Full Paper

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

Petra Herzigová[1], Vera Klimešová[1], Karel Palát[1], Jarmila Kaustová[2], Hans-Martin Dahse[3], and Ute Möllmann[3]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic
[2] Laboratory of Mycobacterial Diagnostics, Regional Institute of Public Health, Ostrava, Czech Republic
[3] Lebniz-Institut für Naturstoff-Forschung und Infektionsbiologie e.V. – Hans-Knöll-Institut, Germany

A set of 4-benzylsulfanylpyridine-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 μmol/L, most often 4 to 32 μmol/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 / Pyridine-2-carbohydrazides

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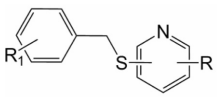
Introduction

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 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].



R = CN, $CSNH_2$; R_1 = H, Cl, Br, F, CH_3, OCH_3,CF_3, CN, NO_2.

Figure 1. Chemical structure of model compounds.

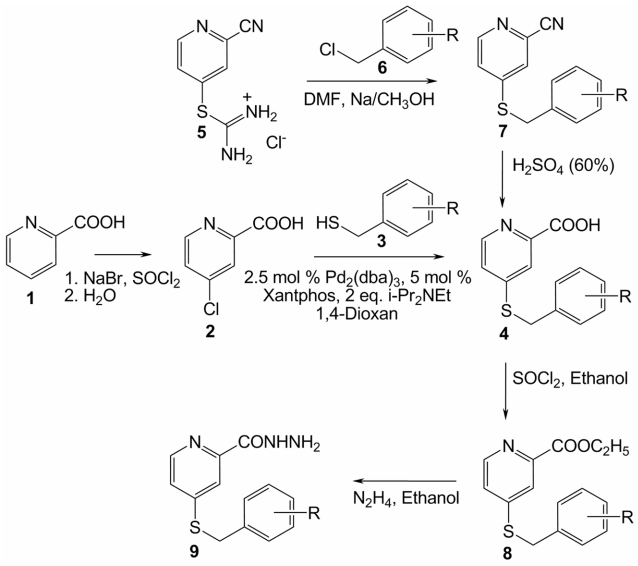
In our previous papers, we have reported the antimycobacterial activity of benzylsulfanyl derivatives of pyridine which were substituted by carbonitrile or carbo-

Correspondence: Vera Klimešová, Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, Hradec Králové, 500 05, Czech Republic.
E-mail: vera.klimesova@faf.cuni.cz
Fax: +420 495 067 166

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 pharmocophore for antituberculotic 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 antituberculotic 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.



4, 7, 8, 9	R
a	H
b	3-Cl
c	4-Cl
d	3-F
e	4-F
f	3-Br
g	4-Br
h	3-CH_3

4, 7, 8, 9	R
i	4-CH_3
j	3-CF_3
k	4-CF_3
l	3-CN
m	3-OCH_3
n	4-NO_2
o	3,5-$(NO_2)_2$

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

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 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-alkylisothiouronium 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_2NEt in the presence of catalyst $Pd_2(dba)_3$ 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-

