147.7, 137.8, 129.9, 123.7, 121.5, 121.2, 114.7, 113.1, 61.5, 55.2, 34.3, 14.3.

**Ethyl 4-(4-nitrobenzylsulfanyl)pyridine-2-carboxylate 8n**

Yield: 72%; m.p.: 106–107°C; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1733 (C=O), 1523 (NO<sub>2</sub>), 1349 (NO<sub>2</sub>);  $^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.48 (dd,  $J = 5.3$  Hz,  $J = 0.6$  Hz, 1H, H<sub>6</sub>), 8.19 (d,  $J = 8.8$  Hz, 2H, Ar-H), 7.87 (dd,  $J = 2.0$  Hz,  $J = 0.6$  Hz, 1H, H<sub>3</sub>), 7.73 (d,  $J = 8.8$  Hz, 2H, Ar-H), 7.56 (ddd,  $J = 5.3$  Hz,  $J = 2.0$  Hz,  $J = 0.7$  Hz, 1H, H<sub>5</sub>), 4.61 (s, 2H, CH<sub>2</sub>), 4.30 (dq,  $J = 7.1$  Hz,  $J = 0.7$  Hz, 2H, CH<sub>2</sub>), 1.30 (dt,  $J = 7.1$  Hz,  $J = 0.7$  Hz, 3H, CH<sub>3</sub>);  $^{13}\text{C}$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 164.6, 149.6, 149.3, 147.8, 147.8, 147.0, 144.8, 130.3, 123.9, 121.8, 61.6, 33.5, 14.3.

**Ethyl 4-(3,5-dinitrobenzylsulfanyl)pyridine-2-carboxylate 8o**

Yield: 72%; m.p.: 136–138°C; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1726 (C=O), 1536 (NO<sub>2</sub>), 1339 (NO<sub>2</sub>);  $^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.78 (d,  $J = 2.1$  Hz, 2H, Ar-H), 8.70 (t,  $J = 2.1$  Hz, 1H, Ar-H), 8.49 (dd,  $J = 5.3$  Hz,  $J = 0.6$  Hz, 1H, H<sub>6</sub>), 7.91 (dd,  $J = 2.0$  Hz,  $J = 0.6$  Hz, 1H, H<sub>3</sub>), 7.61 (ddd,  $J = 5.3$  Hz,  $J = 2.0$  Hz,  $J = 0.6$  Hz, 1H, H<sub>5</sub>), 4.76 (s, 2H, CH<sub>2</sub>), 4.31 (q,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>), 1.30 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>);  $^{13}\text{C}$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 164.5, 149.7, 148.6, 148.3, 147.9, 141.8, 129.4, 124.0, 121.9, 117.9, 61.6, 32.6, 14.2.

### General procedure for the synthesis of compounds **9**

To a solution of **8** (1.3 mmol) in 2–3 mL of absolute ethanol hydrazine hydrate 80% (0.9 mL, 30 mmol) was added and the mixture was stirred at the ambient temperature for 1–2 h. Monitoring by silica gel TLC plates (chloroform – methanol – triethylamine 9 : 1 : 0.25) The mixture was left overnight in the refrigerator. The precipitated solid was collected, then washed by water and crystallized from ethanol.

### 4-(Benzylsulfanyl)pyridine-2-carbohydrazide **9a**

Yield: 63%; m.p.: 132–134°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3301 (N-H), 3202 (N-H), 1664 (C=O), 1627 (NH<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.86 (s, 1H, NH), 8.37 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H<sub>6</sub>), 7.83 (dd, *J* = 1.9 Hz, *J* = 0.5 Hz, 1H, H<sub>3</sub>), 7.48–7.43 (m, 3H, H<sub>5</sub>, Ar-H), 7.36–7.23 (m, 3H, Ar-H), 4.56 (s, 2H, NH<sub>2</sub>), 4.42 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.4, 150.5, 149.8, 148.3, 136.2, 129.1, 128.8, 127.6, 122.7, 118.4, 34.2.

