

Design, Synthesis and Evaluation of N-pyrazinylbenzamides as Potential Antimycobacterial Agents

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Supplementary Material

1. Full results of biological assays

1.1. Full results of antimycobacterial evaluation

Table S1. Antimycobacterial activity of prepared compounds expressed as MIC ($\mu\text{g/mL}$)

Cpd	R ¹	R ²	<i>Mtb</i> H37Rv MIC ($\mu\text{g/mL}$)	<i>M. kans.</i> MIC ($\mu\text{g/mL}$)	<i>M. avium</i> MIC ($\mu\text{g/mL}$)	<i>M. smeg.</i> MIC ($\mu\text{g/mL}$)
1a	H	H	100	>100	>100	≥ 500
1b	H	2-OH	>100	>100	>100	≥ 500
1c-Ac	H	3-OAc	>100	>100	>100	≥ 500
1c	H	3-OH	>100	>100	>100	≥ 500
1d-Ac	H	4-OAc	>100	>100	>100	≥ 500
1e	H	2-OCH ₃	>100	100	>100	125
1f	H	3-OCH ₃	>100	>100	>100	≥ 500
1g	H	4-OCH ₃	25	>100	>100	≥ 500
1h	H	4-CH ₃	>100	>100	>100	≥ 500
1i	H	4-Et	>100	>100	>100	≥ 500
1j	H	2-Cl	>100	>100	>100	250
1k	H	3-Cl	50	50	>100	≥ 500
1l	H	4-Cl	50	50	>100	≥ 500
1l-SP*	H	4-Cl*	50	>100	>100	≥ 500
1n	H	3-CF ₃	>100	>100	>100	≥ 500
2a	5-Cl	H	>100	>100	>100	≥ 500
2b-Ac	5-Cl	2-OAc	12.5	12.5	50	31.25
2b	5-Cl	2-OH	12.5	50	50	15.625
2c-Ac	5-Cl	3-OAc	>100	>100	>100	250
2c	5-Cl	3-OH	>100	>100	>100	≥ 500
2d-Ac	5-Cl	4-OAc	>100	>100	>100	250
2d	5-Cl	4-OH	>100	>100	>100	≥ 500
2e	5-Cl	2-OCH ₃	>100	>100	>100	≥ 500
2f	5-Cl	3-OCH ₃	>100	>100	>100	≥ 500
2g	5-Cl	4-OCH ₃	>100	>100	>100	≥ 500
2h	5-Cl	4-CH ₃	6.25	>100	>100	≥ 500
2i	5-Cl	4-Et	3.13	>100	>100	≥ 500
2j	5-Cl	2-Cl	>100	>100	>100	≥ 500
2k	5-Cl	3-Cl	>100	>100	>100	≥ 500
2l	5-Cl	4-Cl	>100	>100	>100	≥ 500
2m	5-Cl	4-Br	>100	>100	>100	≥ 500
2n	5-Cl	3-CF ₃	25	>100	>100	≥ 500
3a	6-Cl	H	>100 ^a	>100 ^a	>100 ^a	≥ 500 ^a
3b	6-Cl	2-OH	>100	>100	>100	250
3e	6-Cl	2-OCH ₃	>100	>100	>100	≥ 500
3f	6-Cl	3-OCH ₃	50 ^a	100 ^a	100 ^a	≥ 500 ^a
3g	6-Cl	4-OCH ₃	>100	>100	>100	≥ 500 ^a
3h	6-Cl	4-CH ₃	>100 ^a	>100 ^a	>100 ^a	≥ 500 ^a
3i	6-Cl	4-Et	>100 ^a	>100 ^a	>100 ^a	≥ 500 ^a
3j	6-Cl	2-Cl	>100	>100	>100	≥ 500
3k	6-Cl	3-Cl	>100 ^a	>100 ^a	>100 ^a	≥ 500 ^a
3l	6-Cl	4-Cl	>100 ^a	>100 ^a	>100 ^a	≥ 500 ^a
3n	6-Cl	3-CF ₃	25	25	50	125
INH	-	-	0.1–0.39	6.25–12.5	6.25–12.5	15.625
RFM	-	-	-	-	-	1.56
CPX	-	-	-	-	-	0.195

* Diacylated side-product. ^a Precipitate formed in the testing medium. INH - isoniazid; RFM - rifampicin; CPX – ciprofloxacin.

1.2. Full results of antibacterial evaluation

Tested strains											
1. SA <i>Staphylococcus aureus</i> CCM 4516/08											
2. MRSA <i>Staphylococcus aureus</i> H 5996/08		Methicillin resistant									
3. SE <i>Staphylococcus epidermidis</i> H 6966/08											
4. EF <i>Enterococcus sp.</i> J 14365/08											
5. EC <i>Escherichia coli</i> CCM4517											
6. KP <i>Klebsiella pneumoniae</i> D 11750/08											
7. KP-E <i>Klebsiella pneumoniae</i> J 14368/08		ESBL positive									
8. PA <i>Pseudomonas aeruginosa</i> CCM 1961											
Results											
Strain		Tested compound (lab code / publication code) – MIC (μM)									
		OV-6	JZ-AM7	JZ-AM13	JZAM14	JZ-AM6	JZ-AM9	JZ-AM5	JZ-AM3	JZAM10	
		1a	1b	1c-Ac	1c	1d-Ac	1e	1f	1g	1h	
SA	24 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
MRSA	24 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
SE	24 h	>500	>500	>500	>500	>500	>500	>500	>500	500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	500	
EF	24 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
EC	24 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
KP	24 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
KP-E	24 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
PA	72 h	>500	>500	>500	>500	>500	>500	>500	>500	500	
	120 h	>500	>500	>500	>500	>500	>500	>500	>500	500	

Strain		Tested compound (lab code / publication code) – MIC (μM)									
		JZAM15	JZAM11	JZ-AM4	OV-5	JZ-AM2	JZ-AM8	OV-1	OV-14	OV-17	
		1i	1j	1k	1l	1l-SP	1n	2a	2b-Ac	2b	
SA	24 h	>500	250	>125	>125	>125	>125	250	62.5	15.62	
	48 h	>500	250	>125	>125	>125	>125	250	125	15.62	
MRSA	24 h	>500	>500	>125	>125	>125	>125	250	125	62.5	
	48 h	>500	>500	>125	>125	>125	>125	250	250	62.5	
SE	24 h	500	500	>125	>125	>125	62.5	500	125	62.5	
	48 h	500	500	>125	>125	>125	62.5	500	250	62.5	
EF	24 h	500	>500	>125	>125	>125	>125	>500	250	>500	
	48 h	500	>500	>125	>125	>125	>125	>500	>250	>500	
EC	24 h	>500	>500	>125	>125	>125	>125	>500	>250	>500	
	48 h	>500	>500	>125	>125	>125	>125	>500	>250	>500	
KP	24 h	>500	>500	>125	>125	>125	>125	>500	>250	250	
	48 h	>500	>500	>125	>125	>125	>125	>500	>250	250	
KP-E	24 h	>500	>500	>125	>125	>125	>125	>500	>250	250	
	48 h	>500	>500	>125	>125	>125	>125	>500	>250	250	
PA	72 h	>500	250	>125	>125	>125	>125	>500	>250	>500	
	120 h	>500	250	>125	>125	>125	>125	>500	>250	>500	

Strain		Tested compound (lab code / publication code) – MIC (μM)									
		OV-16	OV-19	OV-15	OV-18	OV-9	OV-7	OV-8	OV-11	OV-12	
		2c-Ac	2c	2d-Ac	2d	2e	2f	2g	2h	2i	
SA	24 h	>125	500	0.98	31.25	>125	31.25	>125	>125	>125	
	48 h	>125	500	0.98	31.25	>125	31.25	>125	>125	>125	
MRSA	24 h	>125	>500	>125	>500	>125	>500	>125	>125	>125	
	48 h	>125	>500	>125	>500	>125	>500	>125	>125	>125	
SE	24 h	>125	>500	>125	500	>125	>500	>125	>125	>125	
	48 h	>125	>500	>125	500	>125	>500	>125	>125	>125	
EF	24 h	>125	>500	>125	>500	>125	>500	>125	>125	>125	
	48 h	>125	>500	>125	>500	>125	>500	>125	>125	>125	
EC	24 h	>125	>500	>125	>500	>125	>500	>125	>125	>125	
	48 h	>125	>500	>125	>500	>125	>500	>125	>125	>125	
KP	24 h	>125	>500	>125	>500	>125	>500	>125	>125	>125	
	48 h	>125	>500	>125	>500	>125	>500	>125	>125	>125	
KP-E	24 h	>125	>500	>125	>500	>125	>500	>125	>125	>125	
	48 h	>125	>500	>125	>500	>125	>500	>125	>125	>125	
PA	72 h	>125	>500	>125	>500	>125	>500	>125	>125	>125	
	120 h	>125	>500	>125	>500	>125	>500	>125	>125	>125	

Strain		Tested compound (lab code / publication code) – MIC (μ M)									
		OV-2A	OV-3	OV-4	OV-13	JZ-AM23	JZ-AM25	JZ-AM27			
		2j	2k	2l	2m	3e	3j	3k			
SA	24 h	62.5	>125	>125	>125	>500	>500	>125			
	48 h	62.5	>125	>125	>125	>500	>500	>125			
MRSA	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
SE	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
EF	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
EC	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
KP	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
KP-E	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
PA	72 h	>500	>125	>125	>125	>500	>500	>125			
	120 h	>500	>125	>125	>125	>500	>500	>125			

Compounds **1h**, **1i**, **2n**, **3a**, **3f**, **3g**, **3k**, **3l**, and **3n** precipitated during the preparation of the basic solution of the compound and therefore the testing was discontinued.

