HETEROCYCLES, Vol. 85, No. 9, 2012, pp. 2269 - 2280. © 2012 The Japan Institute of Heterocyclic Chemistry
Received, 8th June, 2012, Accepted, 5th July, 2012, Published online, 13th July, 2012
DOI: 10.3987/COM-12-12524

SYNTHESIS, CHARACTERIZATION, AND TUBERCULOSTATIC ACTIVITY OF NOVEL HETEROCYCLIC COMPOUNDS DERIVED FROM DIMETHYL HETEROAROYLCARBONOHYDRAZONODITHIOATES

Katarzyna Gobis,[1]* Henryk Foks,[1] Ewa Augustynowicz-Kopeć,[2] and Agnieszka Napiórkowska[2]

[1]Department of Organic Chemistry, Medical University of Gdańsk, 107 Gen. J. Hallera, 80-416 Gdańsk, Poland; E-mail: kgobis@gumed.edu.pl
[2]Department of Microbiology, Institute of Tuberculosis and Pulmonary Diseases, 26 Płocka Str., 01-138 Warsaw, Poland

Abstract – A series of dimethyl heteroaroylcarbonohydrazonodithioates (**1-5**) has been synthesized from various heterocyclic carbohydrazides. *N′*-(Cycloalkyl-diamin-2-ylidene)heteroaroylhydrazides (**9-20**) were obtained by the substitution of dimethyl heteroaroylcarbonohydrazonodithioates with respective diamines in EtOH or dioxane. In water the substitution was followed by cyclization to 5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyrimidines (**21-23**). The compounds obtained were tested *in vitro* towards *M. tuberculosis* standard strain (H_{37}Rv) and two "wild" strains, susceptible (Spec. 192) and resistant (Spec. 210). MIC values for all the compounds tested were within 25-100 μg/mL which indicated their activity lower than reference drugs used (INH, PZA).

Tuberculosis is a serious infectious disease which causes 1.4 million deaths per year, not only in developing countries but also in developed countries.[1] Its treatment is associated with long-term therapy, which does not guarantee a success.[2] Multidrug resistance developed by *M. tuberculosis* strains and the lack of discipline among patients, may lead to treatment failure.[3] The disease is particularly dangerous for immunocompromised patients increasing mortality among HIV-positive individuals.[4] The few chemotherapeutics effective in the treatment of tuberculosis cause serious side effects.[5] For this reason, new effective tuberculostatics with reduced toxicity are still searched for. One of research directions is the synthesis of pyridine and pyrazine derivatives as potential tuberculostatics, due to the presence of these heterocyclic systems in the structure of clinically applied chemotherapeutics, isoniazid (INH) and pyrazinamide (PZA).

Previously, we described the synthesis of 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 1,2,4-triazoles and triazole[3,4-*b*][1,3]thiazines from respective carbohydrazides and 2-hetroaroylhydrazinecarbodithioates.[6,7] We also reported an obtaining of dimethyl benzoylcarbonohydrazonodithioates and the usage of such products in the synthesis of 5,6,7,8-tetrahydro[1,2,4]triazol[4,3-*a*]pyrimidines.[8,9] In our research program we have already undertaken the synthesis of methyl 3-isonicotyldithiocarbazates and *S,S′*-dimethyl dithiocarbonate isonicotinoylhydrazone methiodide and their reactions with various amines and hydrazines.[10] Some derivatives of 6-methoxypyrazine-2-carboxylic acid hydrazide and their antibacterial activity have been also reported by us.[11]

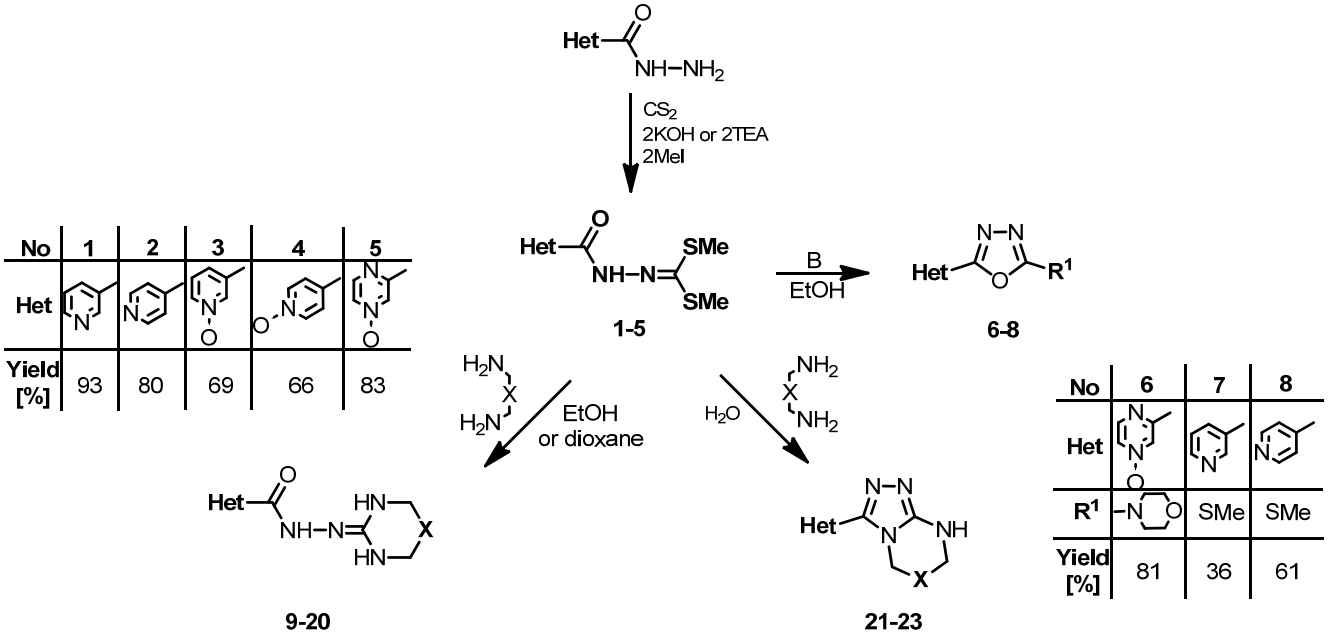
The main aim of this work is the synthesis of dimethyl heteroaroylcarbonohydrazonodithioates from 3- and 4-pyridine, 3- and 4-pyridine-1-oxide, and 3-pyrazine-1-oxide carbohydrazides, and their use for the preparation of other derivatives of pyridine and pyrazine. The reports on better solubility, increased bioavailability and reduced toxicity of 1-oxide derivatives of pyridine and pyrazine prompted us to include these compounds in the study.[12]