### 4-(3-Chlorobenzylsulfanyl)pyridine-2-carbohydrazide **9b**

Yield: 70%; m.p.: 120–122°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3305 (N-H), 3203 (N-H), 1664 (C=O), 1624 (NH<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.86 (s, 1H, NH), 8.39 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H<sub>6</sub>), 7.83 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H<sub>3</sub>), 7.54 (t, *J* = 1.8 Hz, 1H, Ar-H), 7.47 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 7.45–7.33 (m, 3H, Ar-H), 4.56 (s, 2H, NH<sub>2</sub>), 4.46 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.3, 150.0, 149.8, 148.4, 139.1, 133.3, 130.6, 128.9, 127.7, 127.6, 122.7, 118.5, 33.4.

### 4-(4-Chlorobenzylsulfanyl)pyridine-2-carbohydrazide **9c**

Yield: 56%; m.p.: 119–121°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3376 (N-H), 3323 (N-H), 1671 (C=O), 1624 (NH<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.86 (s, 1H, NH), 8.38 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H<sub>6</sub>), 7.82 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H<sub>3</sub>), 7.50–7.45 (m, 3H, H<sub>5</sub>, Ar-H), 7.42–7.37 (m, 2H, Ar-H), 4.55 (s, 2H, NH<sub>2</sub>), 4.45 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.3, 150.1, 149.8, 148.4, 135.5, 132.2, 130.9, 128.8, 122.7, 118.5, 33.4.

### 4-(3-Fluorobenzylsulfanyl)pyridine-2-carbohydrazide **9d**

Yield: 78%; m.p.: 94–95°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3290 (N-H), 1662 (C=O), 1618 (NH<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.86 (s, 1H, NH), 8.39 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H<sub>6</sub>), 7.83 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H<sub>3</sub>), 7.47 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 7.42–7.28 (m, 3H, Ar-H), 7.14–7.06 (m, 1H, Ar-H), 4.56 (s, 2H, NH<sub>2</sub>), 4.47 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.3, 162.3 (d, *J* = 244.1 Hz), 150.0, 149.8, 148.4, 139.4 (d, *J* = 7.6 Hz), 130.7 (d, *J* = 8.6 Hz), 125.2 (d, *J* = 2.9 Hz), 122.7, 118.5, 115.8 (d, *J* = 21.9 Hz), 114.5 (d, *J* = 21.3 Hz), 33.6.

### 4-(4-Fluorobenzylsulfanyl)pyridine-2-carbohydrazide **9e**

Yield: 58%; m.p.: 112–114°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3306 (N-H), 3202 (N-H), 1664 (C=O), 1626 (NH<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.86 (s, 1H, NH), 8.39 (dd, *J* = 5.3 Hz, *J* = 0.7 Hz, 1H, H<sub>6</sub>), 7.82 (dd, *J* = 2.0 Hz, *J* = 0.7 Hz, 1H, H<sub>3</sub>), 7.52–7.46 (m, 3H, H<sub>5</sub>, Ar-H), 7.20–7.13 (m, 2H, Ar-H), 4.56 (s, 2H, NH<sub>2</sub>), 4.44 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.3, 161.6 (d, *J* = 244.0 Hz), 150.2, 149.8, 148.4, 132.5 (d, *J* = 3.0 Hz), 131.1 (d, *J* = 8.2 Hz), 122.7, 118.4, 115.6 (d, *J* = 21.4 Hz), 33.4.

### 4-(3-Bromobenzylsulfanyl)pyridine-2-carbohydrazide **9f**

Yield: 71%; m.p.: 127–129°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3304 (N-H), 3202 (N-H), 1663 (C=O), 1623 (NH<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.86 (s, 1H, NH), 8.39 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H<sub>6</sub>), 7.83 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H<sub>3</sub>), 7.68 (t, *J* = 1.7 Hz, 1H, Ar-H), 7.48–7.45 (m, 3H, H<sub>5</sub>, Ar-H), 7.32–7.27 (m, 1H, Ar-H), 4.57 (s, 2H, NH<sub>2</sub>), 4.46 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.3, 150.0, 149.8, 148.4, 139.3, 131.7, 130.9, 130.5, 128.1, 122.7, 121.9, 118.5, 33.5.