Strain		Tested standards – MIC (μ M)									
		Neom	Bac	Pen	Phen	Cipr					
SA	24 h	3.9	15.62	0.24	0.24	0.98					
	48 h	3.9	31.25	0.24	0.24	0.98					
MRSA	24 h	0.98	15.62	125	250	500					
	48 h	0.98	31.25	125	500	500					
SE	24 h	3.9	15.62	31.25	62.5	250					
	48 h	7.81	31.25	125	250	250					
EF	24 h	250	31.25	7.81	7.81	0.98					
	48 h	250	31.25	15.62	7.81	0.98					
EC	24 h	0.98	>500	125	>500	0.06					
	48 h	0.98	>500	125	>500	0.06					
KP	24 h	0.98	>500	250	>500	0.12					
	48 h	0.98	>500	500	>500	0.12					
KP-E	24 h	0.98	>500	>500	>500	>500					
	48 h	0.98	>500	>500	>500	>500					
PA	72 h	7.81	>500	>500	>500	3.9					
	120 h	15.62	>500	>500	>500	7.81					

Neom – neomycin, Bac – bacitracin, Pen – benzylpenicillin, Phen – phenoxymethylpenicillin, Cipr – ciprofloxacin

1.3. Full results of antifungal evaluation

Tested strains											
1. CA1 - <i>Candida albicans</i> ATCC 44859				5. TA - <i>Trichosporon asahii</i> 1188							
2. CT - <i>Candida tropicalis</i> 156				6. AF - <i>Aspergillus fumigatus</i> 231							
3. CK2 - <i>Candida krusei</i> E28				7. LC - <i>Lichtheimia corymbifera</i> 272							
4. CG - <i>Candida glabrata</i> 20/I				8. TI - <i>Trichophyton interdigitale</i> 445							
Results											
Strain		Tested compound (lab code / publication code) – MIC (μM)									
		OV-6	JZ-AM7	JZ-AM13	JZAM14	JZ-AM6	JZ-AM9	JZ-AM5	JZ-AM3	JZAM10	
		1a	1b	1c-Ac	1c	1d-Ac	1e	1f	1g	1h	
CA	24 h	500	>500	>500	>500	>500	>500	>500	>500	500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	500	
CT	24 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
CK	24 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
CG	24 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
TA	24 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
AF	24 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
LC	24 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
	48 h	>500	>500	>500	>500	>500	>500	>500	>500	>500	
TI	72 h	>500	500	>500	>500	>500	>500	>500	>500	>500	
	120 h	>500	500	>500	>500	>500	>500	>500	>500	>500	

Strain		Tested compound (lab code / publication code) – MIC (μM)								
		JZAM115	JZAM11	JZ-AM4	OV-5	JZ-AM2	JZ-AM8	OV-1	OV-14	OV-17
		1i	1j	1k	1l	1l-SP	1n	2a	2b-Ac	2b
CA	24 h	>500	>500	>125	>125	>125	>125	>500	125	62.5
	48 h	>500	>500	>125	>125	>125	>125	>500	125	62.5
CT	24 h	>500	>500	>125	>125	>125	>125	>500	250	125
	48 h	>500	>500	>125	>125	>125	>125	>500	250	125
CK	24 h	>500	>500	>125	>125	>125	>125	>500	125	125
	48 h	>500	>500	>125	>125	>125	>125	>500	125	125
CG	24 h	>500	>500	>125	>125	>125	>125	>500	250	125
	48 h	>500	>500	>125	>125	>125	>125	>500	250	125
TA	24 h	>500	>500	>125	>125	>125	>125	>500	125	62.5
	48 h	>500	>500	>125	>125	>125	>125	>500	125	125
AF	24 h	>500	>500	>125	>125	>125	>125	>500	250	125
	48 h	>500	>500	>125	>125	>125	>125	>500	250	125
LC	24 h	>500	>500	>125	>125	>125	>125	>500	125	62.5
	48 h	>500	>500	>125	>125	>125	>125	>500	125	125
TI	72 h	>500	>500	>125	>125	>125	>125	>500	250	125
	120 h	>500	>500	>125	>125	>125	>125	>500	250	125

Strain		Tested compound (lab code / publication code) – MIC (μM)								
		OV-16	OV-19	OV-15	OV-18	OV-9	OV-7	OV-8	OV-11	OV-12
		2c-Ac	2c	2d-Ac	2d	2e	2f	2g	2h	2i
CA	24 h	>125	500	>125	500	>125	125	>125	>125	>125
	48 h	>125	>500	>125	>500	>125	500	>125	>125	>125
CT	24 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
	48 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
CK	24 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
	48 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
CG	24 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
	48 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
TA	24 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
	48 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
AF	24 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
	48 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
LC	24 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
	48 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
TI	72 h	>125	>500	>125	>500	>125	>500	>125	>125	>125
	120 h	>125	>500	>125	>500	>125	>500	>125	>125	>125

Strain		Tested compound (lab code / publication code) – MIC (μM)									
		OV-2A	OV-3	OV-4	OV-13	JZ-AM23	JZ-AM25	JZ-AM27			
		2j	2k	2l	2m	3e	3j	3k			
CA	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
CT	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
CK	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
CG	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
TA	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
AF	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
LC	24 h	>500	>125	>125	>125	>500	>500	>125			
	48 h	>500	>125	>125	>125	>500	>500	>125			
TI	72 h	>500	>125	>125	>125	>500	>500	>125			
	120 h	>500	>125	>125	>125	>500	>500	>125			

Compounds **1h**, **1i**, **2n**, **3a**, **3f**, **3g**, **3k**, **3l**, and **3n** precipitated during the preparation of the basic solution of the compound and therefore the testing was discontinued.

Strain		Tested standard drugs (lab code) – MIC (μM)									
		AMPB	NYS	FLU	VOR						
CA	24 h	0.12	0.98	0.24	0.002						
	48 h	0.49	1.95	0.24	0.008						
CT	24 h	1.95	1.95	>500	62.5						
	48 h	1.95	3.9	>500	250						
CK	24 h	1.95	1.95	125	0.49						
	48 h	1.95	3.9	250	1.95						
CG	24 h	0.98	1.95	31.25	0.24						
	48 h	1.95	3.9	250	250						
TA	24 h	0.49	1.95	250	0.98						
	48 h	0.98	1.95	500	7.81						
AF	24 h	1.95	1.95	>500	0.49						
	48 h	1.95	3.9	>500	1.95						
LC	24 h	7.81	15.62	>500	125						
	48 h	7.81	31.25	>500	250						
TI	72 h	1.95	3.9	7.81	0.061						
	120 h	1.95	7.81	125	0.12						

AMPB - amphotericin B, NYS – nystatin, FLU – fluconazole, VOR – voriconazole

2. Experimental

2.1. Analytical data of prepared compounds

In the following interpretation of ^1H NMR spectra, the hydrogens of the benzene core are annotated with non-primed numbers (*e.g.* H2) or non-specifically as ArH. Hydrogens of the pyrazine are annotated with primed numbers (*e.g.* H3').

N-(pyrazin-2-yl)benzamide (**1a**). White solid. Yield: 59%. mp 171.2–172.0 °C. ^1H NMR (500 MHz, CDCl_3) δ 9.73 (d, J = 1.6 Hz, 1H, H3'), 8.78 (bs, 1H, NH), 8.37 (bs, 1H, H5'), 8.22 (bs, 1H, H6'), 7.94 (d, J = 7.5 Hz, 2H, H2, H6), 7.63–7.56 (m, 1H, H4), 7.54–7.48 (m, 2H, H3, H5). ^{13}C NMR (126 MHz, CDCl_3) δ 165.50, 148.29, 141.87, 140.17, 137.20, 133.32, 132.69, 128.93, 127.37. IR (ATR-Ge, cm^{-1}): 3234; 3108; 3063; 2920; 2851; 1676 (C=O, amide); 1531; 1409; 1294; 1263; 1151; 1057; 1014; 844; 802; 705. Anal. calcd. for $\text{C}_{11}\text{H}_9\text{N}_3\text{O}$ (MW 199.21): C, 66.32; H, 4.55; N, 21.09. Found: C, 66.11; H, 4.57; N, 20.83.

2-hydroxy-*N*-(pyrazin-2-yl)benzamide (**1b**). White solid. Yield: 67%. mp 209.9–211.5 °C (lit. 213–216 °C [1]). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.77 (bs, 1H, OH), 11.01 (s, 1H, NH), 9.51 (d, J = 1.4 Hz, 1H, H3'), 8.43 (dd, J = 2.5, 1.5 Hz, 1H, H6'), 8.42 (d, J = 2.6 Hz, 1H, H5'), 8.03 (dd, J = 8.0, 1.8 Hz, 1H, H6), 7.49–7.44 (m, 1H, H4), 7.06 (dd, J = 8.2, 1.1 Hz, 1H, H3), 7.03–6.98 (m, 1H, H5). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 164.47, 156.91, 148.52, 142.99, 140.37, 136.82, 134.46, 130.96, 120.04, 117.84, 117.31. IR (ATR-Ge, cm^{-1}): 3274; 2933; 2566; 1669 (C=O, amide); 1538; 1449; 1417; 1301; 1222; 1149; 1063; 1012; 839; 759; 689. Anal. calcd. for $\text{C}_{11}\text{H}_9\text{N}_3\text{O}_2$ (MW 215.21): C, 61.39; H, 4.22; N, 19.53. Found: C, 61.24; H, 4.12; N, 19.13.

