European Journal of Medicinal Chemistry 45 (2010) 5164–5168

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antituberculosis activity of some new pyridazine derivatives. Part II

Dorina Mantu [a], Mihaela Cătălina Luca [b], Costel Moldoveanu [a], Gheorghita Zbancioc [a], Ionel I. Mangalagiu [a,*]

[a] "Al. I. Cuza" University of Iasi, Organic and Biochemistry Department, Bd. Carol 11, 700506 Iasi, Romania
[b] Gr. T. Popa University of Medicine and Pharmacy, Iasi, School of Medicine, Str. Universitatii 16, 700115 Iasi, Romania

ARTICLE INFO

Article history:
Received 24 March 2010
Received in revised form
9 August 2010
Accepted 10 August 2010
Available online 18 August 2010

Keywords:
Antituberculosis
Pyridazine
Ultrasound
SAR

ABSTRACT

A series of eighteen novel compounds with pyridazine moiety were synthesized and their *in vitro* antituberculosis activities have been evaluated. A fast, general, and facile method for preparation of pyridazine derivatives in moderate to excellent yields is presented. Three compounds were found to be moderate active against *Mycobacterium tuberculosis*. Correlation of structure–biological activity has been done.

1. Introduction

According to the World Health Organization (WHO), tuberculosis (TB) remains the leading infectious disease among humans, with one-third of the world's population being infected with *Mycobacterium tuberculosis* [1]. Most of the world's TB burden exists in less developed countries, but no region of the world is untouched. TB has made a global comeback since the 1980s, with its spread concentrated in South East Asia and sub-Saharan Africa. Much TB's resurgence is directly connected to the explosive spread of the HIV epidemic especially in Africa, where two-thirds of HIV patients also carry TB. During the last two decades, Eastern Europe and Russia are new TB hotspots – a particularly telling indication of the disease's strength since TB was not previously a major public health issue and healthcare systems remain challenged even two decades after the fall of communism.

An other major problem in TB therapy is the fact that *M. tuberculosis* became more and more resistant to the classical antituberculosis (anti-TB) drugs. The appearance of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) strains to *M. tuberculosis* has greatly contributed to the increased incidence of tuberculosis [2,3]. Therefore, there is an urgent need to develop new TB drugs [1,4]. Despite the efforts and resources involved in anti-TB therapy, no new TB drugs have been introduced in therapy in the last 40 years. Many classes of organic compounds have been tested to pursue this goal, a special attention being paid to nitrogen heterocycles, five and six member rings [5–10].

There are two basic approaches to develop a new drug for TB: (i) synthesis of analogues, modifications or derivatives of existing compounds for shortening and improving TB treatment and, (ii) searching novel structures which the TB organism has never encountered with before, especially for the treatment of MDR- and XDR-TB [4].

In previous research work we showed that some pyridazine and pyrimidine compounds have antimicrobial activity, anti-TB including [5,11].

In continuing our work within this area, we decided to design and synthesize new anti-TB compounds having their structure pyridazine moiety. In equal measure we were interested to study the structure–activity relationship (SAR) in the pyridazine series, as we rationalize in Scheme 1.

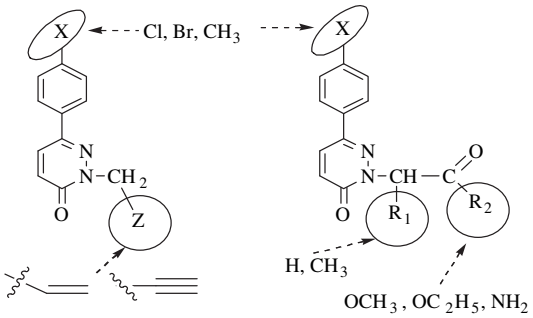
2. Results and discussion

2.1. Chemistry

In accordance with our goal, we synthesize first the *N*-substituted-pyridazinones (Scheme 2) using a straightforward, general and facile pathway, as we described in a preliminary communication [12]. The synthesis of *N*-allyl- (**2a**–**c**) and *N*-propargyl- (**3a**–**c**) pyridazinone was performed by *N*-alkylation of

* Corresponding author. Tel.: +40 232 201343; fax: +40 232 201313.
E-mail address: ionelm@uaic.ro (I.I. Mangalagiu).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.08.029



Scheme 1. SAR consideration for the studied pyridazine derivatives.

pyridazinone **1** with allyl or propargyl bromide, while the synthesis of *N*-(1-alkylcarboxy) pyridazinone **4a**–**c** to **7a**–**c** was achieved by alkylation of pyridazinone **1** with 2-bromoalkyl esters and 2-iodoacetamide respectively (explicitly of the substituents from Scheme 2 is presented in Table 1).

Synthesis was performed both under room temperature and under ultrasound irradiation, the optimization results being summarized in Table 1.

As indicated in Table 1, the desired compounds were obtained in moderate to excellent yields. We may also notice that under ultrasound irradiation the yields are higher by 10–25 percents.

The structure of the compounds was proven by elemental (C, H, N) and spectral analysis. The following spectroscopic methods were used: IR, ^{1}H NMR, ^{13}C NMR, and two-dimensional experiments 2D-COSY, 2D-HETCOR (HMQC), long range 2D-HETCOR (HMBC). Thus, in the ^{1}H and ^{13}C NMR spectra of pyridazinones **2**–**7**, the most important signals are those one furnished by the substituents linked to N1, Schemes 1 and 3.