The starting carbohydrazides were treated with carbon disulfide in the presence of two moles of KOH or TEA (triethylamine). Then formed adduct was methylated with two moles of MeI (Scheme1). In this way, dimethyl heteroaroylcarbonohydrazonodithioates (**1**-**5**) were obtained. Then the reactivity of some of carbonohydrazonodithioates to dimethylamine, morpholine and *N*-aminopiperidine was examined. Compound (**5**) refluxed with morpholine underwent substitution and cyclization to 2-morpholino-1,3,4-oxadiazole (**6**) with methyl mercaptan liberation. Such a course of the reaction was confirmed by disappearance of the band for the carbonyl bond at 1712 cm^{-1} in IR spectrum. Compounds (**1**, **2**) were cyclized to 2-methylthio-1,3,4-oxadiazoles (**7**, **8**) in the ethanol solution of *N*-aminopiperidine. In these cases loss of the band for the carbonyl bond at 1678 cm^{-1} (**7**) and 1646 cm^{-1} (**8**) was found. While absence of *N*-aminopiperidine protons in the ^1H NMR spectra was observed, but at 2.81 ppm (**7**) and 2.78 ppm (**8**) singlets for SMe group were found. The resulting 2-methylthio-1,3,4-oxadiazoles (**7**, **8**) have been already described.[13,14]

Compounds (**1**-**5**) underwent the reaction with diamines (1,2-diaminoethane, 1,3-diaminopropane, 1,3-diamino-2,2-dimethylpropane, 1,3-diaminopropan-2-ol). The structure of the products of these reactions depended on the type of substrate and solvent. Short-term (1 h) refluxing of compounds (**1**-**5**) with a double excess of diamine in ethanol (**1**-**4**) or dioxane (**5**) led to 1,3-diazacycloalkyl-2-ylidene derivatives (**9**-**20**). Derivative (**9**) has been already described in French patent,[15] although it was obtained by other method, the acylation of 2-hydrazonoimidazolidine.

Compound (**5**) reacted with an double excess of the corresponding diamines in the aqueous solution to give in the first stage the derivatives of 1,3-diazacycloalkyl-2-ylidene described above, seen in the reaction mixture in the form of yellow precipitate, which disappeared during the subsequent refluxing to give

bicyclic systems of 5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyrimidines (**21**-**23**). Such a reaction was observed for all the diamines with the exception of 1,2-diaminoethane. In that case the product isolated was 3-carboxypyrazine 1-oxide (mp 212-213 °C).[16] Imidazolidine derivative (**17**) underwent hydrolysis instead of the expected cyclization to the condensed system.



No	1	2	3	4	5
Het					
Yield [%]	93	80	69	66	83

No	6	7	8
Het			
R^1		SMe	SMe
Yield [%]	81	36	61

No	9	10	11	12	13	14	15	16	17	18, 21	19, 22	20, 23
Het												
X	-	CH_2	-	CH_2	-	$C(Me)_2$	-	$C(Me)_2$	-	CH_2	$C(Me)_2$	CH(OH)
Yield [%]	89	70	49	58	59	38	92	85	41	68, 52	43, 56	31, 43

B (basic solution): morpholine or *N*-aminopiperidine

Scheme 1

Described reactions proceeded with moderate and very good yields. The syntheses of dimethyl heteroaroylcarbonohydrazonodithioates (**1**-**5**) were the most efficient (66-93%). Yields obtained for the preparation of 1,3-diazacycloalkyl-2-ylidene derivatives (**9**-**20**) and 5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3-*a*]pyrimidines (**21**-**23**) were more diverse (31-89%).

The newly synthesized compounds were characterized by the IR and NMR spectra as well as the elemental analysis listed in experimental section. The spectral analyses were in accordance with the assigned structures.

Tuberculostatic activity

The newly synthesized heteroaroylcarbonohydrazonodithioates (**1**-**5**), 3-(5-morpholino-1,3,4-oxadiazol-

2-yl)pyrazine 1-oxide (**6**), and *N*′-(cycloalkyldiamin-2-ylidene)pyridinohydrazides (**11**-**20**) were examined *in vitro* for their tuberculostatic activity against *Mycobacterium tuberculosis* $H_{37}Rv$ strain and two "wild" strains isolated from tuberculosis patients.