3-(pyrazin-2-ylcarbamoyl)phenyl acetate (**1c-Ac**). White solid. Yield: 60%. mp 168.4–169.5 °C. ^1H NMR (500 MHz, CDCl_3) δ 9.69 (s, 1H, H3'), 8.63 (s, 1H, NH), 8.38 (d, J = 2.6 Hz, 1H, H5'), 8.25 (s, 1H, H6'), 7.78 (d, J = 7.8 Hz, 1H, H6), 7.68 (s, 1H, H2), 7.52 (t, J = 7.9 Hz, 1H, H5), 7.33 (d, J = 7.7 Hz, 1H, H4), 2.33 (s, 3H, CH_3). ^{13}C NMR (126 MHz, CDCl_3) δ 169.12, 164.41, 151.03, 148.11, 142.08, 140.52, 137.22, 134.90, 130.02, 125.98, 124.51, 121.01, 21.04. IR (ATR-Ge, cm^{-1}): 3244; 1768 (C=O, ester); 1683 (C=O, amide); 1541; 1421; 1304; 1271; 1215; 1194; 1016; 845; 716; 676. Anal. calcd. for $\text{C}_{13}\text{H}_{11}\text{N}_3\text{O}_3$ (MW 257.25): C, 60.7; H, 4.31; N, 16.33. Found: C, 60.67; H, 4.22; N, 16.11.

3-hydroxy-*N*-(pyrazin-2-yl)benzamide (**1c**). White solid. Yield: 58%. mp 192.2–193.7 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 10.98 (bs, 1H, NH), 9.78 (bs, 1H, OH), 9.39 (d, J = 1.5 Hz, 1H, H3'), 8.46 (dd, J = 2.6, 1.5 Hz, 1H, H6'), 8.40 (d, J = 2.5 Hz, 1H, H5'), 7.52–7.47 (m, 1H, ArH), 7.40 (t, J = 2.1 Hz, 1H, ArH), 7.32 (t, J = 7.8 Hz, 1H, ArH), 7.03–6.97 (m, 1H, ArH). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 166.37, 157.56, 149.24, 142.75, 140.07, 137.72, 134.98, 129.65, 119.45, 118.87, 115.17. IR (ATR-Ge, cm^{-1}): 3343; 3158; 2851; 2705; 1679 (C=O, amide); 1539; 1515; 1414; 1297; 1253; 1012; 819; 749; 691. Anal. calcd. for $\text{C}_{11}\text{H}_9\text{N}_3\text{O}_2$ (MW 215.21): C, 61.39; H, 4.22; N, 19.53. Found: C, 61.19; H, 4.23; N, 19.12.

4-(pyrazin-2-ylcarbamoyl)phenyl acetate (**1d-Ac**). White solid. Yield: 14%. mp 147.4–149.4 °C (lit. 158 °C [2]). ^1H NMR (500 MHz, CDCl_3) δ 9.70 (d, J = 1.7 Hz, 1H, H3'), 8.70 (bs, 1H, NH), 8.37 (d, J = 2.6 Hz, 1H, H5'), 8.22 (bs, 1H, H6'), 7.96 (d, J = 8.5 Hz, 2H, H2, H6), 7.24 (d, J = 8.5 Hz, 2H, H3, H5), 2.33 (s, 3H, CH_3). ^{13}C NMR (126 MHz, CDCl_3) δ 168.82, 164.60, 153.95, 148.22, 142.02, 140.41, 137.24, 130.86, 128.90, 122.16, 21.10. IR (ATR-Ge, cm^{-1}): 3171; 3107; 3046; 1753 (C=O, ester); 1672

(C=O, amide); 1541; 1504; 1417; 1300; 1209; 1166; 1015; 916; 843; 757; 704. Anal. calcd. for $C_{13}H_{11}N_3O_3$ (MW 257.25): C, 60.7; H, 4.31; N, 16.33. Found: C, 60.23; H, 4.17; N, 15.98.

2-methoxy-*N*-(pyrazin-2-yl)benzamide (**1e**). White solid. Yield: 11%. mp 100.3–102.4 °C. 1H NMR (500 MHz, $CDCl_3$) δ 10.37 (bs, 1H, NH), 9.74 (d, J = 1.5 Hz, 1H, H3'), 8.33 (d, J = 2.5 Hz, 1H, H5'), 8.30–8.25 (m, 2H, H6', H6), 7.56–7.50 (m, 1H, H4), 7.16–7.11 (m, 1H, H5), 7.05 (d, J = 8.4 Hz, 1H, H3), 4.08 (s, 3H, OCH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.36, 157.52, 148.67, 142.09, 139.96, 137.67, 134.12, 132.60, 121.63, 120.49, 111.58, 56.22. IR (ATR-Ge, cm^{-1}): 3335; 3016; 2955; 1673 (C=O, amide); 1533; 1483; 1414; 1297; 1234; 1016; 844; 745; 685; 669. Anal. calcd. for $C_{12}H_{11}N_3O_2$ (MW 229.24): C, 62.87; H, 4.84; N, 18.33. Found: C, 62.82; H, 4.8; N, 17.91.

3-methoxy-*N*-(pyrazin-2-yl)benzamide (**1f**). White solid. Yield: 22%. mp 148.1–150.1 °C. 1H NMR (500 MHz, $CDCl_3$) δ 9.71 (d, J = 1.5 Hz, 1H, H3'), 8.71 (bs, 1H, NH), 8.36 (d, J = 2.6 Hz, 1H, H5'), 8.24–8.20 (m, 1H, H6'), 7.50–7.43 (m, 2H, ArH), 7.40 (t, J = 7.9 Hz, 1H, ArH), 7.12 (dd, J = 8.7, 2.7 Hz, 1H, ArH), 3.86 (s, 3H, OCH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.35, 160.04, 148.26, 142.02, 140.33, 137.24, 134.79, 129.90, 119.05, 118.88, 112.64, 55.48. IR (ATR-Ge, cm^{-1}): 3171; 3110; 3003; 2976; 1673 (C=O, amide); 1544; 1417; 1300; 1277; 1235; 1033; 1017; 797; 740. Anal. calcd. for $C_{12}H_{11}N_3O_2$ (MW 229.24): C, 62.87; H, 4.84; N, 18.33. Found: C, 63.08; H, 4.83; N, 18.01.

4-methoxy-*N*-(pyrazin-2-yl)benzamide (**1g**). White solid. Yield: 14%. mp 156.0–156.6 °C (lit. 157–159 [3]). 1H NMR (300 MHz, $CDCl_3$) δ 9.70 (d, J = 1.5 Hz, 1H, H3'), 8.68 (bs, 1H, NH), 8.34 (d, J = 2.5 Hz, 1H, H5'), 8.23–8.19 (m, 1H, H6'), 7.90 (d, J = 8.4 Hz, 2H, H2, H6), 6.98 (d, J = 8.4 Hz, 2H, H3, H5), 3.87 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.95, 163.09, 148.49, 141.94, 140.02, 137.24, 129.36, 125.42, 114.10, 55.48. IR (ATR-Ge, cm^{-1}): 3304; 3116; 2927; 2844; 1663 (C=O, amide); 1508; 1414; 1299; 1249; 1173; 1026; 1012; 835; 759; 656. Anal. calcd. for $C_{12}H_{11}N_3O_2$ (MW 229.24): C, 62.87; H, 4.84; N, 18.33. Found: C, 62.58; H, 4.83; N, 17.95.

4-methyl-*N*-(pyrazin-2-yl)benzamide (**1h**). White solid. Yield: 29%. mp 163.3–166.0 °C (lit. [4]). 1H NMR (500 MHz, $CDCl_3$) δ 9.71 (d, J = 1.7 Hz, 1H, H3'), 8.66 (bs, 1H, NH), 8.35 (d, J = 2.6 Hz, 1H, H5'), 8.23 (dd, J = 2.5, 1.6 Hz, 1H, H6'), 7.83 (d, J = 8.2 Hz, 2H, H2, H6), 7.30 (d, J = 7.9 Hz, 2H, H3, H5), 2.43 (s, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.39, 148.40, 143.42, 141.97, 140.15, 137.23, 130.47, 129.59, 127.37, 21.54. IR (ATR-Ge, cm^{-1}): 3245; 3109; 3063; 1676 (C=O, amide); 1529; 1508; 1412; 1298; 1259; 1186; 1057; 1012; 847; 723; 685. Anal. calcd. for $C_{12}H_{11}N_3O$ (MW 213.24): C, 67.59; H, 5.2; N, 19.71. Found: C, 67.37; H, 5.1; N, 19.59.

4-ethyl-*N*-(pyrazin-2-yl)benzamide (**1i**). White solid. Yield: 15%. mp 121.2–122.3 °C. 1H NMR (500 MHz, $CDCl_3$) δ 9.72 (d, J = 1.5 Hz, 1H, H3'), 8.67 (bs, 1H, NH), 8.35 (d, J = 2.6 Hz, 1H, H5'), 8.22 (dd, J = 2.6, 1.6 Hz, 1H, H6'), 7.85 (d, J = 8.3 Hz, 2H, H2, H6), 7.33 (d, J = 8.2 Hz, 2H, H3, H5), 2.72 (q, J = 7.6 Hz, 2H, CH_2), 1.27 (t, J = 7.6 Hz, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.42, 149.58, 148.41, 141.98, 140.17, 137.25, 130.70, 128.41, 127.47, 28.82, 15.17. IR (ATR-Ge, cm^{-1}): 3239; 3107; 3049; 2959; 2933; 2875; 1685 (C=O, amide); 1534; 1509; 1411; 1299; 1269; 1014; 840; 734; 695. Anal. calcd. for $C_{13}H_{13}N_3O$ (MW 227.27): C, 68.7; H, 5.77; N, 18.49. Found: C, 68.89; H, 5.72; N, 18.4.