As it could be seeing, both hydrogen and carbon atoms, are shifted in accordance with the nature of the substituents linked to them. The *N*-methylene group (protons and carbon) appears in *N*-allyl pyridazinone **2a**–**c**, around 4.85 ppm for protons (54.50 ppm for carbon), in *N*-propargyl pyridazinone **3a**–**c**, around 5.00 ppm for protons (41.80 ppm for carbon), in *N*-ester pyridazinone **4a**–**c** and **5a**–**c**, around 4.95 ppm for protons (53.45 ppm for carbon), respectively around 4.72 ppm for protons (54.20 ppm for carbon) in *N*-amide pyridazinone **7a**–**c**. The vinyl (from **2a**–**c**) and ethynyl (from **2a**–**c**) protons and carbons appear characteristic to the class they belong. The carbonyl carbon from esters **4**–**5** and amide **7** appear around 168–169 ppm, again typical to their class. The amide protons appear as two different broadened singlets around 7.25 ppm and 7.62 ppm respectively.

Table 1
Obtaining of *N*-substituted-pyridazinones under room temperature (r.t.) and ultrasound irradiation (US).

Compound	X	Z	R_1	R_2	Yield, %	
					r.t.	US
2a	Cl	$-CH=CH_2$	–	–	55	65
2b	Br	$-CH=CH_2$	–	–	50	65
2c	CH_3	$-CH=CH_2$	–	–	76	64
3a	Cl	$-C\equiv CH$	–	–	72	83
3b	Br	$-C\equiv CH$	–	–	70	82
3c	CH_3	$-C\equiv CH$	–	–	78	89
4a	Cl	–	H	$O-CH_3$	50	68
4b	Br	–	H	$O-CH_3$	48	68
4c	CH_3	–	H	$O-CH_3$	65	88
5a	Cl	–	H	$O-C_2H_5$	60	85
5b	Br	–	H	$O-C_2H_5$	58	82
5c	CH_3	–	H	$O-C_2H_5$	83	89
6a	Cl	–	CH_3	$O-C_2H_5$	50	70
6b	Br	–	CH_3	$O-C_2H_5$	51	69
6c	CH_3	–	CH_3	$O-C_2H_5$	50	71
7a	Cl	–	H	NH_2	65	65
7b	Br	–	H	NH_2	55	60
7c	CH_3	–	H	NH_2	50	60

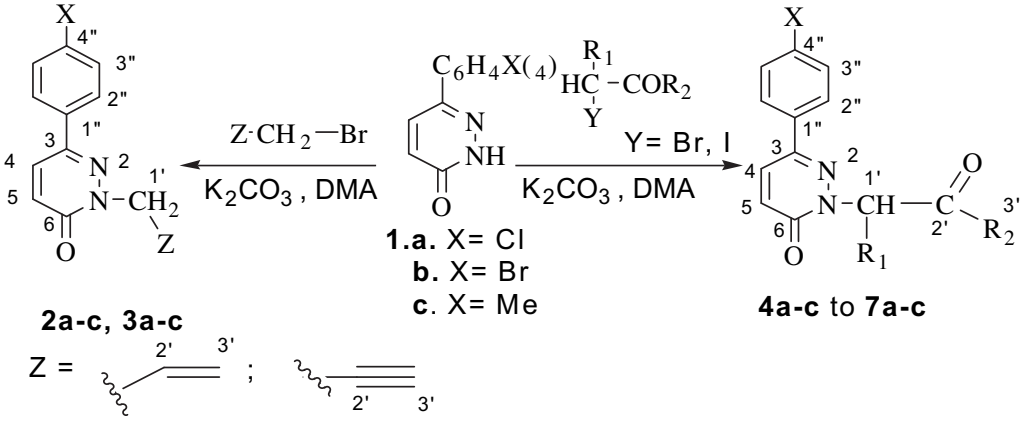
2.2. Biological activity

A standard primary *in vitro* screen was conducted against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) [13]. The results are summarized in Table 2.

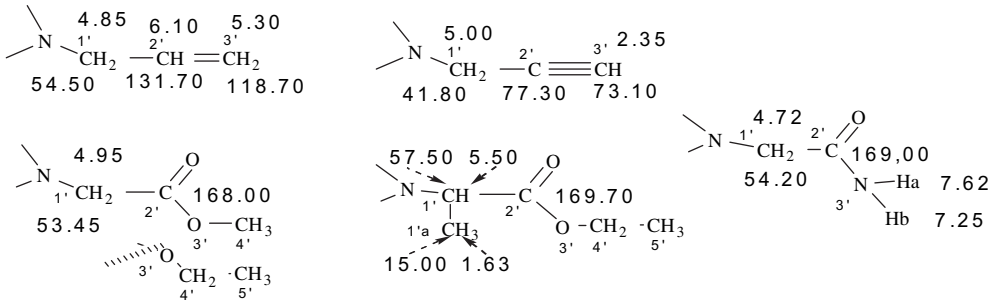
The comparative analysis of the data from Table 2 reveals interesting correlation structure–activity:

- compounds **6a**,**b** and **2b** are the most active, which means that a combined influence of **X** (Br, Cl) and $\mathbf{R_1}$ (methyl) substituents for **6a**,**b**, respectively **X** (Br) and **Z** (vinyl) for **2b**, are favourable for anti-TB activity;
- we may also notice that compounds weakly active are bearing either a **Z** ethynyl (in **3**), either a $\mathbf{R_1}$ hydrogen and $\mathbf{R_2}$ methoxy group (in **4**) [compounds **2a**,**c** and **6c** are on the border between moderate and weakly active];
- there is a certain influence of the $\mathbf{R_1}$ substituent, the most active compounds being those one with $\mathbf{R_1} = CH_3$;
- there is also a certain influence of the **X** substituent from the *para*-position of the benzene ring, activity of these compounds increasing in order $CH_3 < Cl < Br$.

Three compounds among the 18 tested present a moderate activity. Their activity may be due to a cytotoxicity and not



Scheme 2. Reaction pathway to obtain *N*-substituted-pyridazinones.



Scheme 3. Main NMR data of the *N*-substituted-pyridazinones.

Table 2
Anti-TB activity of the *N*-substituted-pyridazinones **2**–**7**.