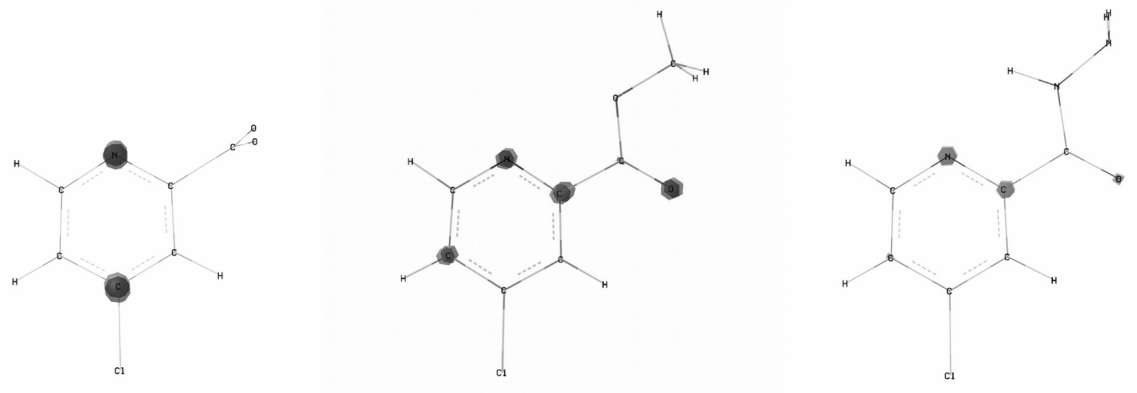


Figure 2. The dislocation of LUMO orbitals in the 4-chloropyridine-2-carboxylate anion, methyl 4-chloropyridine-2-carboxylate, and 4-chloropyridine-2-carbohydrazide (RB3LYP/6-311+G(d,p) level, Isovalue = 0.17).

mediates **4** by hydrolysis in 60% sulfuric acid in good yields. Sulfides **7** were obtained by reacting isothiouronium 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 $SOCl_2$ 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}H-NMR, ^{13}C-NMR, and IR spectral data, and their purity by elemental analysis. For the ^{1}H-NMR spectra of benzylsulfanyl derivatives **4**, **8**, and **9**, the singlet of the S-CH_2 group positioned in the region 4.40 to 4.76 ppm and the signal of the 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}C-NMR signal of the S-CH_2 group can be observed at 32.6 to 34.5 ppm, the signal of the O-CH_2 esters can be found at 61.5 to 61.6 ppm, the signal of the CH_3 group of ethyl esters lies at 14.2 to 14.3 ppm. The carbon signal of the 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 C=O group – acids at 1701 to 1722 cm^{-1}, esters at 1714 to 1735 cm^{-1}, and hydrazides at 1660 to 1699 cm^{-1}; bending vibration of the NH_2 group of hydrazides will be seen in the region 1617 to 1629 cm^{-1}.

Molecular modeling

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

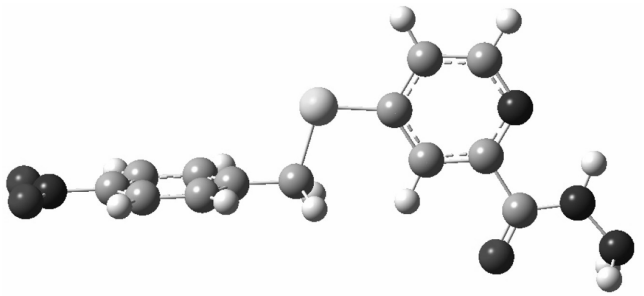


Figure 3. 3D Model of compound **9n**.

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 cm^{-1}).

Calculated	Scaled	Spectrum	Type of vibration
1119	1084	1118	δ(C-H)$_{benzene}$
1285	1244	1256	δ(C-N-H) + ν(C-N)
1357	1314	1301	ν(C-C)$_{benzene}$
1370	1327	1349	ν_s(NO_2)
1527	1479	1467	δ(C-N-H)
1580	1531	1514	ν_{as}(NO_2)
1613	1562	1536	ν(C-C)$_{pyridine}$
1640	1588	1582	ν(C-C)$_{benzene}$
1646	1594	1605	ν(C-C)$_{benzene}$ + ν_{as}(NO_2)
1702	1649	1628	δ(NH_2)
1730	1676	1676	ν(C=O)
3063	2967	2990	ν_{as}(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 μmol/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 μmol/L, most often between 8 to 16 μmol/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 μmol/L, most often between 16 to 32 μmol/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 (μmol/L).

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

Table 3. In vitro antituberculotic activity against MDR *M. tuberculosis* (MIC expressed in μmol/L).

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

[a] Resistant to isoniazid, rifampicin, streptomycin, ethambutol, ofloxacin, ansamycin.
[b] Resistant to isoniazid, rifampicin, streptomycin, ansamycin.
[c] Resistant to isoniazid, rifampicin, streptomycin, ethambutol, ofloxacin, ansamycin.

Table 4. Antiproliferative and cytotoxic effects expressed in μg/mL.