The results of tuberculostatic activity indicated that most of the title compounds showed rather low activity against tested strains *in vitro* and were definitely less active than isoniazid (INH) and pyrazinamide (PZA) used as reference drugs (Table 1). The MIC values for the majority of the tested compounds were ranged from 50 to 100 μg/mL, while for INH from 0.5 to 1.1 μg/mL and for PZA from 25 to 40 μg/mL. There were no differences in sensitivity to tested compounds between sensitive 192 and resistant 210 strains. Six compounds, 3-(2-(bis(methylthio)methylene)hydrazinecarbonyl)pyridine 1-oxide (**3**) with 3-pyridine 1-oxide system, 3-(2-(bis(methylthio)methylene)hydrazinecarbonyl)pyrazine 1-oxide (**5**) and 3-(5-morpholino-1,3,4-oxadiazol-2-yl)pyrazine 1-oxide (**6**) with 3-pyrazine 1-oxide system, and *N*′-(cycloalkyldiamin-2-ylidene)pyridinohydrazides (**18-20**), exhibited a little bit higher tuberculostatic activity *in vitro*. The MIC values for these compounds were 25-50 μg/mL. Interestingly, in the case of 3-(2-(tetrahydropyrimidin-2(1*H*)-ylidene)hydrazinecarbonyl)pyrazine 1-oxide (**18**), a hexahydropyrimidine derivative, obtained MIC values indicated its higher activity towards resistant strain 210 (25 μg/mL) than sensitive one 192 (50 μg/mL). Compound (**3**) with 3-pyridine 1-oxide ring and derivative (**18**) with 3-pyrazine 1-oxide system showed the highest activity in the group of tested compounds.

Table 1. *In vitro* tuberculostatic activity of the newly synthesized compounds [a, b, c]

No	MIC *[μg/mL]*			No	MIC *[μg/mL]*		
	$H_{37}Rv$	Spec. 192	Spec. 210		$H_{37}Rv$	Spec. 192	Spec. 210
1	50	50	100	**13**	100	100	50
2	50	50	50	**14**	100	100	50
3	**25**	**25**	**50**	**17**	25	100	100
4	50	50	50	**18**	**25**	**50**	**25**
5	25	50	50	**19**	25	50	50
6	25	50	50	**20**	25	50	50
11	50	50	50	**INH**	0.5	0.5	1.1
12	50	50	50	**PZA**	25	25	40

[a]Minimum inhibitory concentrations for bacterial strains were determined by two-fold serial dilution method for microdilution plates and for mycobacterial strains by two-fold classical test-tube method of successive dilution.
[b]**INH** isoniazid, **PZA** pyrazinamide.
[c]*M. tuberculosis* $H_{37}Rv$, Spec. 192, Spec. 210.

In summary, a series of *N*′-(cycloalkyldiamin-2-ylidene)pyridinohydrazides and have been synthesized successfully using high reactivity of various dimethyl heteroaroylcarbonohydrazonodithioates *N*′-(Cycloalkyldiamin-2-ylidene)pyridinohydrazides underwent cyclization to 5,6,7,8-tetrahydro-

[1,2,4]triazolo[4,3-*a*]pyrimidines when refluxed in water solution of respective diamines. The obtained compounds exhibited low activity *in vitro* towards *M. tuberculosis*. The most active compounds were 3-(2-(bis(methylthio)methylene)hydrazinecarbonyl)pyridine 1-oxide (**3**) and 3-(2-(tetrahydropyrimidin-2(1*H*)-ylidene)hydrazinecarbonyl)pyrazine 1-oxide (**18**) that inhibited growth of mycobacterial strains in the concentration range 25-50 μg/mL.

EXPERIMENTAL

All materials and solvents were of analytical reagent grade. Thin-layer chromatography was performed on Merck silica gel $60F_{254}$ plates and visualized with UV. The results of elemental analyses (% C, H, N) for all of the obtained compounds were in agreement with calculated values within ± 0.3% range. ^{1}H and ^{13}C NMR spectra in $CDCl_3$, DMSO-d_6 or DMSO-d_6 with the addition of TFA were recorded on Varian Gemini (200 MHz) instrument (Varian, Palo Alto, CA). IR Spectra were determined as KBr pellets of the solids on a Satellite FT-IR spectrophotometer (Mattson Instruments, Madison, WI). Mass spectra for compounds (**19**, **22**) were taken on Finningan MAT 95 spectrometer (ThermoFisher Scientific, Waltham, MA) (15 eV). Melting points were determined with Boethius apparatus (Franz Küstner Nachf. KG, Dresden, Germany) and were uncorrected.

Method for the synthesis of dimethyl pyridinoylcarbonohydrazonodithioates (1-4): The respective carbohydrazide (0.05 mol) was dissolved in the solution of 5.6 g (0.1 mol) of KOH in 20 mL of water and 25 mL of EtOH. Then 3 mL (0.05 mol) of CS_2 was added dropwise to the stirred solution. The mixture was stirred at room temperature for 15 min. Then MeI (6.2 mL, 0.1 mol) was added dropwise to the clear solution and the mixture was stirred for another 30 min. Ethanol was removed under vacuum and 20 g of ice was added to the residue. The precipitate was filtered off, washed with cold water, dried and recrystallized from suitable solvent.

Dimethyl nicotinoylcarbonohydrazonodithioate (1): recrystallized from water to afford 11.2 g (93%) (**1**); mp 88-89 °C; IR (KBr): 3290, 2926, 1678, 1546, 1517, 1299, 1155, 889, 706 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.56 (s, 6H, $2SCH_3$), 7.54 (q, 1H, pyridine, J =4.8 Hz), 8.17 (d, 1H, pyridine, J = 7.6 Hz), 8.73 (d, 1H, pyridine, J = 4.6 Hz), 8.98 (s, 1H, pyridine), 11.03 (s, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ 14.59, 15.07, 123.78, 129.60, 135.51, 148.69, 152.20, 161.25, 164.00 ppm. Anal. Calcd for $C_9H_{11}N_3OS_2$ (241.33): C, 44.79; H, 4.59; N, 17.49. Found: C, 44.68; H, 4.60; N, 17.45.