2-chloro-*N*-(pyrazin-2-yl)benzamide (**1j**). White solid. Yield: 15%. mp 128.2–129.7 °C (lit. 136–138 °C [4]). 1H NMR (500 MHz, $CDCl_3$) δ 9.68 (s, 1H, H3'), 9.07 (bs, 1H, NH), 8.34 (d, J = 2.6 Hz, 1H, H5'), 8.05 (dd, J = 2.6, 1.6 Hz, 1H, H6'), 7.78–7.74 (m, 1H, ArH), 7.47–7.45 (m, 2H, ArH), 7.42–7.37 (m, 1H, ArH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.65, 147.93, 141.91, 140.62, 137.29, 134.06, 132.31,

130.93, 130.58, 130.28, 127.37. IR (ATR-Ge, cm^{-1}): 3306; 3198; 3100; 2971; 1695 (C=O, amide); 1530; 1412; 1299; 1253; 1151; 1048; 1015; 852; 774; 721. Anal. calcd. for $\text{C}_{11}\text{H}_8\text{ClN}_3\text{O}$ (MW 233.66): C, 56.55; H, 3.45; N, 17.98. Found: C, 56.86; H, 3.45; N, 17.51.

3-chloro-*N*-(pyrazin-2-yl)benzamide (**1k**). White solid. Yield: 35%. mp 147.2–149.4 °C. ^1H NMR (500 MHz, CDCl_3) δ 9.69 (d, $J = 1.7$ Hz, 1H, $\text{H}_{3'}$), 8.71 (bs, 1H, NH), 8.38 (d, $J = 2.6$ Hz, 1H, $\text{H}_{5'}$), 8.25 (bs, 1H, $\text{H}_{6'}$), 7.93 (s, 1H, H2), 7.80 (d, $J = 7.7$ Hz, 1H, H6), 7.56 (d, $J = 7.8$ Hz, 1H, H4), 7.45 (t, $J = 7.9$ Hz, 1H, H5). ^{13}C NMR (126 MHz, CDCl_3) δ 164.18, 148.04, 142.08, 140.62, 137.26, 135.21, 135.14, 132.66, 130.22, 127.78, 125.33. IR (ATR-Ge, cm^{-1}): 3178; 3107; 3069; 1680 (C=O, amide); 1545; 1414; 1305; 1262; 1058; 1018; 846; 765; 725. Anal. calcd. for $\text{C}_{11}\text{H}_8\text{ClN}_3\text{O}$ (MW 233.66): C, 56.55; H, 3.45; N, 17.98. Found: C, 56.16; H, 3.44; N, 17.87.

4-chloro-*N*-(pyrazin-2-yl)benzamide (**1l**). White solid. Yield: 11%. mp 182.6–184.3 °C. ^1H NMR (500 MHz, CDCl_3) δ 9.71 (s, 1H, $\text{H}_{3'}$), 8.69 (bs, 1H, NH), 8.40 (d, $J = 2.8$ Hz, 1H, $\text{H}_{5'}$), 8.27 (s, 1H, $\text{H}_{6'}$), 7.89 (d, $J = 8.3$ Hz, 2H, H2, H6), 7.49 (d, $J = 8.2$ Hz, 2H, H3, H5). ^{13}C NMR (126 MHz, CDCl_3) δ 164.38, 148.02, 141.70, 140.30, 139.22, 137.21, 131.58, 129.28, 128.82. IR (ATR-Ge, cm^{-1}): 3330; 3067; 1655 (C=O, amide); 1540; 1488; 1408; 1302; 1262; 1149; 1009; 846; 754; 662. Anal. calcd. for $\text{C}_{11}\text{H}_8\text{ClN}_3\text{O}$ (MW 233.66): C, 56.55; H, 3.45; N, 17.98. Found: C, 56.48; H, 3.43; N, 17.7.

4-chloro-*N*-(4-chlorobenzoyl)-*N*-(pyrazin-2-yl)benzamide (**1l-SP**). White solid. Yield: 61%. mp 153.1–153.8 °C. ^1H NMR (500 MHz, CDCl_3) δ 8.63 (d, $J = 1.5$ Hz, 1H, $\text{H}_{3'}$), 8.45 (d, $J = 2.6$ Hz, 1H, $\text{H}_{5'}$), 8.35 (dd, $J = 2.4, 1.5$ Hz, 1H, $\text{H}_{6'}$), 7.72–7.63 (m, 4H, ArH), 7.38–7.33 (m, 4H, ArH). IR (ATR-Ge, cm^{-1}): 1703 (C=O, amide, široký); 1590; 1403; 1269; 1240; 1113; 1089; 1011; 920; 872; 845; 754. Anal. calcd. for $\text{C}_{18}\text{H}_{11}\text{Cl}_2\text{N}_3\text{O}_2$ (MW 372.21): C, 58.09; H, 2.98; N, 11.29. Found: C, 58.5; H, 2.91; N, 11.15.

N-(pyrazin-2-yl)-3-(trifluoromethyl)benzamide (**1n**). White solid. Yield: 21%. mp 160.9–161.6 °C (lit. 159–161 °C [5]). ^1H NMR (500 MHz, CDCl_3) δ 9.70 (d, $J = 1.6$ Hz, 1H, $\text{H}_{3'}$), 8.80 (bs, 1H, NH), 8.40 (d, $J = 2.6$ Hz, 1H, $\text{H}_{5'}$), 8.28–8.25 (m, 1H, $\text{H}_{6'}$), 8.21 (s, 1H, H2), 8.13 (d, $J = 7.8$ Hz, 1H, ArH), 7.85 (d, $J = 7.7$ Hz, 1H, ArH), 7.67 (t, $J = 7.8$ Hz, 1H, H5). ^{13}C NMR (126 MHz, CDCl_3) δ 164.10, 147.98, 142.11, 140.74, 137.29, 134.26, 131.59 (q, $J = 33.1$ Hz), 130.54, 129.63, 129.19 (q, $J = 3.6$ Hz), 124.49 (q, $J = 3.8$ Hz), 123.46 (q, $J = 272.7$ Hz). IR (ATR-Ge, cm^{-1}): 3177; 3107; 1681 (C=O, amide); 1548; 1419; 1337; 1305; 1257; 1167; 1125; 1074; 1016; 740; 694. Anal. calcd. for $\text{C}_{12}\text{H}_8\text{F}_3\text{N}_3\text{O}$ (MW 267.21): C, 53.94; H, 3.02; N, 15.73. Found: C, 54.02; H, 2.83; N, 15.49.

N-(5-chloropyrazin-2-yl)benzamide (**2a**). White solid. Yield: 48%. mp 162.5–163.5 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.31 (s, 1H, NH), 9.26 (d, $J = 1.4$ Hz, 1H, $\text{H}_{3'}$), 8.62 (d, $J = 1.5$ Hz, 1H, $\text{H}_{6'}$), 8.08–8.01 (m, 2H, H2, H6), 7.66–7.59 (m, 1H, H4), 7.56–7.50 (m, 2H, H3, H5). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 166.37, 148.26, 142.26, 141.96, 136.46, 133.32, 132.58, 128.61, 128.40. IR (ATR-Ge, cm^{-1}): 3342; 1655 (C=O, amide); 1541; 1511; 1484; 1340; 1249; 1131; 1103; 1020; 710; 690; 661. Anal. calcd. for $\text{C}_{11}\text{H}_8\text{ClN}_3\text{O}$ (MW 233.66): C, 56.55; H, 3.45; N, 17.98. Found: C, 56.89; H, 3.23; N, 18.22.

N-(5-chloropyrazin-2-yl)-2-hydroxybenzamide (**2b**). White solid. Yield: 37%. mp 197.7–198.2 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.78 (s, 1H, OH), 11.12 (s, 1H, NH), 9.31 (d, $J = 1.6$ Hz, 1H, $\text{H}_{3'}$), 8.56 (d, $J = 1.4$ Hz, 1H, $\text{H}_{6'}$), 8.01 (dd, $J = 8.0, 1.7$ Hz, 1H, H6), 7.51–7.42 (m, 1H, H4), 7.05 (d, $J = 8.2$ Hz, 1H, H3), 7.00 (t, $J = 7.4$ Hz, 1H, H5). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 164.40, 156.85, 147.47, 142.49, 141.99, 135.59, 134.56, 130.93, 120.05, 117.68, 117.29. IR (ATR-Ge, cm^{-1}): 3383; 3034; 1651

(C=O, amide); 1538; 1515; 1490; 1433; 1339; 1197; 1170; 1136; 1013; 819; 751; 708. Anal. calcd. for C₁₁H₈ClN₃O₂ (MW 249.65): C, 52.92; H, 3.23; N, 16.83. Found: C, 53.2; H, 3.36; N, 16.855.