Comp.	M.W.	X	Z	R_1	R_2	MABA: $H_{37}Rv$ data		
						IC_{50} (μg/mL)	IC_{90} (μg/mL)	Activity
6a	306.5	Cl	–	CH_3	O–C_2H_5	28.58	46.02	Moderate active
2b	291	Br	–CH=CH_2	–	–	37.80	46.80	Moderate active
6b	351	Br	–	CH_3	O–C_2H_5	26.85	47.49	Moderate active
2a	246.5	Cl	–CH=CH_2	–	–	36.63	61.65	Weakly active
2c	226	CH_3	–CH=CH_2	–	–	60.74	76.51	Weakly active
6c	286	CH_3	–	CH_3	O–C_2H_5	64.88	84.03	Weakly active
3c	224	CH_3	–C≡CH	–	–	63.70	89.24	Weakly active
3a	244.5	Cl	–C≡CH	–	–	34.73	>100	Weakly active
3b	289	Br	–C≡CH	–	–	97.81	>100	Weakly active
4a	278.5	Cl	–	H	O–CH_3	94.20	>100	Weakly active
4b	323	Br	–	H	O–CH_3	73.25	>100	Weakly active
4c	258	CH_3	–	H	O–CH_3	>100	>100	Inactive
5a	292.5	Cl	–	H	O–C_2H_5	>100	>100	Inactive
5b	337	Br	–	H	O–C_2H_5	>100	>100	Inactive
5c	272	CH_3	–	H	O–C_2H_5	>100	>100	Inactive
7a	263.5	Cl	–	H	NH_2	>100	>100	Inactive
7b	308	Br	–	H	NH_2	>100	>100	Inactive
7c	243	CH_3	–	H	NH_2	>100	>100	Inactive

necessarily to a specific antituberculosis activity. Further studies including extension of the series and improvement of the activities if possible, together with cytotoxicity assays will clarify these aspects.

3. Conclusion

A series of novel *N*-substituted-pyridazinones 2–7 were synthesized and their *in vitro* anti-TB activities have been evaluated. A fast, general, and facile method for preparation of *N*-substituted-pyridazinones both under conventional conditions and ultrasound irradiation is presented. The desired compounds were obtained in moderate to excellent yields. Three compounds were found to be moderate active against *M. tuberculosis* and other eight weakly active. There was observed a certain influence of substituents of the pyridazine moiety, compounds with $\mathbf{R_1}$ being methyl (CH_3) and **X** being halogen (Br, Cl) having an increasing activity.

4. Experimental protocols

4.1. Chemistry

All the reagents and solvents employed were used without further purification. Melting points were determined using an electrothermal apparatus (MELTEMP II) and were uncorrected. The NMR spectra were recorded on a Bruker Avance 400 DRX spectrometer operating at 400 MHz. The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. The IR spectra were recorded on an FTIR Shimadzu Prestige 8400 s spectrophotometer. The microanalyses were in satisfactory agreement with the calculated values: C, ±0.15; H, ±0.10; N, ±0.30. Some brief spectral data concerning pyridazinone **2a**, **2b**, **3a** and **3g** were prepared in [12]. Ultrasound assisted reactions were carried out using a Sonics reactor (Sonics VCX-130, U.S.A.), with a frequency of 20 kHz and a nominal power of 130 W (5 s pulse/5 s pause, 90% from the full power of the generator).

4.1.1. General procedure for synthesis of pyridazinones (*2* and *3*) under room temperature

To a solution of pyridazinone (**1a**–**c**, 1 mmol) in 10 mL *N*,*N*-dimethylacetamide (DMA), K_2CO_3 (3 mmol) was added. Into the solution, 2-bromoalkyl acid alkyl esters, allyl or propargyl bromide, or 2-iodoacetamide (3 mmol) in 5 mL DMA, was slowly added dropwise (30 min, stirring). The mixture was stirred at room temperature for 24 h (**1a**), 48 h (**1b**), and 72 h (**1c**). The resulting mixture was poured into ice-water, filtered off and washed thoroughly with water. No other purification required, excepting the *N*-amide derivatives **7a**–**c** which were recrystallized from methanol.

4.1.2. General procedure for synthesis of pyridazinones (*2* and *3*) under US irradiation

A solution of 1 mmol of pyridazinone, 3 mmol of K_2CO_3 and 3 mmol of 2-bromoalkyl acid alkyl esters, allyl or propargyl bromide, or 2-iodoacetamide, in 15 mL DMA, was exposed to ultrasound for 60 min. The resulting mixture was poured into ice-water, filtered off and washed thoroughly with water. No other purification required.

*4.1.2.1. 2-Allyl-6-(4-chlorophenyl)pyridazin-3(2H)-one (**2a**).* White crystals; mp 86–88 °C; Anal. $C_{13}H_{11}ClN_2O$ (C, H, N); IR (KBr): υ/cm^{-1}: 1656 (CO_{cet}); ^{1}H NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 4.85–4.83 (false d, J = 6.0 Hz, 2H, $H_{1'}$), 5.32–5.26 (t, J = 10.0, 1.6 Hz, 2H, $H_{3'}$), 6.10–6.01 (m, J = 10.0, 6.0, 1.6 Hz, 1H, $H_{2'}$), 7.04–7.02 (d, J = 9.6 Hz, 1H, H_4), 7.43–7.41 (d, J = 8.4 Hz, 2H, $H_{2''}$), 7.68–7.63 (d, J = 9.6 Hz, 1H, H_5), 7.73–7.71 (d, J = 8.4 Hz, 2H, $H_{3''}$). ^{13}C NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 54.59 ($C_{1'}$), 118.73 ($C_{3'}$), 127.14 ($C_{3''}$), 129.14 ($C_{2''}$), 129.76 (C_5), 130.28 (C_4), 131.71 ($C_{2'}$), 133.14 (C_3), 135.59 ($C_{1''}$), 143.42 ($C_{4''}$), 159.42 (C_6).

