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 (µg/mL)

| Cpd | R¹ | R² | \text{MIC} \text{ (µg/mL)} | \text{MIC} \text{ (µg/mL)} | \text{MIC} \text{ (µg/mL)} | \text{MIC} \text{ (µg/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 | 3-OAc | >100                       | >100                       | >100                       | ≥ 500                      |
| 1e  | H  | 2-OCH₃ | 100                       | >100                       | >100                       | 125                        |
| 1f  | H  | 3-OCH₃ | >100                       | >100                       | >100                       | ≥ 500                      |
| 1g  | H  | 4-OCH₃ | 25                        | >100                       | >100                       | ≥ 500                      |
| 1h  | H  | 4-CH₃ | >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-CF₃ | >100                       | >100                       | >100                       | ≥ 500                      |
| 2a  | 5-Cl | H | >100 a                      | >100 a                     | >100 a                     | ≥ 500 a                    |
| 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-OCH₃ | >100                       | >100                       | >100                       | ≥ 500                      |
| 2f  | 5-Cl | 3-OCH₃ | >100                       | >100                       | >100                       | ≥ 500                      |
| 2g  | 5-Cl | 4-OCH₃ | >100                       | >100                       | >100                       | ≥ 500                      |
| 2h  | 5-Cl | 4-CH₃ | 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-CF₃ | 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                        |
| 3c  | 6-Cl | 2-OCH₃ | >100                       | >100                       | >100                       | ≥ 500                      |
| 3d  | 6-Cl | 3-OCH₃ | 50 a                       | >100 a                     | >100 a                     | ≥ 500 a                    |
| 3e  | 6-Cl | 4-OCH₃ | >100                       | >100                       | >100                       | ≥ 500 a                    |
| 3f  | 6-Cl | 4-CH₃ | >100 a                      | >100 a                     | >100 a                     | ≥ 500 a                    |
| 3g  | 6-Cl | 4-Et | >100 a                      | >100 a                     | >100 a                     | ≥ 500 a                    |
| 3h  | 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-CF₃ | 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**

| Strain | Tested compound (lab code / publication code) – MIC (µM) |
|--------|---------------------------------------------------------|
|        | OV-6 | JZ-AM7 | JZ-AM13 | JZ-AM14 | 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   |
|        | 48 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
| MRSA   |      |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 24 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 48 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
| SE     |      |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 24 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 48 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
| EF     |      |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 24 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 48 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
| EC     |      |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 24 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 48 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
| KP     |      |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 24 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 48 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
| KP-E   |      |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 24 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 48 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
| PA     |      |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 72 h |        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
|        | 120 h|        | >500    | >500   | >500   | >500   | >500   | >500   | >500   |
| Strain | Tested compound (lab code / publication code) – MIC (µM) |
|--------|----------------------------------------------------------|
|        | JZAM15 | JZAM11 | JZ-AM4 | OV-S | JZ-AM2 | JZ-AM8 | OV-1 | OV-14 | OV-17 |
|        | 24 h    |        |        |      |        |        |      |      |      |
|        |        | i     |        |      |        |        |      |      |      |
| SA     | >500    | 250   | >125   | >125 | >125   | >125   | 250  | 62.5 | 15.62 |
|        | 48 h    |        |        |      |        |        |      |      |      |
| MRSA   | >500    | 250   | >125   | >125 | >125   | >125   | 250  | 125  | 15.62 |
|        | 48 h    |        |        |      |        |        |      |      |      |
| SE     | 24 h    | 500   | 500    | >125 | >125   | >125   | 62.5 | 500  | 125  |
|        | 48 h    |        |        |      |        |        |      |      |      |
| EF     | 24 h    | 500   | >500   | >125 | >125   | >125   | >500 | 250  | >500 |
|        | 48 h    |        |        |      |        |        |      |      |      |
| EC     | 24 h    | >500  | >500   | >125 | >125   | >125   | >500 | >250 | >500 |
|        | 48 h    |        |        |      |        |        |      |      |      |
| KP     | 24 h    | >500  | >500   | >125 | >125   | >125   | >500 | >250 | 250  |
|        | 48 h    |        |        |      |        |        |      |      |      |
| KP-E   | 24 h    | >500  | >500   | >125 | >125   | >125   | >500 | >250 | 250  |
|        | 48 h    |        |        |      |        |        |      |      |      |
| PA     | 72 h    | >500  | 250    | >125 | >125   | >125   | >500 | >250 | >500 |
|        | 120 h   |        |        |      |        |        |      |      |      |
| 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   |
| 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 (µM) |
|--------|-----------------------------|
|        | 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 - *Candida albicans* ATCC 44859  
5. TA - *Trichosporon asahii* 1188  
2. CT - *Candida tropicalis* 156  
6. AF - *Aspergillus fumigatus* 231  
3. CK2 - *Candida krusei* E28  
7. LC - *Lichtheimia corymbifera* 272  
4. CG - *Candida glabrata* 20/I  
8. TI - *Trichophyton interdigitale* 445