2-((5-chloropyrazin-2-yl)carbamoyl)phenyl acetate (**2b-Ac**). White solid. Yield: 53%. mp 116.3–117.0 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.35 (s, 1H, NH), 9.18 (d, *J* = 1.5 Hz, 1H, H3'), 8.61 (d, *J* = 1.4 Hz, 1H, H6'), 7.75 (dd, *J* = 7.7, 1.6 Hz, 1H, ArH), 7.65–7.57 (m, 1H, ArH), 7.42–7.38 (m, 1H, ArH), 7.27 (dd, *J* = 8.1, 1.1 Hz, 1H, ArH), 2.22 (s, 3H, CH₃). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 169.02, 165.09, 148.41, 147.90, 142.45, 142.11, 135.98, 132.51, 129.77, 128.09, 125.96, 123.45, 20.87. IR (ATR-Ge, cm⁻¹): 3404; 1766 (C=O, ester); 1685 (C=O, amide); 1534; 1501; 1343; 1192; 1159; 1136; 1017; 916; 748; 682. Anal. calcd. for C₁₃H₁₀ClN₃O₃ (MW 291.69): C, 53.53; H, 3.46; N, 14.41. Found: C, 53.385; H, 3.38; N, 14.39.

N-(5-chloropyrazin-2-yl)-3-hydroxybenzamide (**2c**). White solid. Yield: 48%. mp 190.1–190.8 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.19 (s, 1H, NH), 9.79 (s, 1H, OH), 9.23 (d, *J* = 1.4 Hz, 1H, H3'), 8.61 (d, *J* = 1.4 Hz, 1H, H6'), 7.50–7.45 (m, 1H, ArH), 7.39 (t, *J* = 2.0 Hz, 1H, ArH), 7.32 (t, *J* = 7.9 Hz, 1H, ArH), 7.03–6.99 (m, 1H, ArH). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 166.40, 157.56, 148.25, 142.25, 141.92, 136.50, 134.72, 129.68, 119.58, 118.93, 115.19. IR (ATR-Ge, cm⁻¹): 3322; 1651 (C=O, amide); 1595; 1540; 1494; 1446; 1340; 1296; 1231; 1135; 1030; 827; 804; 750; 712; 684. Anal. calcd. for C₁₁H₈ClN₃O₂ (MW 249.65): C, 52.92; H, 3.23; N, 16.83. Found: C, 53.15; H, 3.235; N, 16.885.

3-((5-chloropyrazin-2-yl)carbamoyl)phenyl acetate (**2c-Ac**). White solid. Yield: 48%. mp 184.1–184.6 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.38 (s, 1H, NH), 9.24 (d, *J* = 1.5 Hz, 1H, H3'), 8.63 (d, *J* = 1.4 Hz, 1H, H6'), 7.95 (d, *J* = 7.8 Hz, 1H, H6), 7.82 (t, *J* = 2.0 Hz, 1H, H2), 7.58 (t, *J* = 7.9 Hz, 1H, H5), 7.43–7.38 (m, 1H, H4), 2.31 (s, 3H, CH₃). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 169.31, 165.36, 150.60, 148.10, 142.32, 142.13, 136.51, 134.70, 129.83, 126.18, 125.81, 121.95, 21.00. IR (ATR-Ge, cm⁻¹): 3357; 3126; 3080; 1758 (C=O, ester); 1668 (C=O, amide); 1542; 1510; 1483; 1351; 1211; 1135; 1020; 822; 746; 690; 661. Anal. calcd. for C₁₃H₁₀ClN₃O₃ (MW 291.69): C, 53.53; H, 3.46; N, 14.41. Found: C, 53.55; H, 3.55; N, 14.455.

N-(5-chloropyrazin-2-yl)-4-hydroxybenzamide (**2d**). White solid. Yield: 36%. mp 210.9–212.6 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.02 (s, 1H, NH), 10.26 (bs, 1H, OH), 9.23 (s, 1H, H3'), 8.59 (s, 1H, H6'), 7.95 (d, *J* = 8.4 Hz, 2H, H2, H6), 6.86 (d, *J* = 8.4 Hz, 2H, H3, H5). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 165.77, 161.57, 148.55, 142.12, 141.53, 136.39, 130.69, 123.68, 115.22. IR (ATR-Ge, cm⁻¹): 3106; 1644 (C=O, amide); 1588; 1532; 1505; 1434; 1336; 1270; 1235; 1139; 1018; 911; 843; 738; 710. Anal. calcd. for C₁₁H₈ClN₃O₂ (MW 249.65): C, 52.92; H, 3.23; N, 16.83. Found: C, 53.025; H, 3.445; N, 16.77.

4-((5-chloropyrazin-2-yl)carbamoyl)phenyl acetate (**2d-Ac**). White solid. Yield: 55%. mp 170.9–171.8 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.36 (s, 1H, NH), 9.25 (d, *J* = 1.4 Hz, 1H, H3'), 8.63 (d, *J* = 1.5 Hz, 1H, H6'), 8.09 (d, *J* = 8.7 Hz, 2H, H2, H6), 7.30 (d, *J* = 8.7 Hz, 2H, H3, H5), 2.31 (s, 3H, CH₃). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 169.07, 165.60, 153.73, 148.23, 142.27, 141.99, 136.42, 130.83, 130.05, 122.09, 21.07. IR (ATR-Ge, cm⁻¹): 3396; 3090; 1761 (C=O, ester); 1683 (C=O, amide); 1535; 1497; 1348; 1171; 1136; 1016; 911; 854; 755. Anal. calcd. for C₁₃H₁₀ClN₃O₃ (MW 291.69): C, 53.53; H, 3.46; N, 14.41. Found: C, 53.92; H, 3.68; N, 14.23.

N-(5-chloropyrazin-2-yl)-2-methoxybenzamide (**2e**). White solid. Yield: 75%. mp 141.2–142.0 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.80 (s, 1H, NH), 9.30 (d, *J* = 1.4 Hz, 1H, H3'), 8.57 (d, *J* = 1.4 Hz, 1H, H6'), 7.83 (dd, *J* = 7.6, 1.8 Hz, 1H, ArH), 7.65–7.51 (m, 1H, ArH), 7.24 (d, *J* = 8.4 Hz, 1H, ArH), 7.15–7.07 (m, 1H, ArH), 3.96 (s, 3H, OCH₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 164.47, 157.26, 147.55,

142.49, 141.99, 135.46, 133.82, 130.75, 121.96, 121.05, 112.58, 56.45. IR (ATR-Ge, cm^{-1}): 3309; 1677 (C=O, amide); 1601; 1531; 1511; 1484; 1344; 1293; 1227; 1116; 1013; 748; 680. Anal. calcd. for $\text{C}_{12}\text{H}_{10}\text{ClN}_3\text{O}_2$ (MW 263.68): C, 54.66; H, 3.82; N, 15.94. Found: C, 54.48; H, 3.77; N, 15.75.

N-(5-chloropyrazin-2-yl)-3-methoxybenzamide (**2f**). White solid. Yield: 79%. mp 124.2–125.7 °C. ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 11.31 (s, 1H, NH), 9.26 (d, J = 1.4 Hz, 1H, H3'), 8.63 (d, J = 1.4 Hz, 1H, H6'), 7.66–7.56 (m, 2H, ArH), 7.44 (t, J = 7.9 Hz, 1H, ArH), 7.18 (dd, J = 8.2, 2.6 Hz, 1H, ArH), 3.84 (s, 3H, OCH_3). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 166.03, 159.31, 148.22, 142.24, 141.98, 136.48, 134.61, 129.76, 120.70, 118.80, 113.12, 55.55. IR (ATR-Ge, cm^{-1}): 3379; 3125; 3100; 2939; 2836; 1680 (C=O, amide); 1585; 1530; 1513; 1484; 1429 1348; 1287; 1272; 1257; 1143; 1048; 1017; 809; 735; 677. Anal. calcd. for $\text{C}_{12}\text{H}_{10}\text{ClN}_3\text{O}_2$ (MW 263.68): C, 54.66; H, 3.82; N, 15.94. Found: C, 54.65; H, 3.825; N, 15.735.

N-(5-chloropyrazin-2-yl)-4-methoxybenzamide (**2g**). White solid. Yield: 83%. mp 177.8–179.0 °C. ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 11.14 (s, 1H, NH), 9.24 (d, J = 1.5 Hz, 1H, H3'), 8.60 (s, 1H, H6'), 8.06 (d, J = 8.8 Hz, 2H, H2, H6), 7.05 (d, J = 8.8 Hz, 2H, H3, H5), 3.84 (s, 3H, OCH_3). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 165.60, 162.74, 148.44, 142.12, 141.66, 136.41, 130.50, 125.25, 113.87, 55.66. IR (ATR-Ge, cm^{-1}): 3384; 3020; 2937; 2841; 1664 (C=O, amide); 1607; 1578; 1543 1495; 1441 1263; 1239; 1188; 1137; 1093; 1026; 1015; 867; 852; 760. Anal. calcd. for $\text{C}_{12}\text{H}_{10}\text{ClN}_3\text{O}_2$ (MW 263.68): C, 54.66; H, 3.82; N, 15.94. Found: C, 54.655; H, 3.855; N, 15.695.

N-(5-chloropyrazin-2-yl)-4-methylbenzamide (**2h**). White solid. Yield: 77%. mp 186.4–187.1 °C. ^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ 11.23 (s, 1H, NH), 9.25 (d, J = 1.5 Hz, 1H, H3'), 8.61 (d, J = 1.4 Hz, 1H, H6'), 7.96 (d, J = 8.1 Hz, 2H, H2, H6), 7.33 (d, J = 7.9 Hz, 2H, H3, H5), 2.38 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$) δ 166.17, 148.35, 142.83, 142.23, 141.84, 136.44, 130.45, 129.18, 128.48, 21.26. IR (ATR-Ge, cm^{-1}): 3336; 2921; 2856; 1654 (C=O, amide); 1541; 1497; 1442; 1340; 1283; 1251; 1137; 1100; 1065; 1019; 869; 837; 748; 709; 692. Anal. calcd. for $\text{C}_{12}\text{H}_{10}\text{ClN}_3\text{O}$ (MW 247.68): C, 58.19; H, 4.07; N, 16.97. Found: C, 58.23; H, 4.13; N, 16.675.