*4.1.2.2. 2-Allyl-6-(4-bromophenyl)pyridazin-3(2H)-one (**2b**).* White crystals; mp 81–83 °C; Anal. $C_{13}H_{11}BrN_2O$ (C, H, N); IR (KBr): υ/cm^{-1}: 1658 (CO_{cet}); ^{1}H NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 4.85–4.83 (d, J = 6.0 Hz, 2H, $H_{1'}$), 5.32–5.26 (dt, J = 10.0, 1.6 Hz, 2H, $H_{3'}$), 6.11–6.01 (m, J = 10.0, 6.0, 1.6 Hz, 1H, $H_{2'}$), 7.04–7.01 (d, J = 9.6 Hz, 1H, H_4), 7.59–7.57 (d, J = 8.4 Hz, 2H, $H_{2''}$), 7.67–7.63 (dd, J = 9.6, 8.4 Hz, 3H, $H_{3''}$, $H_{5,}$). ^{13}C NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 54.51 ($C_{1'}$), 118.50 ($C_{3'}$), 127.32 ($C_{3''}$), 129.65 ($C_{2''}$), 129.78 (C_5), 130.28 (C_4), 131.97 ($C_{2'}$), 131.99 (C_3), 139.58 ($C_{4''}$), 143.42 ($C_{1''}$), 159.56 (C_6).

*4.1.2.3. 2-Allyl-6-p-tolylpyridazin-3(2H)-one (**2c**).* White crystals; mp 62–64 °C; Anal. $C_{14}H_{14}N_2O$ (C, H, N); IR (KBr): υ/cm^{-1}: 1658 (CO_{cet}); ^{1}H NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 2.39 (s, 3H, CH_3 from 4″ position), 4.85–4.84 (dd, J = 6.0 Hz, 2H, $H_{1'}$), 5.32–5.25 (dt, J = 10.0, 1.6 Hz, 2H, $H_{3'}$), 6.12–6.02 (m, J = 10.0, 6.0, 1.6 Hz, 1H, $H_{2'}$), 7.02–6.99 (d, J = 9.6 Hz, 1H, H_4), 7.27–7.25 (d, J = 8.4 Hz, 2H, $H_{2''}$), 7.68–7.64 (dd, J = 9.6, 8.4 Hz, 3H, H_5, $H_{3''}$). ^{13}C NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 21.29 (CH_3 from 4″ position), 54.51 ($C_{1'}$), 118.50 ($C_{3'}$), 125.80 ($C_{3''}$), 129.65 ($C_{2''}$), 130.09 (C_5), 130.14 (C_4), 131.97 ($C_{2'}$), 131.99 (C_3), 139.58 ($C_{4''}$), 144.61 ($C_{1''}$), 159.58 (C_6).

*4.1.2.4. 6-(4-Chlorophenyl)-2-(prop-2-ynyl)pyridazin-3(2H)-one (**3a**).* Pink crystals; mp 123–125 °C; Anal. $C_{13}H_9ClN_2O$ (C, H, N); IR (KBr): υ/cm^{-1}: 1661 (CO_{cet}); ^{1}H NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 2.37–2.35 (false t, 1H, $H_{3'}$), 4.99 (s, 2H, $H_{1'}$), 7.05–7.03 (d, J = 10.0 Hz, 1H, H_4), 7.44–7.42 (d, J = 8.4 Hz, 2H, $H_{2''}$), 7.67–7.65 (d, J = 10.0, Hz, 1H, H_5), 7.75–7.73 (d, J = 8.4 Hz, 2H, $H_{3''}$). ^{13}C NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 41.84 ($C_{1'}$), 73.14 ($C_{3'}$), 77.23 ($C_{2'}$), 127.23 ($C_{3''}$), 129.19 ($C_{2''}$), 130.32 (C_4), 130.44 (C_5), 132.83 (C_3), 135.84 ($C_{1''}$), 143.79 ($C_{4''}$), 158.86 (C_6).

*4.1.2.5. 6-(4-Bromophenyl)-2-(prop-2-ynyl)pyridazin-3(2H)-one (**3b**).* White crystals; mp 132–134 °C; Anal. $C_{13}H_9BrN_2O$ (C, H, N); IR (KBr): υ/cm^{-1}: 1665 (CO_{cet}); ^{1}H NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 2.36–2.35 (false t, 1H, $H_{3'}$), 4.98 (s, 2H, $H_{1'}$), 7.04–7.01 (d, J = 10.0 Hz, 1H, H_4), 7.58–7.56 (d, J = 8.8 Hz, 2H, $H_{2''}$), 7.67–7.63 (dd, J = 10.0, 8.8 Hz, 3H, $H_{3''}$, H_5). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 41.81 ($C_{1'}$), 73.15 ($C_{3'}$), 77.18 ($C_{2'}$), 124.06 (C_3), 127.43 ($C_{3''}$), 130.23 (C_5), 130.38 (C_4), 132.08 ($C_{2''}$), 133.21 ($C_{4''}$), 143.77 ($C_{1''}$), 158.80 (C_6).

*4.1.2.6. 2-(Prop-2-ynyl)-6-p-tolylpyridazin-3(2H)-one (**3c**).* White crystals; mp 94–96 °C; Anal. $C_{14}H_{12}N_2O$ (C, H, N); IR (KBr): υ/cm^{-1}: 1665 (CO_{cet}); ^{1}H NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 2.36–2.34 (false t, 1H, $H_{3'}$), 2.40 (s, 3H, CH_3 from 4″ position), 5.00–4.99 (false d, 2H, $H_{1'}$), 7.03–7.01 (d, J = 9.6 Hz, 1H, H_4), 7.27–7.25 (d, J = 8.0 Hz, 2H, $H_{2''}$), 7.70–7.66 (dd, J = 9.6, 8.0 Hz, 3H, H_5, $H_{3''}$). ^{13}C NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 21.31 (CH_3 from 4″ position), 41.83 ($C_{1'}$), 72.95 ($C_{3'}$), 77.49 ($C_{2'}$), 125.90 ($C_{3''}$), 129.69 ($C_{2''}$), 130.24 (C_4), 130.73 (C_5), 131.66 (C_3), 139.84 ($C_{4''}$), 145.02 ($C_{1''}$), 159.05 (C_6).