**Results**

| Strain | Tested compound (lab code / publication code) – MIC (µM) |
|--------|----------------------------------------------------------|
| CA     | OV-6 | JZ-AM7 | JZ-AM13 | JZAM14 | JZ-AM6 | JZ-AM9 | JZ-AM5 | JZ-AM3 | JZAM10 |
| CA     | 24 h  | 500    | >500    | >500   | >500   | >500   | >500   | >500   | >500   | 500    |
| CA     | 48 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | 500    |
| CT     | 24 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| CT     | 48 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| CK     | 24 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| CK     | 48 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| CG     | 24 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| CG     | 48 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| TA     | 24 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| TA     | 48 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| AF     | 24 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| AF     | 48 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| LC     | 24 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| LC     | 48 h  | >500   | >500    | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| TI     | 72 h  | >500   | 500     | >500   | >500   | >500   | >500   | >500   | >500   | >500   |
| TI     | 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   |
| CA     | 24 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 62.5  |
|        | 48 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 62.5  |
| CT     | 24 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 125   |
|        | 48 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 125   |
| CK     | 24 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 125   |
|        | 48 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 125   |
| CG     | 24 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 125   |
|        | 48 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 125   |
| TA     | 24 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 62.5  | 125   |
|        | 48 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 125   |
| AF     | 24 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 125   |
|        | 48 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 125   |
| LC     | 24 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 62.5  |
|        | 48 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 125   |
| TI     | 72 h   | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 125   |
|        | 120 h  | >500   | >500   | >125 | >125   | >125   | >500 | 125   | 125   | 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 |
|        | 48 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
| CT     | 24 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
|        | 48 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
| CK     | 24 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
|        | 48 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
| CG     | 24 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
|        | 48 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
| TA     | 24 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
|        | 48 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
| AF     | 24 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
|        | 48 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
| LC     | 24 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
|        | 48 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
| TI     | 72 h   | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
|        | 120 h  | >125  | >500  | >125  | >500 | >125 | >500 | >125 | >125 |
| Strain | Tested compound (lab code / publication code) – MIC (µM) | 2j | 2k | 2l | 2m | 3e | 3j | 3k |
|--------|-------------------------------------------------|----|----|----|----|----|----|----|
| CA     | OV-2A | OV-3 | OV-4 | OV-13 | JZ-AM23 | JZ-AM25 | JZ-AM27 |
| 24 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| 48 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| CT     | OV-2A | OV-3 | OV-4 | OV-13 | JZ-AM23 | JZ-AM25 | JZ-AM27 |
| 24 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| 48 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| CK     | OV-2A | OV-3 | OV-4 | OV-13 | JZ-AM23 | JZ-AM25 | JZ-AM27 |
| 24 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| 48 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| CG     | OV-2A | OV-3 | OV-4 | OV-13 | JZ-AM23 | JZ-AM25 | JZ-AM27 |
| 24 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| 48 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| TA     | OV-2A | OV-3 | OV-4 | OV-13 | JZ-AM23 | JZ-AM25 | JZ-AM27 |
| 24 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| 48 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| AF     | OV-2A | OV-3 | OV-4 | OV-13 | JZ-AM23 | JZ-AM25 | JZ-AM27 |
| 24 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| 48 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| LC     | OV-2A | OV-3 | OV-4 | OV-13 | JZ-AM23 | JZ-AM25 | JZ-AM27 |
| 24 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| 48 h   | >500  | >125 | >125 | >125  | >500   | >500   | >125 |
| TI     | OV-2A | OV-3 | OV-4 | OV-13 | JZ-AM23 | JZ-AM25 | JZ-AM27 |
| 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     | AMPB | 0.12 | 0.98 | 0.24 | 0.002 |
|        | 48 h | 0.49 | 1.95 | 0.24 | 0.008 |
| CT     | AMPB | 1.95 | 1.95 | >500 | 62.5 |
|        | 48 h | 1.95 | 3.9  | >500 | 250  |
| CK     | AMPB | 1.95 | 1.95 | 125  | 0.49 |
|        | 48 h | 1.95 | 3.9  | 250  | 1.95 |
| CG     | AMPB | 0.98 | 1.95 | 31.25| 0.24 |
|        | 48 h | 1.95 | 3.9  | 250  | 250  |
| TA     | AMPB | 0.49 | 1.95 | 250  | 0.98 |
|        | 48 h | 0.98 | 1.95 | 500  | 7.81 |
| AF     | AMPB | 1.95 | 1.95 | >500 | 0.49 |
|        | 48 h | 1.95 | 3.9  | >500 | 1.95 |
| LC     | AMPB | 7.81 | 15.62| >500 | 125 |
|        | 48 h | 7.81 | 31.25| >500 | 250 |
| TI     | AMPB | 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, CDCl3) 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). 13C NMR (126 MHz, CDCl3) 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; 842; 802; 705. Anal. calcd. for C11H9N3O (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, DMSO-d6) 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). 13C NMR (126 MHz, DMSO-d6) 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 C11H9N3O2 (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, CDCl3) 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, CH3). 13C NMR (126 MHz, CDCl3) 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); 1630; 1511; 1421; 1304; 1271; 1215; 1194; 1016; 845; 716; 676. Anal. calcd. for C13H11N3O3 (MW 257.25): C, 60.7; H, 4.31; N, 16.33. Found: C, 60.67; H, 4.22; N, 19.13.