N-(5-chloropyrazin-2-yl)-4-ethylbenzamide (**2i**). White solid. Yield: 85%. mp 150.0–150.8 °C. ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 11.22 (s, 1H, NH), 9.26 (d, J = 1.5 Hz, 1H, H3'), 8.61 (d, J = 1.4 Hz, 1H, H6'), 7.98 (d, J = 8.3 Hz, 2H, H2, H6), 7.36 (d, J = 8.1 Hz, 2H, H3, H5), 2.68 (q, J = 7.6 Hz, 2H, CH_2), 1.21 (t, J = 7.6 Hz, 3H, CH_3). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 166.18, 148.88, 148.32, 142.20, 141.82, 136.41, 130.72, 128.55, 127.99, 28.27, 15.39. IR (ATR-Ge, cm^{-1}): 3356; 2972; 2928; 1670 (C=O, amide); 1539; 1493; 1339; 1247; 1132; 1104; 1015; 905; 869; 846. Anal. calcd. for $\text{C}_{13}\text{H}_{12}\text{ClN}_3\text{O}$ (MW 261.71): C, 59.66; H, 4.62; N, 16.06. Found: C, 59.46; H, 4.61; N, 15.8.

2-chloro-*N*-(5-chloropyrazin-2-yl)benzamide (**2j**). White solid. Yield: 68%. mp 158.9–159.7 °C. ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 11.58 (s, 1H, NH), 9.25 (d, J = 1.5 Hz, 1H, H3'), 8.60 (d, J = 1.4 Hz, 1H, H6'), 7.62 (dd, J = 7.5, 1.7 Hz, 1H, ArH), 7.59–7.50 (m, 2H, ArH), 7.46 (td, J = 7.4, 1.4 Hz, 1H, ArH). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 166.01, 147.69, 142.56, 142.29, 135.69, 131.78, 130.15, 129.83, 129.34, 127.34. IR (ATR-Ge, cm^{-1}): 3208; 3064; 1687 (C=O, amide); 1541; 1496; 1433; 1335; 1293; 1258; 1122; 1020; 774; 745; 684; 657. Anal. calcd. for $\text{C}_{11}\text{H}_7\text{Cl}_2\text{N}_3\text{O}$ (MW 268.1): C, 49.28; H, 2.63; N, 15.67. Found: C, 49.53; H, 2.63; N, 15.47.

3-chloro-*N*-(5-chloropyrazin-2-yl)benzamide (**2k**). White solid. Yield: 54%. mp 150.7–151.9 °C. ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 11.44 (s, 1H, NH), 9.23 (d, J = 1.4 Hz, 1H, H3'), 8.63 (d, J = 1.4 Hz, 1H, H6'), 8.08 (t, J = 1.9 Hz, 1H, H2), 8.01–7.95 (m, 1H, H6), 7.71–7.66 (m, 1H, H4), 7.56 (t, J = 7.9 Hz, 1H, H5). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 165.00, 148.02, 142.32, 142.18, 136.43, 135.31, 133.41,

132.32, 130.55, 128.21, 127.13. IR (ATR-Ge, cm^{-1}): 3366; 3074; 2925; 1672 (C=O, amide); 1541; 1506; 1473; 1345; 1289; 1243; 1136; 1018; 763; 741; 692; 677. Anal. calcd. for $\text{C}_{11}\text{H}_7\text{Cl}_2\text{N}_3\text{O}$ (MW 268.1): C, 49.28; H, 2.63; N, 15.67. Found: C, 49.29; H, 2.71; N, 15.375.

4-chloro-*N*-(5-chloropyrazin-2-yl)benzamide (**2l**). White solid. Yield: 8%. mp 225.0–226.3 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.41 (s, 1H, NH), 9.24 (d, J = 1.5 Hz, 1H, H3'), 8.63 (d, J = 1.6 Hz, 1H, H6'), 8.05 (d, J = 8.5 Hz, 2H, H2, H6), 7.60 (d, J = 8.5 Hz, 2H, H3, H5). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 165.37, 148.11, 142.29, 142.09, 137.47, 136.44, 132.09, 130.36, 128.69. IR (ATR-Ge, cm^{-1}): 3374; 3127; 3101; 1679 (C=O, amide); 1530; 1515; 1480; 1440; 1344; 1295; 1249; 1136; 1097; 1016; 840; 746. Anal. calcd. for $\text{C}_{11}\text{H}_7\text{Cl}_2\text{N}_3\text{O}$ (MW 268.1): C, 49.28; H, 2.63; N, 15.67. Found: C, 49.44; H, 2.565; N, 15.585.

4-bromo-*N*-(5-chloropyrazin-2-yl)benzamide (**2m**). White solid. Yield: 43%. mp 229.2–230.5 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.42 (s, 1H, NH), 9.24 (d, J = 1.4 Hz, 1H, H3'), 8.63 (d, J = 1.4 Hz, 1H, H6'), 7.97 (d, J = 8.6 Hz, 2H, H2, H6), 7.74 (d, J = 8.5 Hz, 2H, H3, H5). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 165.52, 148.11, 142.31, 142.10, 136.45, 132.47, 131.65, 130.51, 126.51. IR (ATR-Ge, cm^{-1}): 3313; 1650 (C=O, amide); 1544; 1514; 1484; 1335; 1287; 1139; 1105; 1072; 1021; 1007; 868; 848; 658. Anal. calcd. for $\text{C}_{11}\text{H}_7\text{BrClN}_3\text{O}$ (MW 312.55): C, 42.27; H, 2.26; N, 13.44. Found: C, 42.23; H, 2.27; N, 13.29.

N-(5-chloropyrazin-2-yl)-3-(trifluoromethyl)benzamide (**2n**). White solid. Yield: 65%. mp 155.4–156.4 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.62 (s, 1H, NH), 9.26 (d, J = 1.4 Hz, 1H, H3'), 8.64 (d, J = 1.3 Hz, 1H, H6'), 8.40 (bs, 1H, H2), 8.31 (d, J = 8.2 Hz, 1H, H6), 7.98 (d, J = 7.8 Hz, 1H, H4), 7.78 (t, J = 7.8 Hz, 1H, H5). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 165.00, 148.04, 142.34, 142.24, 136.45, 134.27, 132.52, 129.90, 129.35 (q, J = 32.1 Hz), 129.02 (q, J = 3.8 Hz), 125.13 (q, J = 3.9 Hz), 124.05 (q, J = 272.6 Hz). IR (ATR-Ge, cm^{-1}): 3367; 1679 (C=O, amide); 1541; 1512; 1454; 1329; 1237; 1181; 1131; 1073; 1018; 916; 791; 750; 720; 694; 669. Anal. calcd. for $\text{C}_{12}\text{H}_7\text{ClF}_3\text{N}_3\text{O}$ (MW 301.65): C, 47.78; H, 2.34; N, 13.93. Found: C, 48.23; H, 2.25; N, 13.78.

N-(6-chloropyrazin-2-yl)benzamide (**3a**). White to pale beige solid. Yield: 28%. mp 152.8–153.6 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.41 (bs, 1H, NH), 9.42 (s, 1H, H3'), 8.53 (s, 1H, H5'), 8.07–8.03 (m, 2H, H2, H6), 7.66–7.60 (m, 1H, H4), 7.53 (m, 2H, H3, H5). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 166.39, 148.65, 145.58, 138.64, 135.30, 133.12, 132.70, 128.61, 128.48. IR (ATR-Ge, cm^{-1}): 3209; 3066; 1686 (C=O, amide); 1542; 1398; 1292; 1267; 1164; 1008; 882. Anal. calcd. for $\text{C}_{11}\text{H}_8\text{ClN}_3\text{O}$ (MW 233.66): C, 56.55; H, 3.45; N, 17.98. Found: C, 56.59; H, 3.42; N, 17.85.

N-(6-chloropyrazin-2-yl)-2-methoxybenzamide (**3e**). White to pale beige solid. Yield: 20%. mp 149.1–150.7 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 10.86 (bs, 1H, NH), 9.44 (s, 1H, H3'), 8.53 (s, 1H, H5'), 7.78 (dd, J = 7.7, 1.7 Hz, 1H, H6), 7.62–7.54 (m, 1H, H4), 7.23 (d, J = 8.4 Hz, 1H, H3), 7.10 (t, J = 7.5 Hz, 1H, H5), 3.95 (s, 3H, OCH_3). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 164.88, 157.23, 147.92, 145.69, 138.82, 134.50, 133.69, 130.57, 122.32, 120.91, 112.50, 56.39. IR (ATR-Ge, cm^{-1}): 3320; 3071; 2951; 1676 (C=O, amide); 1526; 1483; 1395; 1282; 1152; 1012; 1001; 756; 673. Anal. calcd. for $\text{C}_{12}\text{H}_{10}\text{ClN}_3\text{O}_2$ (MW 263.68): C, 54.66; H, 3.82; N, 15.94. Found: C, 54.28; H, 3.64; N, 15.61.