Dimethyl isonicotinoylcarbonohydrazonodithioate (2): recrystallized from water to afford 9.65 g (80%) (**2**); mp 50-52 °C; IR (KBr): 3257, 2930, 1646, 1528, 1408, 1298, 1065, 1052, 840, 694 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.56 (s, 6H, $2SCH_3$), 7.74 (d, 2H, pyridine, J = 4.7 Hz), 8.45 (d, 2H, pyridine, J = 4.6 Hz), 11.11 (s, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ 14.53, 15.09, 121.67, 140.84, 150.50,

161.25, 165.22 ppm. Anal. Calcd for $C_9H_{13}N_3OS_2$ (241.33): C, 44.79; H, 4.59; N, 17.41. Found: C, 44.81; H, 4.58; N, 17.38.

3-(2-(Bis(methylthio)methylene)hydrazinecarbonyl)pyridine 1-oxide (3): recrystallized from water to afford 0.89 g (69%) (**3**); mp 164-166 °C; IR (KBr): 3261, 3078, 2927, 1674, 1502, 1299, 1218, 1129, 1057, 846, 672 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.54 (s, 6H, $2SCH_3$), 7.52 (t, 1H, pyridine, J = 4.8 Hz), 7.67 (d, 1H, pyridine, J = 7.8 Hz), 8.36 (d, 1H, pyridine J = 4.7 Hz), 8.50 (s, 1H, pyridine), 11.10 (s, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ 14.57, 15.10, 124.34, 126.81, 133.17, 137.84, 141.07, 159.13, 164.87 ppm. Anal. Calcd for $C_9H_{11}N_3O_2S_2$ (257.33): C, 42.01; H, 4.31; N, 16.33. Found: C, 42.08; H, 4.29; N, 16.29.

4-(2-(Bis(methylthio)methylene)hydrazinecarbonyl)pyridine 1-oxide (4): recrystallized from toluene to afford 8.49 g (66%) (**4**); mp 143-146 °C; IR (KBr): 3258, 3092, 1667, 1534, 1500, 1476, 1295, 1235, 1174, 1047, 862, 648, 511 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.58 (s, 6H, SCH_3), 7.90 (d, 2H, pyridine, J = 5.1 Hz), 8.42 (d, 2H, pyridine, J = 5.0 Hz), 9.20 (brs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 14.59, 15.09, 125.31, 129.04, 139.15, 159.00, 164.75 ppm. Anal. Calcd for $C_9H_{11}N_3O_2S_2$ (257.33): C, 42.01; H, 4.31; N, 16.33. Found: C, 41.98; H, 4.32; N, 16.35.

3-(2-(Bis(methylthio)methylene)hydrazinecarbonyl)pyrazine 1-oxide (5): 3-(Hydrazinecarbonyl)-pyrazine 1-oxide (6.16 g, 0.04 mol) was dissolved in the mixture of 40 mL of DMF, 60 mL of water, 12 mL (0.13 mol) of TEA and 4 mL (0.068 mol) of CS_2. The mixture was stirred at room temperature until a clear solution. Then 6.2 mL (0.1 mol) of MeI was added dropwise. The mixture was stirred for 0.5 h. Then the suspension was cooled in ice-bath, the precipitate was filtered off, washed with coldwater, dried and recrystallized from MeOH to afford 8.58 g (83%) (**5**); mp 221-223 °C; IR (KBr): 3295, 3072, 1712, 1520, 1502, 1324, 1001, 923, 856 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.53 (s, 6H, $2SCH_3$), 8.10-8.13 (m, 1H, pyrazine), 8.42-8.45 (m, 1H, pyrazine), 8.87-8.90 (m, 1H, pyrazine), 9.25 (brs, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ 15.02, 15.23, 133.22, 136.36, 147.17, 149.16, 156.26, 158.37 ppm. Anal. Calcd for $C_8H_{10}N_4O_2S_2$ (258.32): C, 37.20; H, 3.90; N, 21.69. Found: C, 37.09; H, 3.91; N, 21.64.

3-(5-Morpholino-1,3,4-oxadiazol-2-yl)pyrazine 1-oxide (6): Dimethyl carbonohydrazonodithioate (**5**) (1.29 g, 5 mmol) and morpholine (2 mL, 23 mmol) were refluxed for 2 h. Then the mixture was cooled down and diluted with 10 mL of water. The resulting solution was extracted with $CHCl_3$ (3 x 10 mL). Organic fraction were combined and dried with $MgSO_4$. Then the solvent was removed under vacuum and the residue was washed with dry Et_2O to afford 1.01 g (81%) (**6**); mp 248-250 °C; IR (KBr): 2920, 1615, 1464, 1403, 1323, 1083, 912, 856 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.55-3.65 (m, 2H, NCH_2), 3.40-3.50 (m, 2H, NCH_2), 3.70-3.80 (m, 2H, OCH_2), 3.85-4.00 (m, 2H, OCH_2), 8.50-8.53 (m, 1H, pyrazine), 8.75-8.80 (m, 1H, pyrazine), 8.85-9.00 (m, 1H, pyrazine) ppm; ^{13}C NMR (DMSO-d_6): δ 45.93, 65.42, 131.76, 134.76,

148.36, 155.25, 164.79 ppm. Anal. Calcd for $C_{10}H_{11}N_5O_3$ (249.23): C, 48.19; H, 4.45; N, 28.10. Found: C, 48.27; H, 4.43; N, 28.14.