### 4-(4-Bromobenzylsulfanyl)pyridine-2-carbohydrazide **9g**

Yield: 73%; m.p.: 137–138°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3375 (N-H), 3319 (N-H), 1669 (C=O), 1618 (NH<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.86 (s, 1H, NH), 8.38 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H<sub>6</sub>), 7.83 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H<sub>3</sub>), 7.55–7.51 (m, 3H, H<sub>5</sub>, Ar-H), 7.43–7.39 (m, 2H, Ar-H), 4.57 (s, 2H, NH<sub>2</sub>), 4.46 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.3, 150.7, 149.5, 148.3, 135.8, 131.7, 131.3, 122.9, 120.8, 118.8, 33.5.

### 4-(3-Methylbenzylsulfanyl)pyridine-2-carbohydrazide **9h**

Yield: 79%; m.p.: 104–105°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3308 (N-H), 3197 (N-H), 1678 (C=O), 1618 (NH<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.86 (s, 1H, NH), 8.38 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H<sub>6</sub>), 7.83 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H<sub>3</sub>), 7.46 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 7.27–7.19 (m, 3H, Ar-H), 7.09–7.06 (m, 1H, Ar-H), 4.56 (s, 2H, NH<sub>2</sub>), 4.38 (s, 2H, CH<sub>2</sub>), 2.26 (s, 3H, CH<sub>3</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.4, 150.6, 149.7, 148.3, 138.0, 136.0, 129.7, 128.7, 128.3, 126.2, 122.6, 118.3, 34.3, 21.1.

### 4-(4-Methylbenzylsulfanyl)pyridine-2-carbohydrazide **9i**

Yield: 62%; m.p.: 124–126°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3338 (N-H), 1693 (C=O), 1617 (NH<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.85 (s, 1H, NH), 8.37 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H<sub>6</sub>), 7.82 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H<sub>3</sub>), 7.46 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 7.33 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.14 (d, *J* = 7.9 Hz, 2H, Ar-H), 4.56 (s, 2H, NH<sub>2</sub>), 4.38 (s, 2H, CH<sub>2</sub>), 2.26 (s, 3H, CH<sub>3</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.4, 150.5, 149.7, 148.3, 136.9, 133.0, 129.4, 129.0, 122.7, 118.4, 34.0, 20.9.

### 4-(3-Trifluoromethylbenzylsulfanyl)pyridine-2-carbohydrazide **9j**

Yield: 87%; m.p.: 96–98°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3332 (N-H), 1678 (C=O), 1617 (NH<sub>2</sub>), 1331 (CF<sub>3</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.87 (s, 1H, NH), 8.39 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H<sub>6</sub>), 7.85–7.83 (m, 2H, H<sub>3</sub>, Ar-H), 7.77 (d, *J* = 7.4 Hz, 1H, Ar-H), 7.66–7.55 (m, 2H, Ar-H), 7.49 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 4.57 (s, 4H, NH<sub>2</sub>, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.3, 149.9, 149.8, 148.4, 138.1, 133.2, 129.9, 129.5 (q, *J* = 31.8 Hz), 125.6 (q, *J* = 3.9 Hz), 124.4 (q, *J* = 3.9 Hz), 124.3 (q, *J* = 272.3 Hz), 122.8, 118.5, 33.5.

### 4-(4-Trifluoromethylbenzylsulfanyl)pyridine-2-carbohydrazide **9k**

Yield: 45%; m.p.: 159–161°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3323 (N-H), 1699 (C=O), 1617 (NH<sub>2</sub>), 1324 (CF<sub>3</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.87 (s, 1H, NH), 8.39 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H<sub>6</sub>), 7.83 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H<sub>3</sub>), 7.72–7.65 (m, 4H, Ar-H), 7.49 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H<sub>5</sub>), 4.57 (s, 2H, NH<sub>2</sub>), 4.56 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.3, 149.9, 149.7, 148.4,

141.5, 129.8, 128.3 (q,  $J = 31.8$  Hz), 125.6 (q,  $J = 3.9$  Hz), 124.4 (q,  $J = 272.9$  Hz), 122.9, 118.4, 33.6.