*4.1.2.7. Methyl 2-(3-(4-chlorophenyl)-6-oxopyridazin-1(6H)-yl) acetate (**4a**).* White crystals; mp 127–129 °C; Anal. $C_{13}H_{11}ClN_2O_3$ (C, H, N); IR (KBr): υ/cm^{-1}: 1747 (CO_{est}), 1666 (CO_{cet}); ^{1}H NMR (400 MHz, DMSO-d_6): δ$_{ppm}$: 3.70 (s, 3H, $H_{4'}$), 4.97 (s, 2H, $H_{1'}$), 7.15–7.13 (d, J = 9.6 Hz, 1H, H_4), 7.58–7.56 (d, J = 8.4 Hz, 2H, $H_{2''}$), 7.93–7.90 (d, J = 8.4 Hz, 2H, $H_{3''}$), 8.15–8.12 (d, J = 9.6 Hz, 1H, H_5). ^{13}C NMR (400 MHz, DMSO-d_6): δ$_{ppm}$: 52.27 ($C_{4'}$), 53.46 ($C_{1'}$), 127.63 ($C_{3''}$), 128.94 ($C_{2''}$), 129.75 (C_4), 131.43 (C_5), 132.79 (C_3), 134.36 ($C_{4''}$), 142.86 ($C_{1''}$), 158.73 (C_6), 167.97 ($C_{2'}$).

*4.1.2.8. Methyl 2-(3-(4-bromophenyl)-6-oxopyridazin-1(6H)-yl)acetate (**4b**).* White crystals; mp 142–144 °C; Anal. $C_{13}H_{11}BrN_2O_3$ (C, H, N); IR (KBr): υ/cm^{-1}: 1747 (CO_{est}), 1666 (CO_{cet}); ^{1}H NMR (400 MHz, DMSO-d_6): δ$_{ppm}$: 3.70 (s, 3H, $H_{4'}$), 4.97 (s, 2H, $H_{1'}$), 7.15–7.13 (d, J = 9.6 Hz, 1H, H_4), 7.58–7.56 (d, J = 8.4 Hz, 2H, $H_{2''}$), 7.93–7.90 (d, J = 8.4 Hz, 2H, $H_{3''}$), 8.15–8.12 (d, J = 9.6 Hz, 1H, H_5). ^{13}C NMR (400 MHz, DMSO-d_6): δ$_{ppm}$: 52.27 ($C_{4'}$), 53.46 ($C_{1'}$), 127.63 ($C_{3''}$), 128.94 ($C_{2''}$), 129.75 (C_4), 131.43 (C_5), 132.79 (C_3), 134.36 ($C_{4''}$), 142.86 ($C_{1''}$), 158.73 (C_6), 167.97 ($C_{2'}$).

*4.1.2.9. Methyl 2-(6-oxo-3-p-tolylpyridazin-1(6H)-yl)acetate (**4c**).* White crystals; mp 100–102 °C; Anal. $C_{14}H_{14}N_2O_3$ (C, H, N); IR (KBr): υ/cm^{-1}: 1743 (CO_{est}), 1670 (CO_{cet}); ^{1}H NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 2.39 (s, 3H, CH_3 from 4″ position), 3.79 (s, 3H, $H_{4'}$), 4.97 (s, 2H, $H_{1'}$), 7.05–7.02 (d, J = 9.6 Hz, 1H, H_4), 7.26–7.24 (d, J = 8.4 Hz, 2H, $H_{3''}$), 7.66–7.64 (d, J = 8.4 Hz, 2H, $H_{2''}$), 7.71–7.69 (d, J = 9.6 Hz, 1H, H_5). ^{13}C NMR (400 MHz, $CDCl_3$): δ$_{ppm}$: 21.30 (CH_3 from 4″ position), 52.58 ($C_{4'}$), 53.65 ($C_{1'}$), 125.99 ($C_{2''}$), 129.67 ($C_{3''}$), 130.11 (C_4), 131.08 (C_5), 131.66 ($C_{1''}$), 139.83 ($C_{4''}$), 145.14 (C_3), 159.71 (C_6), 167.92 ($C_{2'}$).

*4.1.2.10. Ethyl 2-(3-(4-chlorophenyl)-6-oxopyridazin-1(6H)-yl)acetate (**5a**).* White crystals; mp 164–166 °C; Anal. $C_{14}H_{13}ClN_2O_3$ (C, H, N); IR (KBr): υ/cm^{-1}: 1755 (CO_{est}), 1670 (CO_{cet}); ^{1}H NMR (400 MHz, DMSO-d_6): δ$_{ppm}$: 1.23–1.19 (t, J = 7.2 Hz, 3H, $H_{5'}$), 4.19–4.14 (q, J = 7.2 Hz, 2H, $H_{4'}$), 4.95 (s, 2H, $H_{1'}$), 7.15–7.12 (d, J = 10.0 Hz, 1H, H_4), 7.58–7.56 (d, J = 8.8 Hz, 2H, $H_{2''}$), 7.93–7.90 (d, J = 8.8 Hz, 2H, $H_{3''}$), 8.14–8.12 (d, J = 10.0 Hz, 1H, H_5). ^{13}C NMR (400 MHz, DMSO-d_6): δ$_{ppm}$: 13.96 ($C_{5'}$), 53.56 ($C_{1'}$), 61.14 ($C_{4'}$), 127.63 ($C_{3''}$), 128.95 ($C_{2''}$), 129.75 (C_4), 131.41 (C_5), 132.82 (C_3), 134.35 ($C_{4''}$), 142.82 ($C_{1''}$), 158.74 (C_6), 167.46 ($C_{2'}$).