Method for the synthesis of 2-methylthio-1,3,4-oxadiazoles (7, 8): The respective dimethyl pyridinoylcarbonohydrazonodithioate (**1**, **2**) (0.96 g, 4 mmol) was dissolved in 5 mL of EtOH and refluxed with 0.54 mL (5 mmol) of *N*-aminopiperidine for 2 h. Then the solvent was removed under vacuum and 10 g of ice was added to the residue. The precipitate was filtered off, washed with water, dried and recrystallized.

2-(Methylthio)-5-(pyridin-3-yl)-1,3,4-oxadiazole (7): recrystallized from water to afford 0.28 g (36%) (**7**); characteristics identical with described earlier (mp 81-83 °C).[11]

2-(Methylthio)-5-(pyridin-4-yl)-1,3,4-oxadiazole (8): recrystallized from MeOH to afford 0.47 g (61%) (**8**); characteristics identical with described earlier (mp 101-103 °C).[12]

***N′*-(Cycloalkyldiamin-2-ylidene)pyridinohydrazides (9-16):** The respective dimethyl carbono-hydrazonodithioate (**2**, **3**) (5 mmol) was dissolved in 5 mL of EtOH and corresponding alkyldiamine was added (10 mmol). The mixture was refluxed for 1 h, cooled down and the product was filtered off, washed with cold EtOH, dried and recrystallized from MeOH.

***N′*-(Imidazolidin-2-ylidene)nicotinohydrazide (9):** obtained in 89% yield (0.91 g); mp 229-231 °C; IR (KBr): 3166, 3043, 1699, 1589, 1541, 1471, 1365, 1308, 1151, 1028, 744, 700, 672 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.50 (s, 4H, NCH_2), 7.20 (brs, 2H, 2NH + D_2O exchangeable), 7.37 (q, 1H, pyridine, J = 5.0 Hz), 8.16-8.22 (m, 1H, pyridine), 8.53 (dd, 1H, pyridine, J_1 = 1.6 Hz, J_2 = 3.2 Hz), 9.08 (d, 1H, pyridine, J = 1.2 Hz), 10.10 (s, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ 42.74, 123.06, 133.25, 134.48, 148.73, 149.76, 158.88, 160.83 ppm. Anal. Calcd for $C_9H_{11}N_5O$ (205.22): C, 52.67; H, 5.40; N, 34.13. Found: C, 52.58; H, 5.41; N, 34.06.

***N′*-(Tetrahydropyrimidin-2(1*H*)-ylidene)nicotinohydrazide (10):** obtained in 70% yield (0.77 g); mp 244-246 °C; IR (KBr): 3199, 3058, 2977, 2872, 1673, 1630, 1588, 1543, 1363, 1323, 1174, 1150, 740, 687 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.85-1.91 (m, 2H, CH_2)3.31-3.36 (m, 4H, $2NCH_2$), 7.28-7.34 (m, 1H, pyridine), 7.70 (brs, 2H, 2NH + D_2O exchangeable), 8.18-8.24 (m, 1H, pyridine), 8.44-8.47 (m, 1H, pyridine), 9.14-9.15 (m, 1H, pyridine) 9.95 (brs, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ 19.50, 38.03, 125.97, 129.96, 141.68, 147.78, 154.26, 157.68, 163.96 ppm. Anal. Calcd for $C_{10}H_{13}N_5O$ (219.24): C, 54.78; H, 5.98; N, 31.94. Found: C, 54.87; H, 5.99; N, 31.89.

***N′*-(Imidazolidin-2-ylidene)isonicotinohydrazide (11):** obtained in 49% yield (0.50 g); mp 254-256 °C; IR (KBr): 3173, 2999, 2927, 1694, 1597, 1569, 1527, 1466, 1413, 1324, 1194, 1147, 1055, 989, 840, 828, 699, 665 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.54 (s, 4H, $2NCH_2$), 6.90 (brs, 2H, 2NH + D_2O exchangeable), 7.81 (d, 2H, pyridine, J = 5.7 Hz), 8.54 (d, 2H, pyridine, J = 4.8 Hz), 10.00 (brs, 1H, NH + D_2O

exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ 42.78, 121.57, 145.75, 149.42, 158.00, 160.57 ppm. Anal. Calcd for $C_9H_{11}N_5O$ (205.22): C, 52.67; H, 5.40; N, 34.12. Found: C, 52.71; H, 5.39; N, 34.15.

***N'*-(Tetrahydropyrimidin-2(1*H*)-ylidene)isonicotinohydrazide (12):** obtained in 58% yield (0.64 g); mp 235-237 °C; IR (KBr): 3216, 2969, 2862, 1673, 1632, 1594, 1579, 1526, 1466, 1413, 1363, 1195, 1057, 992, 843, 755, 699, 664 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.86-1.91 (m, 2H, CH_2), 3.32-3.34 (m, 4H, 2NCH_2), 7.77 (brs, 2H, 2NH + D_2O exchangeable), 7.86 (d, 2H, pyridine, J = 5.9 Hz), 8.51 (d, 2H, pyridine, J = 6.0 Hz), 10.05 (brs, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6 + TFA): δ 20.77, 38.97, 121.37, 146.60, 149.23, 149.89, 158.87 ppm. Anal. Calcd for $C_{10}H_{13}N_5O$ (219.24): C, 54.78; H, 5.98; N, 31.94. Found: C, 54.75; H, 5.97; N, 31.96.