Compound	Antiproliferative effect		Cytotoxicity
	Huvec GI_{50}	K-562 GI_{50}	HeLa CC_{50}
9a	>50	50	35.7
9c	47.2	12.1	37.6
9d	>50	37.9	38.9
9g	>50	21.0	31.8
9i	>50	18.4	>50
9j	42.7	12.9	31.8
9m	>50	>50	48.4
9n	>50	>50	50.0
9o	>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 μmol/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_{50}: concentration which inhibits cell proliferation by 50% compared to control), and against HeLa cells for their cytotoxic effects (CC_{50}: 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_{50} are ranging from 12 to >50 μg/mL and values of CC_{50} from 31 to 50 μg/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_4O_{10} at 78°C or 25°C and 2.4–2.6 kPa for 2 h. Elemental analyses were performed on CHNS-O CE instrument, FISONS EA 1110 (Fisions, Milan,

Italy) and are within ± 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}H and 75 MHz for ^{13}C) in DMSO-d_6; the solutions were at ambient temperature. Chemical shifts were recorded as δ 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 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*-Pr_2NEt (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 $Pd_2(dba)_3$ (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 H_2SO_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°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°C for two hours. After cooling to ambient temperature, the reaction mixture was neutralized by an aqueous solution of Na_2CO_3 to pH 6. Precipitated crystals were recrystallized from ethanol.