N-(6-chloropyrazin-2-yl)-3-methoxybenzamide (**3f**). White to pale beige solid. Yield: 27%. mp 131.6–132.7 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.41 (s, 1H, NH), 9.42 (s, 1H, H3'), 8.53 (s, 1H, H5'), 7.67–7.58 (m, 2H, ArH), 7.44 (t, J = 7.9 Hz, 1H, ArH), 7.18 (dd, J = 8.2, 2.5 Hz, 1H, ArH), 3.84 (s, 3H, OCH_3). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 166.07, 159.31, 148.62, 145.57, 138.67, 135.33,

134.41, 129.76, 120.80, 119.03, 113.09, 55.56. IR (ATR-Ge, cm^{-1}): 3228; 3072; 2947; 2836; 1686 (C=O, amide); 1541; 1488; 1415; 1398; 1283; 1160; 1047; 1007; 733; 704; 680. Anal. calcd. for $\text{C}_{12}\text{H}_{10}\text{ClN}_3\text{O}_2$ (MW 263.68): C, 54.66; H, 3.82; N, 15.94. Found: C, 54.36; H, 3.72; N, 15.7.

N-(6-chloropyrazin-2-yl)-4-methoxybenzamide (**3g**). White to pale beige solid. Yield: 8%. mp 196.9-198.8 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.25 (bs, 1H, NH), 9.41 (s, 1H, H3'), 8.51 (s, 1H, H5'), 8.07 (d, J = 8.8 Hz, 2H, H2, H6), 7.06 (d, J = 8.9 Hz, 2H, H3, H5), 3.85 (s, 3H, OCH_3). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 165.64, 162.85, 148.86, 145.52, 138.30, 135.29, 130.60, 125.08, 113.90, 55.69. IR (ATR-Ge, cm^{-1}): 3065; 2922; 2843; 1679 (C=O, amide); 1550; 1509; 1416; 1399; 1260; 1187; 1164; 1008; 838; 738; 694. Anal. calcd. for $\text{C}_{12}\text{H}_{10}\text{ClN}_3\text{O}_2$ (MW 263.68): C, 54.66; H, 3.82; N, 15.94. Found: C, 54.83; H, 3.6; N, 15.79.

N-(6-chloropyrazin-2-yl)-4-methylbenzamide (**3h**). White to pale beige solid. Yield: 29%. mp 169.1-170.0 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.32 (bs, 1H, NH), 9.41 (s, 1H, H3'), 8.51 (s, 1H, H5'), 7.97 (d, J = 8.2 Hz, 2H, H2, H6), 7.33 (d, J = 7.9 Hz, 2H, H3, H5), 2.38 (s, 3H, CH_3). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 166.19, 148.73, 145.55, 142.95, 138.48, 135.29, 130.27, 129.16, 128.53, 21.24. IR (ATR-Ge, cm^{-1}): 3205; 3063; 1684 (C=O, amide); 1541; 1398; 1293; 1271; 1159; 1106; 1008; 831; 736; 689. Anal. calcd. for $\text{C}_{12}\text{H}_{10}\text{ClN}_3\text{O}$ (MW 247.68): C, 58.19; H, 4.07; N, 16.97. Found: C, 57.89; H, 3.99; N, 16.53.

N-(6-chloropyrazin-2-yl)-4-ethylbenzamide (**3i**). White to pale beige solid. Yield: 26%. mp 142.5-143.3 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.33 (bs, 1H, NH), 9.42 (s, 1H, H3'), 8.52 (s, 1H, H5'), 7.99 (d, J = 8.3 Hz, 2H, H2, H6), 7.37 (d, J = 8.3 Hz, 2H, H3, H5), 2.69 (q, J = 7.6 Hz, 2H, CH_2), 1.21 (t, J = 7.6 Hz, 3H, CH_3). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 166.21, 149.04, 148.73, 145.55, 138.50, 135.29, 130.53, 128.63, 128.00, 28.27, 15.36. IR (ATR-Ge, cm^{-1}): 3203; 3063; 2969; 2882; 1682 (C=O, amide); 1545; 1398; 1293; 1271; 1152; 1008; 845; 726. Anal. calcd. for $\text{C}_{13}\text{H}_{12}\text{ClN}_3\text{O}$ (MW 261.71): C, 59.66; H, 4.62; N, 16.06. Found: C, 59.22; H, 4.49; N, 15.74.

2-chloro-*N*-(6-chloropyrazin-2-yl)benzamide (**3j**). White to pale beige solid. Yield: 23%. mp 182.4-183.4 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.67 (s, 1H, NH), 9.40 (s, 1H, H3'), 8.56 (s, 1H, H5'), 7.62 (dd, J = 7.5, 1.8 Hz, 1H, ArH), 7.59-7.50 (m, 2H, ArH), 7.49-7.42 (m, 1H, ArH). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 166.07, 147.98, 145.76, 139.14, 135.46, 134.59, 131.88, 130.17, 129.84, 129.37, 127.32. IR (ATR-Ge, cm^{-1}): 3145; 3008; 2928; 1699 (C=O, amide); 1537; 1417; 1401; 1287; 1152; 1124; 1005; 873; 745. Anal. calcd. for $\text{C}_{11}\text{H}_7\text{Cl}_2\text{N}_3\text{O}$ (MW 268.1): C, 49.28; H, 2.63; N, 15.67. Found: C, 49.1; H, 2.49; N, 15.37.

3-chloro-*N*-(6-chloropyrazin-2-yl)benzamide (**3k**). White to pale beige solid. Yield: 32%. mp 166.0-167.1 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.53 (s, 1H, NH), 9.40 (s, 1H, H3'), 8.55 (s, 1H, H5'), 8.10 (t, J = 1.9 Hz, 1H, H2), 8.02-7.96 (m, 1H, H6), 7.71-7.68 (m, 1H, ArH), 7.56 (t, J = 7.9 Hz, 1H, ArH). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 165.05, 148.40, 145.61, 138.91, 135.26, 135.13, 133.43, 132.45, 130.57, 128.29, 127.20. IR (ATR-Ge, cm^{-1}): 3202; 3070; 1691 (C=O, amide); 1542; 1415; 1397; 1296; 1256; 1166; 1009; 775; 732; 690. Anal. calcd. for $\text{C}_{11}\text{H}_7\text{Cl}_2\text{N}_3\text{O}$ (MW 268.1): C, 49.28; H, 2.63; N, 15.67. Found: C, 49.45; H, 2.47; N, 15.63.

4-chloro-*N*-(6-chloropyrazin-2-yl)benzamide (**3l**). White to pale beige solid. Yield: 36%. mp 193.8-194.7 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.50 (s, 1H, NH), 9.40 (s, 1H, H3'), 8.54 (s, 1H, H5'), 8.06 (d, J = 8.6 Hz, 2H, H2, H6), 7.60 (d, J = 8.7 Hz, 2H, H3, H5). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ

165.40, 148.50, 145.58, 138.79, 137.60, 135.27, 131.92, 130.43, 128.70. IR (ATR-Ge, cm^{-1}): 3196; 3065; 1687 (C=O, amide); 1546; 1401; 1292; 1268; 1167; 1103; 1009; 842; 750; 682. Anal. calcd. for $\text{C}_{11}\text{H}_7\text{Cl}_2\text{N}_3\text{O}$ (MW 268.1): C, 49.28; H, 2.63; N, 15.67. Found: C, 49.41; H, 2.54; N, 15.56.

N-(6-chloropyrazin-2-yl)-3-(trifluoromethyl)benzamide (**3n**). White to pale beige solid. Yield: 46%. mp 124.2–125.0 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.70 (bs, 1H, NH), 9.42 (s, 1H, H3'), 8.55 (s, 1H, H5'), 8.42 (bs, 1H, H2), 8.31 (d, $J = 7.8$ Hz, 1H, H6), 7.99 (d, $J = 7.8$ Hz, 1H, H4), 7.77 (t, $J = 7.8$ Hz, 1H, H5). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 165.05, 148.42, 145.61, 138.96, 135.25, 134.08, 132.59, 129.92, 129.35 (q, $J = 32.3$ Hz), 129.14 (q, $J = 3.8$ Hz), 125.22 (q, $J = 3.8$ Hz), 124.04 (q, $J = 272.6$ Hz). IR (ATR-Ge, cm^{-1}): 3303; 3040; 1655 (C=O, amide); 1537; 1400; 1330; 1256; 1157; 1118; 1076; 1008; 826; 702; 677. Anal. calcd. for $\text{C}_{12}\text{H}_7\text{ClF}_3\text{N}_3\text{O}$ (MW 301.65): C, 47.78; H, 2.34; N, 13.93. Found: C, 47.55; H, 2.01; N, 13.87.