*4.1.2.11. Ethyl 2-(3-(4-bromophenyl)-6-oxopyridazin-1(6H)-yl) acetate (**5b**).* White crystals; mp 164–166 °C; Anal. $C_{14}H_{13}BrN_2O_3$ (C, H, N); IR (KBr): υ/cm^{-1}: 1753 (CO_{est}), 1670 (CO_{cet}); ^{1}H NMR (400 MHz, DMSO-d_6): δ$_{ppm}$: 1.23–1.20 (t, J = 7.2 Hz, 3H, $H_{5'}$), 4.20–4.15 (q, J = 7.2 Hz, 2H, $H_{4'}$), 4.95 (s, 2H, $H_{1'}$), 7.15–7.13 (d, J = 10.0 Hz, 1H, H_4), 7.72–7.70 (d, J = 8.8 Hz, 2H, $H_{2''}$), 7.86–7.84 (d, J = 8.8 Hz, 2H, $H_{3''}$), 8.14–8.12 (d, J = 10.0 Hz, 1H, H_5). ^{13}C NMR (400 MHz, DMSO-d_6): δ$_{ppm}$: 13.98 ($C_{5'}$), 53.58 ($C_{1'}$), 61.16 ($C_{4'}$), 123.13 (C_3), 127.89 ($C_{3''}$), 129.76 (C_4), 131.37 (C_5), 131.89 ($C_{2''}$), 133.19 ($C_{4''}$), 142.91 ($C_{1''}$), 158.76 (C_6), 167.47 ($C_{2'}$).

*4.1.2.12. Ethyl 2-(6-oxo-3-p-tolylpyridazin-1(6H)-yl)acetate (**5c**).* White crystals; mp 134–135 °C; Anal. $C_{15}H_{16}N_2O_3$ (C, H, N); IR (KBr): υ/cm^{-1}: 1755 (CO_{est}), 1670 (CO_{cet}); ^{1}H NMR (400 MHz, DMSO-d_6): δ$_{ppm}$: 1.24–1.20 (t, J = 7.2 Hz, 3H, $H_{5'}$), 2.36 (s, 3H, CH_3 from 4″ position), 4.20–4.15 (q, J = 7.2 Hz, 2H, $H_{4'}$), 4.94 (s, 2H, $H_{1'}$), 7.12–7.10 (d, J = 10.0 Hz, 1H, H_4), 7.32–7.30 (d, J = 8.4 Hz, 2H, $H_{2''}$), 7.80–7.78 (d, J = 8.4 Hz, 2H, $H_{3''}$), 8.11–8.09 (d, J = 10.0 Hz, 1H, H_5). ^{13}C NMR (400 MHz, DMSO-d_6): δ$_{ppm}$: 13.96 ($C_{5'}$), 20.78 (CH_3 from 4″ position), 53.52 ($C_{1'}$), 61.10 ($C_{4'}$), 125.73 ($C_{3''}$), 129.48 ($C_{2''}$), 129.63 (C_4), 131.20 ($C_{1''}$), 131.48 (C_5), 139.20 ($C_{4''}$), 143.87 (C_3), 158.78 (C_6), 167.54 ($C_{2'}$).

*4.1.2.13. Ethyl 2-(3-(4-chlorophenyl)-6-oxopyridazin-1(6H)-yl)propanoate (**6a**).* White crystals; mp 68–70 °C; Anal. $C_{15}H_{15}ClN_2O_3$ (C, H, N); IR (KBr): υ/cm^{-1}: 1739 (CO_{est}), 1670 (CO_{cet}); ^{1}H NMR (400 MHz, DMSO-d_6): δ$_{ppm}$: 1.16–1.13 (t, J = 7.2 Hz, 3H, $H_{5'}$), 1.63–1.62 (d, J = 7.2 Hz, 3H, $H_{1'a}$), 4.17–4.09 (m, J = 7.2 Hz, 2H, $H_{4'}$),

5.52–5.47 (q, $J = 7.2$ Hz, 1H, $H_{1'}$), 7.13–7.11 (d, $J = 9.6$ Hz, 1H, H_4), 7.58–7.56 (d, $J = 8.4$ Hz, 2H, $H_{2''}$), 7.93–7.91 (d, $J = 8.4$ Hz, 2H, $H_{3''}$), 8.13–8.11 (d, $J = 9.6$ Hz, 1H, H_5). ^{13}C NMR (400 MHz, DMSO-d_6): δ_{ppm}: 13.93 ($C_{5'}$), 15.06 ($C_{1'a}$), 57.44 ($C_{1'}$), 61.01 ($C_{4'}$), 127.52 ($C_{3''}$), 128.97 ($C_{2''}$), 129.60 (C_4), 130.80 (C_5), 133.05 (C_3), 134.30 ($C_{4''}$), 142.47 ($C_{1''}$), 158.48 (C_6), 169.71 ($C_{2'}$).

*4.1.2.14. Ethyl 2-(3-(4-bromophenyl)-6-oxopyridazin-1(6H)-yl)propanoate (**6b**).* White crystals; mp 96–98 °C; Anal. $C_{15}H_{15}BrN_2O_3$ (C, H, N); IR (KBr): υ/cm^{-1}: 1740 (CO_{est}), 1670 (CO_{cet}); ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 1.25–1.22 (t, $J = 7.2$ Hz, 3H, $H_{5'}$), 1.76–1.74 (d, $J = 7.2$ Hz, 3H, $H_{1'a}$), 4.24–4.18 (m, $J = 7.2$ Hz, 2H, $H_{4'}$), 5.67–5.61 (q, $J = 7.2$ Hz, 1H, $H_{1'}$), 7.05–7.02 (d, $J = 10.0$ Hz, 1H, H_4), 7.43–7.41 (d, $J = 8.4$ Hz, 2H, $H_{2''}$), 7.68–7.66 (d, $J = 10.0$ Hz, 2H, H_5), 7.73–7.71 (d, $J = 8.4$ Hz, 1H, $H_{3''}$). ^{13}C NMR (400 MHz, $CDCl_3$): δ_{ppm}: 13.90 ($C_{5'}$), 15.06 ($C_{1'a}$), 57.48 ($C_{1'}$), 61.00 ($C_{4'}$), 127.52 ($C_{3''}$), 128.97 ($C_{2''}$), 129.75 (C_4), 130.80 (C_5), 133.05 (C_3), 134.30 ($C_{4''}$), 142.48 ($C_{1''}$), 158.48 (C_6), 169.83 ($C_{2'}$).