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

Yield: 67%; m.p.: 170 – 172°C; IR (KBr) λ_{max} (cm^{-1}): 3500 – 2400 (COOH), 1718 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.46 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.88 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H_3), 7.53 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H_5), 7.46 – 7.43 (m, 2H, Ar-H), 7.36 – 7.26 (m, 3H, Ar-H), 4.44 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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 – 145°C; IR (KBr) λ_{max} (cm^{-1}): 3500 – 2400 (COOH), 1718 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.47 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H_6), 7.88 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H_3), 7.54 – 7.52 (m, 2H, H_5, Ar-H), 7.43 – 7.33 (m, 3H, Ar-H), 4.46 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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 – 161°C; IR (KBr) ν_{max} (cm^{-1}): 3500 – 400 (COOH), 1701 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.46 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.86 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H_3), 7.52 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H_5), 7.49 – 7.46 (m, 2H, Ar-H), 7.40 – 7.37 (m, 2H, Ar-H), 4.44 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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 – 162°C; IR (KBr) ν_{max} (cm^{-1}): 3500 – 2400 (COOH), 1719 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.50 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H_6), 7.88 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H_3), 7.55 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H_5), 7.41 – 7.28 (m, 3H, Ar-H), 7.14 – 7.06 (m, 1H, Ar-H), 4.47 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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 – 174°C; IR (KBr) ν_{max} (cm^{-1}): 3500 – 2400 (COOH), 1722 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.50 (dd, *J* = 5.5 Hz, *J* = 0.6 Hz, 1H, H_6), 7.96 (dd, *J* = 1.8 Hz, *J* = 0.6 Hz, 1H, H_3), 7.65 (dd, *J* = 5.5 Hz, *J* = 1.8 Hz, 1H, H_5), 7.52 – 7.48 (m, 2H, Ar-H), 7.20 – 7.14 (m, 2H, Ar-H), 4.49 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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 – 158°C; IR (KBr) ν_{max} (cm^{-1}): 3600 – 2500 (COOH), 1717 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.47 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.87 (dd, *J* = 1.9 Hz, *J* = 0.5 Hz, 1H, H_3), 7.67 – 7.66 (m, 1H, Ar-H), 7.52 (dd, *J* = 5.3 Hz, *J* = 1.9 Hz, 1H, 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, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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 – 168°C; IR (KBr) ν_{max} (cm^{-1}): 3600 – 2500 (COOH), 1718 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.46 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H_6), 7.86 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H_3), 7.54 – 7.51 (m, 3H, H_5, Ar-H), 7.42 – 7.39 (m, 2H, Ar-H), 4.43 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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 – 150°C; IR (KBr) ν_{max} (cm^{-1}) 3600 – 2400 (COOH), 1717 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.47 (dd, *J* = 5.3 Hz, *J* = 0.7 Hz, 1H, H_6), 7.89 (dd, *J* = 2.0 Hz, *J* = 0.7 Hz, 1H, H_3), 7.54 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H_5), 7.27 – 7.22 (m, 3H, Ar-H), 7.10 – 7.08 (m, 1H, Ar-H), 4.40 (s, 2H, CH_2), 2.28 (s, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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 – 185°C; IR (KBr) ν_{max} (cm^{-1}): 3600-2500 (COOH), 1716 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.47 (dd, *J* = 5.4 Hz, *J* = 0.6 Hz, 1H, H_6), 7.91 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H_3), 7.57 (dd, *J* = 5.4 Hz, *J* = 2.0 Hz, 1H, H_5), 7.34 – 7.31 (m, 2H, Ar-H), 7.15 – 7.12 (m, 2H, Ar-H), 4.40 (s, 2H, CH_2), 2.25 (s, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3600 – 2500 (COOH), 1717 (C=O), 1332 (CF_3); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.48 (dd, J = 5.3 Hz, J = 0.6 Hz, 1H, H_6), 7.92 (dd, J = 2.0 Hz, J = 0.6 Hz, 1H, H_3), 7.84 (s, 1H, Ar-H), 7.77 (d, J = 7.5 Hz, 1H, Ar-H), 7.65 – 7.57 (m, 3H, H_5, Ar-H), 4.58 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3600 – 2500 (COOH), 1718 (C=O), 1326 (CF_3); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.47 (dd, J = 5.3 Hz, J = 0.6 Hz, 1H, H_6), 7.88 (dd, J = 2.0 Hz, J = 0.6 Hz, 1H, H_3), 7.73 – 7.66 (m, 4H, Ar-H), 7.54 (dd, J = 5.3 Hz, J = 2.0 Hz, 1H, H_5), 4.56 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3600 – 2500 (COOH), 2230 (CN), 1716 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.48 (dd, J = 5.3 Hz, J = 0.5 Hz, 1H, H_6), 7.93 (s, 1H, Ar-H), 7.88 (dd, J = 2.0 Hz, J = 0.5 Hz, 1H, H_3), 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_5, Ar-H), 4.52 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3600 – 2500 (COOH), 1718 (C=O), 1270 (OCH_3), 1040 (OCH_3); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.50 (dd, J = 5.6 Hz, J = 0.6 Hz, 1H, H_6), 8.01 (dd, J = 2.0 Hz, J = 0.6 Hz, 1H, H_3), 7.68 (dd, J = 5.6 Hz, J = 2.0 Hz, 1H, H_5), 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_2), 3.73 (s, 3H, OCH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3600 – 2500 (COOH), 1718 (C=O), 1519 (NO_2), 1347 (NO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.47 (dd, J = 5.3 Hz, J = 0.6 Hz, 1H, H_6), 8.20 – 8.17 (m, 2H, Ar-H), 7.88 (dd, J = 2.0 Hz, J = 0.6 Hz, 1H, H_3), 7.74 – 7.71, (m, 2H, Ar-H), 7.54 (dd, J = 5.3 Hz, J = 2.0 Hz, 1H, H_5), 4.61 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3600 – 2500 (COOH), 1709 (C=O), 1544 (NO_2), 1529 (NO_2), 1342 (NO_2), 1320 (NO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (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_6), 7.92 (dd, J = 2.0 Hz, J = 0.5 Hz, 1H, H_3), 7.59 (dd, J = 5.3 Hz, J = 2.0 Hz, 1H, H_5), 4.76 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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 isothiouronium 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 exceeded 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 alkalized by the saturated aqueous solution of $NaHCO_3$ 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) ν_{max} (cm^{-1}): 1720 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.45 (dd, J = 5.3 Hz, J = 0.5 Hz, 1H, H_6), 7.88 (dd, J = 2.0 Hz, J = 0.5 Hz, 1H, H_3), 7.51 (dd, J = 5.3 Hz, J = 2.0 Hz, 1H, H_5), 7.48 – 7.43 (m, 2H, Ar-H), 7.37 – 7.23 (m, 3H, Ar-H), 4.42 (s, 2H, CH_2), 4.30 (q, J = 7.1 Hz, 2H, CH_2), 1.30 (t, J = 7.1 Hzv, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 1735 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.48 (dd, J = 5.2 Hz, J = 0.5 Hz, 1H, H_6), 7.87 (dd, J = 1.9 Hz, J = 0.5 Hz, 1H, H_3), 7.56 – 7.53 (m, 2H, H_5, Ar-H), 7.44 – 7.31 (m, 3H, Ar-H), 4.46 (s, 2H, CH_2), 4.31 (q, J = 7.1 Hz, 2H, CH_2), 1.31 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}) 1730 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.47 (dd, J = 5.3 Hz, J = 0.7 Hz, 1H, H_6), 7.86 (dd, J = 2.0 Hz, J = 0.7 Hz, 1H, H_3), 7.54 (dd, J = 5.3 Hz, J = 2.0 Hz, 1H, H_5), 7.49 – 7.46 (m, 2H, Ar-H), 7.40 – 7.37 (m, 2H, Ar-H), 4.45 (s, 2H, CH_2), 4.31 (q, J = 7.1 Hz, 2H, CH_2), 1.30 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 1724 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.48 (dd, J = 5.3 Hz, J = 0.7 Hz, 1H, H_6), 7.87 (dd, J = 2.0 Hz, J = 0.7 Hz, 1H, H_3), 7.55 (dd, J = 5.3 Hz, J = 2.0 Hz, 1H, H_5), 7.41 – 7.28 (m, 3H, Ar-H), 7.13 – 7.06 (m, 1H, Ar-H), 4.47 (s, 2H, CH_2), 4.31 (q, J = 7.1 Hz, 2H, CH_2), 1.30 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}) 1728 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.48 (dd, *J* = 5.2 Hz, *J* = 1.1 Hz, 1H, H_6), 7.86 (dd, *J* = 1.7 Hz, *J* = 1.1 Hz, 1H, H_3), 7.56-7.47 (m, 2H, H_5, Ar-H), 7.36 – 7.32 (m, 1H, Ar-H), 7.20 – 7.13 (m, 2H, Ar-H), 4.44 (s, 2H, CH_2), 4.31 (dq, *J* = 7.1 Hz, *J* = 1.7 Hz, 2H, CH_2), 1.30 (dt, *J* = 7.1 Hz, *J* = 1.7 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 1733 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.47 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.86 (dd, *J* = 1.8 Hz, *J* = 0.5 Hz, 1H, H_3), 7.67 (s, 1H, Ar-H), 7.54 (dd, *J* = 5.3 Hz, *J* = 1.8 Hz, 1H, H_5), 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_2), 4.31 (q, *J* = 7.1 Hz, 2H, CH_2), 1.30 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 1730 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.47 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.85 (dd, *J* = 1.9 Hz, *J* = 0.5 Hz, 1H, H_3), 7.55 – 7.51 (m, 3H, H_5, Ar-H), 7.43 – 7.40 (m, 2H, Ar-H), 4.43 (s, 2H, CH_2), 4.31 (q, *J* = 7.1 Hz, 2H, CH_2), 1.30 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 1720 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.47 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H_6), 7.87 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H_3), 7.53 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H_5), 7.26 – 7.18 (m, 3H, Ar-H), 7.08 – 7.06 (m, 1H, Ar-H), 4.38 (s, 2H, CH_2), 4.31 (q, *J* = 7.1 Hz, 2H, CH_2), 2.27 (s, 3H, CH_3), 1.31 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 1722 