2.2. Evaluation of *in vitro* antimycobacterial activity

A microdilution panel method was used. Tested strains *Mycobacterium tuberculosis* H37Rv CNCTC My 331/88 (ATCC 27294), *M. kansasii* CNCTC My 235/80 (ATCC12478) and *M. avium* ssp. *avium* CNCTC My 80/72 (ATCC 15769) were obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health (Prague, Czech Republic). Middlebrook 7H9 broth (Sigma-Aldrich, Steinheim, Germany) enriched with the 0.4% of glycerol (Sigma-Aldrich) and 10% of OADC supplement (oleic acid, albumin, dextrose, catalase; Himedia, Mumbai, India) of declared pH = 6.6 was used for cultivation. Tested compounds were dissolved and diluted in DMSO and mixed with broth (25 μL of DMSO solution in 4.475 mL of broth) and placed (100 μL) into microplate wells. Mycobacterial inocula were suspended in isotonic saline solution and the density was adjusted to 0.5–1.0 McFarland. These suspensions were diluted by 10^{-1} and used to inoculate the testing wells, adding 100 μL of suspension to 100 μL of the DMSO/broth solution of tested compound. Final concentrations of tested compounds in wells were 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 $\mu\text{g}\cdot\text{mL}^{-1}$. Isoniazid (INH) was used as positive control (inhibition of growth). Negative control consisted of broth plus DMSO. A total of 30 μL of Alamar Blue working solution (1:1 mixture of 0.01% resazurin sodium salt (aq. sol.) and 10% Tween 80) was added after five days of incubation. Results were then determined after 24 h of incubation. The minimum inhibitory concentration (MIC; $\mu\text{g}\cdot\text{mL}^{-1}$) was determined as the lowest concentration that prevented the blue to pink colour change. MIC values of INH were 6.25–12.5 $\mu\text{g}\cdot\text{mL}^{-1}$ against *M. avium*, 3.13–12.5 $\mu\text{g}\cdot\text{mL}^{-1}$ against *M. kansasii*, and 0.1–0.2 $\mu\text{g}\cdot\text{mL}^{-1}$ against *M. tbc*.

2.3. Antimycobacterial *in vitro* activity screening against *Mycobacterium smegmatis*

The antimycobacterial assay was performed with fast growing *Mycobacterium smegmatis* CCM 4622 (ATCC 607) from the Czech Collection of Microorganisms (Brno, Czech Republic). The technique used for activity determination was microdilution broth panel method using 96-well microtitration plates. The culturing medium was Middlebrook 7H9 (MB) broth (Sigma-Aldrich), enriched with 0.4% of glycerol (Sigma-Aldrich, Steinheim, Germany) and 10% of Middlebrook OADC growth supplement (Himedia). Tested compounds were dissolved in DMSO (Sigma-Aldrich), and the MB broth was then added to achieve a concentration of 2000 $\mu\text{g}\cdot\text{mL}^{-1}$. Standards

used for activity determination were INH, rifampicin (RIF), and ciprofloxacin (CPX) (Sigma-Aldrich). Final concentrations were reached by binary dilution followed by the addition of mycobacterial suspension, and were set as 500, 250, 125, 62.5, 31.25, 15.625, 7.81, and 3.91 $\mu\text{g}\cdot\text{mL}^{-1}$, except for the standards of ciprofloxacin and rifampicin, where the final concentrations were 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, and 0.098 $\mu\text{g}\cdot\text{mL}^{-1}$. The final concentration of DMSO did not exceed 2.5% (v/v) and did not affect the growth of *M. smegmatis*. Plates were also sealed with polyester adhesive film and incubated in the dark at 37 °C, without agitation. The addition of 0.01% solution of resazurin sodium salt followed after 48 h. This stain was prepared by dissolving resazurin sodium salt (Sigma-Aldrich) in deionised water, producing a 0.02% solution. Then, a 10% aqueous solution of Tween 80 (Sigma-Aldrich) was prepared. Both liquids were mixed up making use of the same volumes and filtered through a syringe membrane filter. Microtitration panels were then further incubated for 4 h. Antimycobacterial activity was expressed as the minimal inhibition concentration (MIC) and the value was read on the basis of stain colour change (blue colour—active compound; pink colour—not active compound). The MIC values for the standards were in the range of 7.81–15.625 $\mu\text{g}\cdot\text{mL}^{-1}$ for INH, 0.78–1.56 $\mu\text{g}\cdot\text{mL}^{-1}$ for RIF, and 0.098–0.195 $\mu\text{g}\cdot\text{mL}^{-1}$ for CPX. All experiments were conducted in duplicate.

2.4. Evaluation of *in vitro* antibacterial activity

Microdilution broth method was used. Antibacterial evaluation was performed against eight bacterial strains from the Czech Collection of Microorganisms (*Staphylococcus aureus* CCM 4516/08, *Escherichia coli* CCM 4517, *Pseudomonas aeruginosa* CCM 1961) or clinical isolates from the Department of Clinical Microbiology, University Hospital and Faculty of Medicine in Hradec Králové, Charles University in Prague, Czech Republic (*Staphylococcus aureus* H 5996/08-methicilin resistant (MRSA), *Staphylococcus epidermidis* H 6966/08, *Enterococcus* sp. J 14365/08, *Klebsiella pneumoniae* D 11750/08, *Klebsiella pneumoniae* J 14368/08-ESBL positive). All strains were subcultured on Mueller-Hinton agar (MHA) (Difco/Becton Dickinson, Detroit, MI, USA) at 35 °C and maintained on the same medium at 4 °C. The compounds were dissolved in DMSO, and the antibacterial activity was determined in Mueller-Hinton liquid broth (Difco/Becton Dickinson), and buffered to pH 7.0. Controls consisted of medium and DMSO alone. The final concentration of DMSO in the test medium did not exceed 1% (v/v) of the total solution composition. The minimum inhibitory concentration (MIC), defined as the minimum concentration to prevent the visible growth compared to control, was determined after 24 and 48 h of static incubation at 35 °C. The standards were neomycin, bacitracin, penicillin G, ciprofloxacin, and phenoxymethylpenicillin.

2.5. Evaluation of *in vitro* antifungal activity

Antifungal evaluation was performed using a microdilution broth method against eight fungal strains (*Candida albicans* ATCC 44859, *C. tropicalis* 156, *C. krusei* E28, *C. glabrata* 20/I, *Trichosporon asahii* 1188, *Aspergillus fumigatus* 231, *Lichtheimia corymbifera* 272 and *Trichophyton interdigitale* 445). Compounds were dissolved in DMSO and diluted in a twofold manner with RPMI 1640 medium, with glutamine buffered to pH 7.0 (3-morpholinopropane-1-sulfonic acid). The final concentration of DMSO in the tested medium did not exceed 2.5% (v/v) of the total solution composition. Static incubation was performed in the dark and humidity, at 35 °C, for 24 and 48 h

(72 and 120 h for *Trichophyton interdigitale*). Drug-free controls were included. The standards were amphotericin B, voriconazole, nystatin, and fluconazole.

2.6. HepG2 cytotoxicity determination

The human liver hepatocellular carcinoma cell line HepG2 (passage 32–34 for compounds **2b-Ac**, **2b**, **2h**, and **2i**; and passage 17–18 for **2d-Ac** and **3n**) purchased from Health Protection Agency Culture Collections (ECACC, Salisbury, UK) was routinely cultured in Minimum Essential Eagle Medium (MEM; Sigma-Aldrich) supplemented with 10% (v/v) fetal bovine serum (PAA, Austria), 1% (v/v) L-glutamine solution (Sigma-Aldrich) and 1% (v/v) non-essential amino acid solution (Sigma-Aldrich) in a humidified atmosphere containing 5% CO₂ at 37 °C. For subculturing, the cells were harvested after trypsin/EDTA (Sigma-Aldrich) treatment at 37°C. To evaluate the cytotoxicity, the HepG2 cells treated with the tested substances were used as experimental groups whereas untreated HepG2 cells served as control groups.

HepG2 cells were seeded in a density of 1×10⁴ cells per well on a 96-well plate. Next day (24 h after seeding) the cells were treated with tested substances dissolved in DMSO at different concentrations ranging from 0.1 to 1000 µM (depending on the solubility, see Table 2). Maximal incubation concentration of DMSO in a well did not exceed 1% (v/v). The treatment was carried out in triplicates in a humidified atmosphere containing 5% of CO₂ at 37 °C. The controls representing 100% cell viability (untreated cells), 0% cell viability (cells treated with 10% DMSO), no-cell controls and vehiculum controls were incubated in triplicates simultaneously. After 24 h exposure to tested compounds, CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA) reagent was added to each well according to the manufacturer's recommendations. After 2 h incubation at 37 °C in humidified, 5% of CO₂ containing atmosphere, the absorbance was recorded at 490 nm. Inhibitory curves were constructed for each compound plotting incubation concentrations *vs.* percentage of absorbance relative to untreated control. The standard toxicological parameter IC₅₀ was calculated by nonlinear regression analysis of the inhibitory curves using GraphPad Prism software, version 6 (GraphPad Software, Inc., CA, USA).

2.7. Confirmatory test of HepG2 cytotoxicity

The HepG2 cells (passage 35–36) were seeded in density 1×10⁴ cells per well on a 96-well plate. Next day (24 h after seeding), they were treated with tested substances dissolved in DMSO (maximal incubation concentration of DMSO was 1% v/v). The tested substances were prepared according to their solubility in DMSO at incubation concentrations 0.1–500 µM (see Table 3). The treatment was carried out in a humidified atmosphere containing 5% CO₂ at 37 °C in triplicates for 24 h and 48 h. The controls representing 100% cell viability, 0% cell viability (the cells treated with 10% DMSO and the cells treated with Lysis Solution 1:25), no-cell controls and vehiculum controls were incubated in triplicates simultaneously. After 24 h exposure, the reagent from the kit CellTox™ Green Cytotoxicity Assay (Promega, Madison, WI, USA) was prepared and added according to the recommendation of the manufacturer. After 15 min incubation at room

temperature, the fluorescence was measured at 485nm_{Ex}/520nm_{Em}. The measurement was repeated after 24 h to get results for the exposure period 48 h. Inhibitory curves were constructed for each compound plotting incubation concentrations vs. percentage of fluorescence relative to untreated control. The standard toxicological parameter IC₅₀ was calculated by nonlinear regression analysis of the inhibitory curves using GraphPad Prism software version 6 (GraphPad Software, Inc., CA, USA).

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