*4.1.2.15. Ethyl 2-(6-oxo-3-p-tolylpyridazin-1(6H)-yl)propanoate (**6c**).* White crystals; mp 61–63 °C; Anal. $C_{16}H_{18}N_2O_3$ (C, H, N); IR (KBr): υ/cm^{-1}: 1740 (CO_{est}), 1670 (CO_{cet}); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.24–1.21$ (t, $J = 7.2$ Hz, 3H, $H_{5'}$), 1.75–1.73 (d, $J = 7.2$ Hz, 3H, $H_{1'a}$), 2.39 (s, 3H, CH_3 from 4″ position), 4.25–4.16 (m, $J = 7.2$ Hz, 2H, $H_{4'}$), 5.67–5.61 (q, $J = 7.2$ Hz, 1H, $H_{1'}$), 7.02–7.00 (d, $J = 9.6$ Hz, 1H, H_4), 7.26–7.24 (d, $J = 8.4$ Hz, 2H, $H_{2''}$), 7.69–7.65 (dd, $J = 9.6$, 8.4 Hz, 3H, $H_{3''}$, H_5). ^{13}C NMR (400 MHz, $CDCl_3$): δ_{ppm}: 14.13 ($C_{5'}$), 15.47 ($C_{1'a}$), 21.29 (CH_3 from 4″ position), 57.42 ($C_{1'}$), 61.56 ($C_{4'}$), 125.80 ($C_{3''}$), 129.63 ($C_{2''}$), 129.80 (C_4), 130.19 (C_5), 131.93 ($C_{1''}$), 139.66 ($C_{4''}$), 144.40 (C_3), 159.48 (C_6), 170.22 ($C_{2'}$).

*4.1.2.16. 2-(3-(4-Chlorophenyl)-6-oxopyridazin-1(6H)-yl)acetamide (**7a**).* White crystals; mp 217–218 °C; Anal. $C_{12}H_{10}ClN_3O_2$ (C, H, N); IR (KBr): υ/cm^{-1}: 3384, 3261 ($NH_{asim,\ sim}$), 1690 (CO_{amide}), 1662 (CO_{cet}); ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm}: 4.73 (s, 2H, $H_{1'}$), 7.09–7.06 (d, $J = 10.0$ Hz, 1H, H_4), 7.26 (brs, 1H, NH_b), 7.56–7.54 (d, $J = 8.4$ Hz, 1H, $H_{6''}$), 7.63 (brs, 1H, NH_a), 7.70–7.68 (d, $J = 8.4$ Hz, 1H, $H_{2''}$), 7.84–7.82 (d, $J = 8.4$ Hz, 1H, $H_{3''}$), 7.91–7.89 (d, $J = 8.4$ Hz, 1H, $H_{5''}$), 8.08–8.05 (dd, $J = 10.0$ Hz, 1H, H_5). ^{13}C NMR (400 MHz, DMSO-d_6): δ_{ppm}: 54.25 ($C_{1'}$), 127.56 ($C_{5'''}$), 127.81 ($C_{3''}$), 128.90 ($C_{6''}$), 129.69 (C_4), 130.87 (C_5), 131.83 ($C_{2''}$), 133.49 (C_3), 134.13 ($C_{4''}$), 142.43 ($C_{1''}$), 158.91 (C_6), 168.07 ($C_{2'}$).

*4.1.2.17. 2-(3-(4-Bromophenyl)-6-oxopyridazin-1(6H)-yl)acetamide (**7b**).* White crystals; mp 223–225 °C; Anal. $C_{12}H_{10}BrN_3O_2$ (C, H, N); IR (KBr): υ/cm^{-1}: 3360, 3246 ($NH_{asim,\ sim}$), 1689 (CO_{amide}), 1662 (CO_{cet}); ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm}: 4.73 (s, 2H, $H_{1'}$), 7.09–7.06 (d, $J = 10.0$ Hz, 1H, H_4), 7.26 (brs, 1H, NH_b), 7.63 (brs, 1H, NH_a), 7.70–7.67 (d, $J = 8.8$ Hz, 1H, $H_{2''}$), 7.84–7.81 (d, $J = 8.8$ Hz, 1H, $H_{3''}$), 8.07–8.05 (d, $J = 10.0$ Hz, 1H, H_5). ^{13}C NMR (400 MHz, DMSO-d_6): δ_{ppm}: 54.23 ($C_{1'}$), 122.84 (C_3), 127.77 ($C_{3''}$), 129.66 (C_4), 130.77 (C_5), 131.79 ($C_{2''}$), 133.46 ($C_{4''}$), 142.41 ($C_{1''}$), 158.89 (C_6), 168.04 ($C_{2'}$).