3-(2-(Imidazolidin-2-ylidene)hydrazinecarbonyl)pyridine 1-oxide (13): obtained in 59% yield (0.65 g); mp 265-267 °C; IR (KBr): 3268, 2916, 1690, 1359, 1133, 1014, 972, 869, 736, 669, 589 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 3.34 (s, 4H, 2CH_2), 6.81 (brs, 2H, 2NH + D_2O exchangeable), 7.30 (t, 1H, pyridine, J = 4.8 Hz), 7.70 (d, 1H, pyridine, J = 7.6 Hz), 8.11 (d, 1H, pyridine, J =4.6 Hz), 8.62 (s, 1H, pyridine), 10.10 (brs, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6 + TFA): δ 43.25, 123.80, 127.29, 129.20, 131.76, 138.83, 142.12, 162.92 ppm. Anal. Calcd for $C_9H_{11}N_5O_2$ (221.22): C, 48.86; H, 5.01; N, 31.66. Found: C, 48.73; H, 5.02; N, 31.62.

3-(2-(5,5-Dimethyltetrahydropyrimidin-2(1*H*)-ylidene)hydrazinecarbonyl)pyridine 1-oxide (14): obtained in 38% yield (0.50 g); mp 255-257 °C; IR (KBr): 3268, 2959, 1678, 1550, 1377, 1309, 1128, 1051, 892, 748, 671 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.03 (s, 6H, 2CH_3), 3.00 (s, 4H, 2NCH_2), 6.35 (brs, 2H, 2NH + D_2O exchangeable), 7.31 (t, 1H, pyridine, J = 4.8 Hz), 7.73 (d, 1H, pyridine, J = 7.5 Hz), 8.05 (d, 1H, pyridine, J = 4.7 Hz), 8.71 (s, 1H, pyridine), 10.06 (brs, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6 + TFA): δ 23.17, 26.77, 49.03, 123.90, 127.23, 129.20, 131.79, 138.89, 142.07, 163.27 ppm. Anal. Calcd for $C_{12}H_{17}N_5O_2$ (263.30): C, 54.74; H, 6.51; N, 26.60. Found: C, 54.59; H, 6.52; N, 26.57.

4-(2-(Imidazolidin-2-ylidene)hydrazinecarbonyl)pyridine 1-oxide (15): obtained in 92% yield (1.01 g); mp 209-211 °C; IR (KBr): 3331, 3049, 1679, 1634, 1566, 1532, 1480, 1327, 1241, 1174, 840, 753, 630 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 3.55 (s, 6H, 2CH_2), 6.90 (brs, 2H, 2NH + D_2O exchangeable), 7.83 (d, 2H, pyridine, J = 5.7 Hz), 8.14 (d, 2H, pyridine, J = 5.8 Hz), 10.13 (brs, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6): δ 42.79, 123.91, 138.04, 140.10, 143.00, 159.27 ppm. Anal. Calcd for $C_9H_{11}N_5O_2$ (221.22): C, 48.86; H, 5.01; N, 31.66. Found: C, 48.81; H, 5.00; N, 31.72.

4-(2-(5,5-Dimethyltetrahydropyrimidin-2(1*H*)-ylidene)hydrazinecarbonyl)pyridine 1-oxide (16): obtained in 85% yield (1.12 g); mp 245-247 °C; IR (KBr): 3203, 2962, 2875, 1674, 1633, 1570, 1526, 1482, 1359, 1170, 851, 756, 685, 638 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.02 (s, 6H, 2CH_3), 3.03 (s, 4H, 2NCH_2), 7.72 (brs, 2H, 2NH + D_2O exchangeable), 7.87 (d, 2H, pyridine, J = 6.6 Hz), 8.12 (d, 2H, pyridine, J = 6.3 Hz),

8.05 (d, 1H, pyridine, J = 4.7 Hz), 10.16 (brs, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6 + TFA): δ 23.38, 26.89, 49.06, 125.63, 127.66, 140.10, 164.05 ppm. Anal. Calcd for $C_{12}H_{17}N_5O_2$ (263.30): C, 54.74; H, 6.51; N, 26.60. Found: C, 54.83; H, 6.49; N, 26.65.

General method for the synthesis of 3-(2-(cycloalkyldiamin-2-ylidene)hydrazinecarbonyl)pyrazine 1-oxides (17-20): Dimethyl carbonohydrazonodithioate (**5**) (1.29 g, 5 mmol) was dissolved in 10 mL of dioxane and corresponding alkyldiamine (20 mmol) was added. The mixture was refluxed for 1 h and cooled down. The precipitate was filtered off, washed with water, dried and recrystallized from water.

3-(2-(Imidazolidin-2-ylidene)hydrazinecarbonyl)pyrazine 1-oxide (17): obtained in 41% yield (0.46 g); mp 257-259 °C; IR (KBr): 3224, 2957, 1692, 1615, 1552, 1455, 1144, 977, 886 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 3.48 (s, 4H, 2CH_2), 6.75 (brs, 2H, 2NH + D_2O exchangeable), 8.31-8.34 (m, 1H, pyrazine), 8.47-8.50 (m, 1H, pyrazine), 8.68-8.71 (m, 1H, pyrazine), 10.15 (brs, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6 + TFA): δ 42.97, 133.53, 136.39, 147.09, 148.69, 160.95, 161.86 ppm. Anal. Calcd for $C_8H_{10}N_6O_2$ (222.20): C, 43.24; H, 4.54; N, 37.82. Found: C, 43.16; H, 4.53; N, 37.84.