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.45 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.90 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H_3), 7.56 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H_5), 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_2), 4.31 (q, *J* = 7.1 Hzv, CH_2), 2.25 (s, 3H, CH_3), 1.30 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 1716 (C=O), 1331 (CF_3); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.48 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.88 (dd, *J* = 1.9 Hz, *J* = 0.5 Hz, 1H, H_3), 7.83 (s, 1H, Ar-H), 7.78 – 7.76 (m, 1H, Ar-H), 7.64 – 7.54 (m, 3H, H_5, Ar-H), 4.56 (s, 2H, CH_2), 4.31 (q, *J* = 7.1 Hz, 2H, CH_2), 1.30 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 1728 (C=O), 1327 (CF_3); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.48 (dd, *J* = 5.2 Hz, *J* = 0.4 Hz, 1H, H_6), 7.87 (dd, *J* = 1.2 Hz, *J* = 0.4 Hz, 1H, H_3), 7.70 – 7.65 (m, 4H, Ar-H), 7.56 (dd, *J* = 5.2 Hz, *J* = 1.2 Hz, 1H, H_5), 4.56 (s, 2H, CH_2), 4.31 (q, *J* = 7.1 Hz, 2H, CH_2), 1.30 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 2230 (CN), 1735 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.48 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.93 (s, 1H, Ar-H), 7.86 (dd, *J* = 1.9 Hz, *J* = 0.5 Hz, 1H, H_3), 7.82 – 7.73 (m, 2H, Ar-H), 7.57 – 7.52 (m, 2H, H_5, Ar-H), 4.51 (s, 2H, CH_2), 4.31 (q, *J* = 7.1 Hz, 2H, CH_2), 1.31 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 1714 (C=O), 1266 (OCH_3), 1044 (OCH_3); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm) 8.47 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.87 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H_3), 7.54 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H_5), 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_2), 4.31 (q, *J* = 7.1 Hz, 2H, CH_2), 3.72 (s, 3H, OCH_3), 1.30 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 1733 (C=O), 1523 (NO_2), 1349 (NO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.48 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H_6), 8.19 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.87 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H_3), 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_5), 4.61 (s, 2H, CH_2), 4.30 (dq, *J* = 7.1 Hz, *J* = 0.7 Hz, 2H, CH_2), 1.30 (dt, *J* = 7.1 Hz, *J* = 0.7 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 1726 (C=O), 1536 (NO_2), 1339 (NO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (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_6), 7.91 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H_3), 7.61 (ddd, *J* = 5.3 Hz, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H_5), 4.76 (s, 2H, CH_2), 4.31 (q, *J* = 7.1 Hz, 2H, CH_2), 1.30 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3301 (N-H), 3202 (N-H), 1664 (C=O), 1627 (NH_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm) 9.86 (s, 1H, NH), 8.37 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.83 (dd, *J* = 1.9 Hz, *J* = 0.5 Hz, 1H, H_3), 7.48 – 7.43 (m, 3H, H_5, Ar-H), 7.36 – 7.23 (m, 3H, Ar-H), 4.56 (s, 2H, NH_2), 4.42 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3305 (N-H), 3203 (N-H), 1664 (C=O), 1624 (NH_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm) 9.86 (s, 1H, NH), 8.39 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H_6), 7.83 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H_3), 7.54 (t, *J* = 1.8 Hz, 1H, Ar-H), 7.47 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H_5), 7.45 – 7.33 (m, 3H, Ar-H), 4.56 (s, 2H, NH_2), 4.46 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3376 (N-H), 3323 (N-H), 1671 (C=O), 1624 (NH_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 9.86 (s, 1H, NH), 8.38 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.82 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H_3), 7.50 – 7.45 (m, 3H, H_5, Ar-H), 7.42 – 7.37 (m, 2H, Ar-H), 4.55 (s, 2H, NH_2), 4.45 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3290 (N-H), 1662 (C=O), 1618 (NH_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 9.86 (s, 1H, NH), 8.39 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H_6), 7.83 (dd, *J* = 2.0 Hz, *J* = 0.6 Hz, 1H, H_3), 7.47 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H_5), 7.42 – 7.28 (m, 3H, Ar-H), 7.14 – 7.06 (m, 1H, Ar-H), 4.56 (s, 2H, NH_2), 4.47 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3306 (N-H), 3202 (N-H), 1664 (C=O), 1626 (NH_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 9.86 (s, 1H, NH), 8.39 (dd, *J* = 5.3 Hz, *J* = 0.7 Hz, 1H, H_6), 7.82 (dd, *J* = 2.0 Hz, *J* = 0.7 Hz, 1H, H_3), 7.52 – 7.46 (m, 3H, H_5, Ar-H), 7.20 – 7.13 (m, 2H, Ar-H), 4.56 (s, 2H, NH_2), 4.44 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3304 (N-H), 3202 (N-H), 1663 (C=O), 1623 (NH_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 9.86 (s, 1H, NH), 8.39 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.83 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H_3), 7.68 (t, *J* = 1.7 Hz, 1H, Ar-H), 7.48 – 7.45 (m, 3H, H_5, Ar-H), 7.32 – 7.27 (m, 1H, Ar-H), 4.57 (s, 2H, NH_2), 4.46 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3375 (N-H), 3319 (N-H), 1669 (C=O), 1618 (NH_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 9.86 (s, 1H, NH), 8.38 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.83 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H_3), 7.55 – 7.51 (m, 3H, H_5, Ar-H), 7.43 – 7.39 (m, 2H, Ar-H), 4.57 (s, 2H, NH_2), 4.46 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3308 (N-H), 3197 (N-H), 1678 (C=O), 1618 (NH_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 9.86 (s, 1H, NH), 8.38 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.83 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H_3), 7.46 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H_5), 7.27 – 7.19 (m, 3H, Ar-H), 7.09 – 7.06 (m, 1H, Ar-H), 4.56 (s, 2H, NH_2), 4.38 (s, 2H, CH_2), 2.28 (s, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3338 (N-H), 1693 (C=O), 1617 (NH_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 9.85 (s, 1H, NH), 8.37 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.82 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H_3), 7.46 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H_5), 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_2), 4.38 (s, 2H, CH_2), 2.26 (s, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3332 (N-H), 1678 (C=O), 1617 (NH_2), 1331 (CF_3); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.87 (s, 1H, NH), 8.39 (dd, *J* = 5.3 Hz, *J* = 0.6 Hz, 1H, H_6), 7.85 – 7.83 (m, 2H, H_3, 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_5), 4.57 (s, 4H, NH_2, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}) 3323 (N-H), 1699 (C=O), 1617 (NH_2), 1324 (CF_3); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 9.87 (s, 1H, NH), 8.39 (dd, *J* = 5.3 Hz, *J* = 0.5 Hz, 1H, H_6), 7.83 (dd, *J* = 2.0 Hz, *J* = 0.5 Hz, 1H, H_3), 7.72-7.65 (m, 4H, Ar-H), 7.49 (dd, *J* = 5.3 Hz, *J* = 2.0 Hz, 1H, H_5), 4.57 (s, 2H, NH_2), 4.56 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3351 (N-H), 3313 (N-H), 2229 (CN), 1660 (C=O), 1629 (NH_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 9.87 (s, 1H, NH), 8.39 (dd, J = 5.3 Hz, J = 0.4 Hz, 1H, H_6), 7.94 (t, J = 1.4 Hz, 1H, Ar-H), 7.83 – 7.73 (m, 3H, H_3, 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_5), 4.56 (s, 2H, NH_2), 4.52 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3300 (N-H), 3201 (N-H), 1664 (C=O), 1626 (NH_2), 1270 (OCH_3), 1049 (OCH_3); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 9.86 (s, 1H, NH), 8.38 (dd, J = 5.3 Hz, J = 0.6 Hz, 1H, H_6), 7.84 (dd, J = 2.0 Hz, J = 0.6 Hz, 1H, H_3), 7.46 (dd, J = 5.3 Hz, J = 2.0 Hz, 1H, H_5), 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_2), 4.40 (s, 2H, CH_2), 3.74 (s, 3H, OCH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3390 (N-H), 3314 (N-H), 1676 (C=O), 1628 (NH_2), 1514 (NO_2), 1349 (NO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 9.85 (s, 1H, NH), 8.38 (dd, J = 5.3 Hz, J = 0.5 Hz, 1H, H_6), 8.19 (d, J = 8.9 Hz, 2H, Ar-H), 7.82 (dd, J = 2.0 Hz, J = 0.5 Hz, 1H, H_3), 7.73 (d, J = 7.9 Hz, 2H, Ar-H), 7.48 (dd, J = 5.3 Hz, J = 2.0 Hz, 1H, H_5), 4.61 (s, 2H, CH_2), 4.55 (s, 2H, NH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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) ν_{max} (cm^{-1}): 3334 (N-H), 3256 (N-H), 1660 (C=O), 1626 (NH_2), 1534 (NO_2), 1530 (NO_2), 1342 (NO_2), 1329 (NO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (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_6), 7.85 (dd, J = 2.0 Hz, J = 0.5 Hz, 1H, H_3), 7.54 (dd, J = 5.3 Hz, J = 2.0 Hz, 1H, H_5), 4.76 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (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 μmol/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 multidrug-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 μm. 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_2EDTA: 0.38 g/L; KCl: 0.4 g/L; NaH_2PO_4 monohydrate: 0.22 g/L; NaH_2PO_4 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_{50} values (concentration which inhibited cell growth by 50%) were calculated with CASYSTAT. The GI_{50} 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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