*4.1.2.18. 2-(6-Oxo-3-p-tolylpyridazin-1(6H)-yl)acetamide (**7c**).* Yellowish white crystals; mp 180–183 °C; Anal. $C_{13}H_{13}N_3O_2$ (C, H, N); IR (KBr): υ/cm^{-1}: 3386, 3263 ($NH_{asim,\ sim}$), 1691 (CO_{amide}), 1670 (CO_{cet}); ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm}: 2.35 (s, 3H, CH_3 from 4″ position), 4.72 (s, 2H, $H_{1'}$), 7.06–7.03 (d, $J = 10.0$ Hz, 1H, H_4), 7.24 (brs, 1H, NH_b), 7.31–7.29 (d, $J = 8.0$ Hz, 2H, $H_{2''}$), 7.66 (brs, 1H, NH_a), 7.78–7.76 (d, $J = 8.0$ Hz, 2H, $H_{3''}$), 8.05–8.03 (d, $J = 10.0$ Hz, 1H, H_5). ^{13}C NMR (400 MHz, DMSO-d_6): δ_{ppm}: 20.80 (CH_3 from 4″ position), 54.19 ($C_{1'}$), 125.68 ($C_{3''}$), 129.46 ($C_{2''}$), 129.61 (C_4), 130.98 (C_5), 131.50 (C_3), 138.97 ($C_{4''}$), 143.46 ($C_{1''}$), 158.98 (C_6), 168.21 ($C_{2'}$).

4.2. Microbiology

4.2.1. Determination of a 90% inhibitory concentration (IC_{90}) and 50% inhibitory concentration (IC_{50})

The initial *in vitro* screen was conducted against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) [13]. Compounds are tested in ten 2-fold dilutions (dissolved in dimethyl sulfoxide and subsequently diluted with distilled water), typically from 100 μg/mL to 0.19 μg/mL. The IC_{90} is defined as the concentration effecting a reduction in fluorescence of 90% relative to controls (analogous for IC_{50}). This value is determined from the dose–response curve using a curve-fitting program. Any IC_{90} value of $\leq$10 μg/mL is considered "Active" for antitubercular activity; compounds with IC_{90} value between 11 and 50 μg/mL are considered "Moderate active", between 51 and 99 μg/mL are considered "Weakly active", while those one with IC_{90} higher than 100 μg/mL is considered "Inactive".

Acknowledgements

Authors are thankful to the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), USA, for the *in vitro* evaluation of antimycobacterial activity and to CNCSIS Bucuresti, Romania, grant BD no. 308/2008, for financial support.

Appendix. Supporting information

Supporting information associated with this article could be found, in the online version, at doi:10.1016/j.ejmech.2010.08.029.

References

[1] World Health Organization, Tuberculosis Programme (2009) Available from: http://www.who.int/tb/en/June.
[2] S. Shenoi, G. Friedland, Annu. Rev. Med. 60 (2009) 307–320.
[3] A. Imramovsky, S. Polanc, J. Vinsova, M. Kocevar, J. Jampilek, Z. Reckova, J. Kaustova, Bioorg. Med. Chem. 15 (2007) 2551–2559.
[4] C. Crabb, Bull. World Health Organ. 80 (2002) 517.
[5] C. Moldoveanu, G. Mangalagiu, G. Drochioiu, M. Caprosu, M. Petrovanu, I.I. Mangalagiu, An. Stiint. Univ. "Al.I.Cuza" Iasi 11 (2003) 367–374 (Chem. Abstr. 142 (2004) 56249).
[6] Y.L. Janin, Bioorg. Med. Chem. 15 (2007) 2479–2513.
[7] (a) S. Eswarana, A.V. Adhikarib, R.A. Kumarc, Eur. J. Med. Chem. 45 (2010) 957–966;
(b) S. Eswarana, A.V. Adhikarib, N.K. Palc, I.H. Chowdhury, Bioorg. Med. Chem. Lett. 20 (2010) 1040–1044.
[8] M.J. Hearn, M.H. Cynamon, M.F. Chen, R. Coppins, J. Davis, H. Joo-On Kang, A. Noble, B. Tu-Sekine, M.S. Terrot, D. Trombino, M. Thai, E.R. Webster, R. Wilson, Eur. J. Med. Chem. 44 (2009) 4169–4178.
[9] G. Turan-Zitouni, A. Ozdemir, Z.A. Kaplancikli, K. Benkli, P. Chevallet, G. Akalin, Eur. J. Med. Chem. 43 (2008) 981–985.
[10] M. Johar, T. Manning, D.Y. Kunimoto, R. Kumar, Bioorg. Med. Chem. 13 (2005) 6663–6671.
[11] (a) R. Butnariu, I.I. Mangalagiu, Bioorg. Med. Chem. 17 (2009) 2823–2829;
(b) A.M. Balan, O. Florea, C. Moldoveanu, Ghe Zbancioc, D. Iurea, I.I. Mangalagiu, Eur. J. Med. Chem. 44 (2009) 2275–2279;
(c) R. Butnariu, M. Caprosu, V. Bejan, M. Ungureanu, A. Poiata, C. Tuchilus, M. Florescu, I.I. Mangalagiu, J. Heterocyclic Chem. 44 (2007) 1149–1152;
(d) M. Ungureanu, C. Moldoveanu, A. Poiata, G. Drochioiu, M. Petrovanu, I.I. Mangalagiu, Ann. Pharm. Fr. 64 (2006) 287–288;
(e) M. Caprosu, R. Butnariu, I.I. Mangalagiu, Heterocycles 65 (2005) 1871–1879.
[12] D. Mantu, C. Moldoveanu, A. Nicolescu, C. Deleanu, I.I. Mangalagiu, Ultrason. Sonochem. 16 (2009) 452–454.
[13] (a) J. Secrist, S. Anathan, C. Kwong, J. Maddry, R. Reynolds, A. Poffenberger, M. Michael, L. Miller, J. Krahenbuhl, L. Adams, A. Biswas, S. Franzblau, D. Rouse, D. Winfield, J. Brooks, I. Orme, Antimicrob. Agents Chemother. 45 (2001) 1943–1946;
(b) L.A. Collins, S.G. Franzblau, Antimicrob. Agents Chemother. 41 (1997) 1004–1009.