3-(2-(Tetrahydropyrimidin-2(1*H*)-ylidene)hydrazinecarbonyl)pyrazine 1-oxide (18): obtained in 68% yield (0.80 g); mp 275-277 °C; IR (KBr): 3163, 2961, 1671, 1634, 1558, 1363, 1141, 976, 870, 790 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.75-2.15 (m, 2H, CH_2), 3.21-3.62 (m, 4H, 2NCH_2), 6.53 (brs, 2H, 2NH + D_2O exchangeable), 8.31-8.35 (m, 1H, pyrazine), 8.52-8.55 (m, 1H, pyrazine), 8.87-8.90 (m, 1H, pyrazine), 10.12 (brs 1H, NH) ppm; ^{13}C NMR (DMSO-d_6 + TFA): δ 19.54, 38.05, 133.45, 136.33, 147.12, 148.90, 154.00, 162.28 ppm. Anal. Calcd for $C_9H_{12}N_6S_2$ (236.23): C, 45.76; H, 5.12; N, 35.58. Found: C, 45.85; H, 5.10; N, 35.53.

3-(2-(5,5-Dimethyltetrahydropyrimidin-2(1*H*)-ylidene)hydrazinecarbonyl)pyrazine 1-oxide (19): obtained in 43% yield (0.57 g); mp 255-257 °C; IR (KBr): 3264, 2958, 1664, 1632, 1552, 1367, 1140, 961, 862, 788 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.11 (s, 6H, 2CH_3), 3.25 (s, 2H, NCH_2), 4.20 (s, 2H, NCH_2), 6.64 (brs, 2H, 2NH + D_2O exchangeable), 8.45-8.48 (m, 1H, pyrazine), 8.62-8.65 (m, 1H, pyrazine), 8.97-9.00 (m, 1H, pyrazine), 10.08 (brs, 1H, NH + D_2O exchangeable) ppm; ^{13}C NMR (DMSO-d_6 + TFA): δ 23.19, 26.76, 49.08, 133.46, 136.34, 147.12, 148.84, 153.51, 162.29 ppm; MS (EI) *m/z* (%): 264 (M^+, 100), 231 (M^+-O-Me-2H, 65.94), 230 (M^+-O-Me-3H, 26.76), 215 (M^+-O-2Me-3H, 17.06). Anal. Calcd for $C_{11}H_{16}N_6O_2$ (264.28): C, 49.99; H, 6.10; N, 31.80. Found: C, 49.89; H, 6.12; N, 31.84.

3-(2-(5-Hydroxytetrahydropyrimidin-2(1*H*)-ylidene)hydrazinecarbonyl)pyrazine 1-oxide (20): obtained in 31% yield (0.39 g); mp 241-243 °C; IR (KBr): 3232, 2960, 1669, 1632, 1563, 1360, 1323, 1138, 978, 880, 778 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 3.11-3.59 (m, 4H, 2CH_2), 4.11-4.17 (m, 1H, CH), 5.25 (s, 1H, OH + D_2O exchangeable), 6.72 (brs, 2H, 2NH + D_2O exchangeable), 8.35-8.38 (m, 1H, pyrazine), 8.62-8.65 (m, 1H, pyrazine), 8.86-8.90 (m, 1H, pyrazine), 10.11 (brs, 1H, NH + D_2O exchangeable) ppm;

^{13}C NMR (DMSO-d_6 + TFA): δ 44.40, 58.06, 133.45, 136.28, 147.07, 148.90, 153.66, 162.27 ppm. Anal. Calcd for $C_9H_{12}N_6O_3$ (252.23): C, 42.86; H, 4.80; N, 33.32. Found: C, 42.78; H, 4.81; N, 33.27.

Method for the synthesis of 5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyrimidines (21-23): Dimethyl carbonohydrazonodithioate (**5**) (1.29 g, 5 mmol) was suspended in 5 mL of water and corresponding alkyldiamine (10 mmol) was added. The suspension was refluxed for 6 h, then cooled down. The precipitate was filtered off, washed with cold water, dried and recrystallized from water.

3-(5,6,7,8-Tetrahydro[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)pyrazine 1-oxide (21): obtained in 52% yield (0.57 g); mp 284-286 °C; IR (KBr): 3244, 3191, 3054, 2977, 1629, 1591, 1529, 1440, 1267, 1055, 914, 862, 732 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.94-2.02 (m, 2H, CH_2), 3.25-3.32 (m, 2H, NCH2), 4.32-4.38 (t, 2H, NCH_2, J = 5.8 Hz), 7.28 (brs, 1H, NH + D_2O exchangeable), 8.28-8.31 (m, 1H, pyrazine), 8.54-8.57 (m, 1H, pyrazine), 8.66-8.67 (m, 1H, pyrazine) ppm; ^{13}C NMR (DMSO-d_6 + TFA): δ 19.15, 37.77, 44.38, 132.72, 135.23, 143.94, 145.28, 147.62, 149.59 ppm. Anal. Calcd for $C_9H_{10}N_6O$ (218.22): C, 49.54; H, 4.62; N, 38.51. Found: C, 49.61; H, 4.61; N, 38.42.

3-(6,6-Dimethyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)pyrazine 1-oxide (22): obtained in 56% yield (0.69 g); mp 267-269 °C; IR (KBr): 3235, 3197, 2960, 1643, 1584, 1520, 1452, 1273, 1047, 927, 854, 741 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.00 (s, 6H, $2CH_3$), 3.21 (s, 2H, NCH_2), 4.15 (s, 2H, NCH_2), 7.25 (brs, 1H, NH + D_2O exchangeable), 8.32-8.35 (m, 1H, pyrazine), 8.61-8.64 (m, 1H, pyrazine), 8.93-8.96 (m, 1H, pyrazine) ppm; ^{13}C NMR (DMSO-d_6 + TFA): δ 23.15, 27.57, 48.76, 54.41, 132.81, 135.19, 143.93, 145.16, 147.54, 148.98 ppm; MS (EI) *m/z* (%): 246 (M^+, 100), 231 (M^+-Me, 61.07), 230 (M^+-O, 13.15). Anal. Calcd for $C_{11}H_{14}N_6O$ (246.27): C, 53.65; H, 5.73; N, 34.13. Found: C, 53.72; H, 5.74; N, 34.03.