#### 4-(3-Cyanobenzylsulfanyl)pyridine-2-carbohydrazide **9l**

Yield: 76%; m.p.: 130–132°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3351 (N-H), 3313 (N-H), 2229 (CN), 1660 (C=O), 1629 (NH<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.87 (s, 1H, NH), 8.39 (dd,  $J = 5.3$  Hz,  $J = 0.4$  Hz, 1H, H<sub>6</sub>), 7.94 (t,  $J = 1.4$  Hz, 1H, Ar-H), 7.83–7.73 (m, 3H, H<sub>3</sub>, Ar-H), 7.56 (t,  $J = 7.7$  Hz, 1H, Ar-H), 7.48 (dd,  $J = 5.3$  Hz,  $J = 2.0$  Hz, 1H, H<sub>5</sub>), 4.56 (s, 2H, NH<sub>2</sub>), 4.52 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.3, 149.9, 149.7, 148.5, 138.4, 134.0, 132.6, 131.5, 130.1, 122.8, 118.7, 118.5, 111.7, 33.3.

#### 4-(3-Methoxybenzylsulfanyl)pyridine-2-carbohydrazide **9m**

Yield: 60%; m.p.: 94–95°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3300 (N-H), 3201 (N-H), 1664 (C=O), 1626 (NH<sub>2</sub>), 1270 (OCH<sub>3</sub>), 1049 (OCH<sub>3</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.86 (s, 1H, NH), 8.38 (dd,  $J = 5.3$  Hz,  $J = 0.6$  Hz, 1H, H<sub>6</sub>), 7.84 (dd,  $J = 2.0$  Hz,  $J = 0.6$  Hz, 1H, H<sub>3</sub>), 7.46 (dd,  $J = 5.3$  Hz,  $J = 2.0$  Hz, 1H, H<sub>5</sub>), 7.28–7.22 (m, 1H, Ar-H), 7.04–7.01 (m, 2H, Ar-H), 6.85–6.82 (m, 1H, Ar-H), 4.57 (s, 2H, NH<sub>2</sub>), 4.40 (s, 2H, CH<sub>2</sub>), 3.74 (s, 3H, OCH<sub>3</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.4, 159.5, 150.5, 149.8, 148.3, 137.8, 129.9, 122.7, 121.2, 118.4, 114.7, 113.1, 55.2, 34.2.

#### 4-(4-Nitrobenzylsulfanyl)pyridine-2-carbohydrazide **9n**

Yield: 76%; m.p.: 150–152°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3390 (N-H), 3314 (N-H), 1676 (C=O), 1628 (NH<sub>2</sub>), 1514 (NO<sub>2</sub>), 1349 (NO<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.85 (s, 1H, NH), 8.38 (dd,  $J = 5.3$  Hz,  $J = 0.5$  Hz, 1H, H<sub>6</sub>), 8.19 (d,  $J = 8.9$  Hz, 2H, Ar-H), 7.82 (dd,  $J = 2.0$  Hz,  $J = 0.5$  Hz, 1H, H<sub>3</sub>), 7.73 (d,  $J = 7.9$  Hz, 2H, Ar-H), 7.48 (dd,  $J = 5.3$  Hz,  $J = 2.0$  Hz, 1H, H<sub>5</sub>), 4.61 (s, 2H, CH<sub>2</sub>), 4.55 (s, 2H, NH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.2, 149.9, 149.5, 148.4, 146.9, 144.8, 130.3, 123.9, 122.8, 118.6, 33.4.

#### 4-(3,5-Dinitrobenzylsulfanyl)pyridine-2-carbohydrazide **9o**

Yield: 69%; m.p.: 230–232°C; IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3334 (N-H), 3256 (N-H), 1660 (C=O), 1626 (NH<sub>2</sub>), 1534 (NO<sub>2</sub>), 1530 (NO<sub>2</sub>), 1342 (NO<sub>2</sub>), 1329 (NO<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 9.85 (s, 1H, NH), 8.78 (d,  $J = 2.1$  Hz, 2H, Ar-H), 8.71 (t,  $J = 2.1$  Hz, 1H, Ar-H), 8.40 (dd,  $J = 5.3$  Hz,  $J = 0.5$  Hz, 1H, H<sub>6</sub>), 7.85 (dd,  $J = 2.0$  Hz,  $J = 0.5$  Hz, 1H, H<sub>3</sub>), 7.54 (dd,  $J = 5.3$  Hz,  $J = 2.0$  Hz, 1H, H<sub>5</sub>), 4.76 (s, 2H, CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 162.2, 150.0, 148.9, 148.6, 148.3, 141.8, 129.4, 122.9, 118.8, 117.9, 32.6.