3-(6-Hydroxy-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)pyrazine 1-oxide (23): obtained in 43% yield (0.50 g); mp 264-266 °C; IR (KBr): 3260, 1620, 1452, 1303, 1264, 1045, 842, 799 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 3.32-3.37 (m, 4H, $2NCH_2$), 4.11-4.40 (m, 1H, CH), 5.31 (s, 1H, OH + D_2O exchangeable), 7.30 (brs, 1H, NH + D_2O exchangeable), 8.36-8.39 (m, 1H, pyrazine), 8.58-8.61 (m, 1H, pyrazine), 8.92-8.95 (m, 1H, pyrazine) ppm; ^{13}C NMR (DMSO-d_6): δ 44.47, 50.22, 59.69, 130.32, 132.95, 144.03, 147.29, 147.82, 155.05 ppm. Anal. Calcd for $C_9H_{10}N_6O_2$ (234.21): C, 46.15; H, 4.30; N, 35.88. Found: C, 46.09; H, 4.29; N, 35.94.

Tuberculostatic activity

Compounds obtained were examined *in vitro* for their tuberculostatic activity against *Mycobacterium tuberculosis* H_{37}Rv strain and two "wild" strains isolated from tuberculosis patients: one (Spec. 210) resistant to *p*-aminosalicylic acid (PAS), isonicotinic acid hydrazide (INH), etambutol (ETB) and rifampicine (RMP) and the another (Spec. 192) fully sensitive to the administered tuberculostatics.

Investigations were performed by a classical test-tube method of a successive dilution in Youmans modification of Proskauer and Beck's liquid medium containing 10% of bovine serum.[17,18] Bacterial suspensions were prepared from 14-days-old cultures of slowly growing strains and from 48-hours-old cultures of saprophytic strains.[19,20] Solutions of compounds in ethylene glycol were tested. Stock solutions contained 10 mg of compounds in one millilitre. Dilutions (in a geometric progression) were prepared in Youmans' medium. The medium containing no investigated substances and containing isoniazid (INH) or pyrazinamide (PZA) as reference drug were used for comparison.

REFERENCES

1. WHO 2011/2012 Tuberculosis Global Facts http://www.who.int/tb/publications/2011/factsheet_tb_2011.pdf
2. S. D. Lawn and A. I. Zumla, *Lancet*, 2011, **378**, 57.
3. G. B. Migliori, M. D'Arcy Richardson, G. Sotgiu, and C. Lange, *Clin. Chest Med.*, 2009, **30**, 637.
4. C. Dye and B. G.Williams, *Science*, 2010, **328**, 856.
5. H. Izzedine, V. Launay-Vacher, T. Storme, and G. Deray, *Am. J. Gastroenterol.*, 2001, **96**, 3208.
6. K. Gobis, H. Foks, Z. Zwolska, and E. Augustynowicz-Kopeć, *Heterocycles*, 2010, **81**, 917.
7. K. Gobis, H. Foks, E. Augustynowicz-Kopeć, and A. Napiórkowska, *Heterocycles*, 2012, **83**, 871.
8. K. Gobis, H. Foks, Z. Zwolska, E. Augustynowicz-Kopeć, M. L. Główka, A. Olczak, and M. Sabisz, *Monatsh. Chem*., 2011, **142**, 1137.
9. M. Sitarz, H. Foks, M. Janowiec, Z. Zwolska, and E. Augustynowicz-Kopeć, *Chem. Heterocycl. Compd*., 2005, **41**, 200.
10. H. Foks, J. Mieczkowska, M. Janowiec, Z. Zwolska, and Z. Andrzejczak, *Chem. Heterocycl. Compd.*, 2002, **38**, 810.
11. K. Gobis, H. Foks, A. Żuralska, and A. Kędzia, *Heterocycles*, 2006, **68**, 2615.
12. M. Tarao, K. Kurasawa, N. Tanaka, H. Yonehera, and H. Umezawa, *J. Antibiotics Ser. A*, 1960, **13**, 401.
13. R. H. Omar and B. Abd El-Fattah, *Egypt. J. Pharm. Sci.*, 1985, **24**, 49.
14. K. K. Oza and H. S. Patel, *Bulg. Chem. Commun*., 2010, **42**, 103.
15. Ferlux-Chemie, N1-2-Imidazolinyl carbohydrazide, *Fr. Demande*, 1974, FR 2186238 A1 (*Chem. Abstr.*, 1975, **82**, 16861).
16. H. Foks and J. Sawlewicz, *Acta Polon. Pharm.*, 1964, **21**, 429.
17. G. P. Youmans, *Am. Rev. Tuberc.*, 1947, **56**, 376.
18. G. P. Youmans and A. S. Youmans, *J. Bacteriol.*, 1949, **58**, 247.
19. R. M. Atlas and J. W. Singler, 'Handbook of Media for Clinical Microbiology', CRC Press, Boca Raton,

1995, pp. 313-326.

20. H. Foks, M. Buraczewska, W. Manowska, and J. Sawlewicz, *Dissert. Pharm. Pharmacol.*, 1971, **23**, 49.