## Biology

### Antimycobacterial evaluation

*In-vitro* antimycobacterial activity of the compounds was evaluated against *Mycobacterium tuberculosis* CNCTC My 331/88, *Mycobacterium kansasii* CNCTC My 235/80, *Mycobacterium kansasii* 6509/96, and *Mycobacterium avium* CNCTC My 330/88 using the micromethod for the determination of the minimum inhibitory concentration (MIC). All strains were obtained from the Czech National Collection of Type Cultures (CNCTC), with the exception of *M. kansasii* 6509/96, which was a clinical isolate. The activities of the compounds were determined in the Šula semisynthetic medium (SEVAC, Prague). The compounds were added to the medium in dimethylsulfoxide solutions. The following concentrations were used: 1000, 500, 250, 125, 62, 32, 16, 8, 4, and

2  $\mu$ mol/L. MICs were determined after incubation at 37°C for 14 and 21 days, for *M. kansasii* for 7, 14, and 21 days. MIC was the lowest concentration of a substance, at which the inhibition of the growth of mycobacteria occurred. INH was used as a standard.

The selected compounds were evaluated against three multi-drug-resistant strains of *M. tuberculosis* using the micromethod for the determination of the minimum inhibitory concentration (MIC) under the same conditions as described above. The characterization of resistant strains of *M. tuberculosis* is following: *Mycobacterium tuberculosis* 7357/98 resistant to isoniazid, rifampicin, streptomycin, ethambutol, ofloxacin, and ansamycin; *Mycobacterium tuberculosis* 9449/06 resistant to isoniazid, rifampicin, streptomycin, and ansamycin; *Mycobacterium tuberculosis* 2092/05 resistant to isoniazid, rifampicin, streptomycin, ethambutol, ofloxacin, and ansamycin.

### Antiproliferative and cytotoxic assay

The target compounds were assayed against cell lines K-562 and HUVEC for their antiproliferative effects and against HeLa for their cytotoxic effects. The cells were incubated with ten concentrations of the test compounds [22].

Suspension cultures of K-562 in micro plates were analyzed by an electronic cell analyzer system CASY 1 (Schärfe, Reutlingen, Germany) using an aperture of 150  $\mu$ m. The software for data evaluation CASYSTAT (Schärfe) offers fast graphical evaluation of the measurement parameters, e.g. as diagrams of cell diameter distributions, overlays of different curves, and cell volume distributions. The 0.2 mL-content of each well in the micro plate was diluted 1 : 50 with CASYTON (NaCl: 7.93 g/L; Na<sub>2</sub>EDTA: 0.38 g/L; KCl: 0.4 g/L; NaH<sub>2</sub>PO<sub>4</sub> monohydrate: 0.22 g/L; NaH<sub>2</sub>PO<sub>4</sub> dihydrate: 2.45 g/L; NaF: 0.3 g/L; Schärfe). Every count/mL was automatically calculated from the arithmetic mean of three successive counts of 0.4 mL each. From the dose-response curves, the GI<sub>50</sub> values (concentration which inhibited cell growth by 50%) were calculated with CASYSTAT. The GI<sub>50</sub> value was defined as being where the concentration-response curve intersected the 50% line, determined by means of the cell counts/mL, compared to control.

The monolayers of the adherent HUVEC and HeLa cells were fixed by glutaraldehyde and stained with a 0.05% solution of methylene blue for 15 min. After gently washing, the stain was eluted by 0.2 mL of 0.33 M HCl in the wells. The optical densities were measured at 630 nm in a DYNATECH MR 7000 microplate reader (Dynatech Laboratories, Chantilly, USA). Comparisons of the different values were performed with Microsoft Excel.

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