3-hydroxy-N-(pyrazin-2-yl)benzamide (1c). White solid. Yield: 58%. mp 192.2–193.7 °C. 1H NMR (500 MHz, DMSO-d6) 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). 13C NMR (126 MHz, DMSO-d6) 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 C11H9N3O2 (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, CDCl3) 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, CH3). 13C NMR (126 MHz, CDCl3) 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
2-methoxy-N-(pyrazin-2-yl)benzamide (1e). White solid. Yield: 11%. mp 100.3–102.4 °C. 1H NMR (500 MHz, CDCl3) 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, OCH3). 13C NMR (126 MHz, CDCl3) 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 C13H11N3O3 (MW 257.25): C, 60.7; H, 4.31; N, 16.33. Found: C, 60.23; H, 4.17; N, 15.98.

3-methoxy-N-(pyrazin-2-yl)benzamide (1f). White solid. Yield: 22%. mp 148.1–150.1 °C. 1H NMR (500 MHz, CDCl3) 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, OCH3). 13C NMR (126 MHz, CDCl3) 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; 1033; 1017; 797; 740. Anal. calcd. for C12H11N3O2 (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, CDCl3) 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, OCH3). 13C NMR (75 MHz, CDCl3) 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; 1173; 1026; 1012; 835; 759; 656. Anal. calcd. for C12H11N3O2 (MW 229.24): C, 62.87; H, 4.84; N, 18.33. Found: C, 63.08; H, 4.83; N, 18.01.

4-methyl-N-(pyrazin-2-yl)benzamide (1h). White solid. Yield: 29%. mp 163.3–166.0 °C (lit. 157–159 [3]). 1H NMR (500 MHz, CDCl3) δ 9.71 (d, J = 1.7 Hz, 1H, H3'), 8.66 (bs, 1H, NH), 8.35 (d, J = 2.6 Hz, 1H, H5'), 8.23–8.19 (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, OCH3). 13C NMR (126 MHz, CDCl3) 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 C12H11N3O (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, CDCl3) 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), 7.30 (d, J = 7.9 Hz, 2H, H3, H5), 2.43 (s, 3H, CH3). 13C NMR (126 MHz, CDCl3) 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 C13H13N3O (MW 227.24): C, 67.59; H, 5.2; N, 19.71. Found: C, 67.37; H, 5.1; N, 19.59.

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, CDCl3) 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). 13C NMR (126 MHz, CDCl3) δ 164.65, 147.93, 141.91, 140.62, 137.29, 134.06, 132.31,
3-chloro-N-(pyrazin-2-yl)benzamide (1k). White solid. Yield: 35%. mp 147.2–149.4 °C. 1H NMR (500 MHz, CDCl₃) δ 9.69 (d, J = 1.7 Hz, 1H, H₃´), 8.71 (bs, 1H, NH), 8.38 (d, J = 2.6 Hz, 1H, H₅´), 8.25 (bs, 1H, H₆´), 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). 13C NMR (126 MHz, CDCl₃) 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 C₁₁H₈ClN₃O (MW 233.66): C, 56.55; H, 3.45; N, 17.98. Found: C, 56.86; H, 3.45; N, 17.51.

4-chloro-N-(pyrazin-2-yl)benzamide (1l). White solid. Yield: 11%. mp 182.6–184.3 °C. 1H NMR (500 MHz, CDCl₃) δ 9.71 (s, 1H, H₃´), 8.69 (bs, 1H, NH), 8.40 (d, J = 2.8 Hz, 1H, H₅´), 8.27 (s, 1H, H₆´), 7.89 (d, J = 8.3 Hz, 2H, H₂, H₆), 7.49 (d, J = 8.2 Hz, 2H, H₃, H₅). 13C NMR (126 MHz, CDCl₃) 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 C₁₁H₈ClN₃O (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, CDCl₃) δ 8.63 (d, J = 1.5 Hz, 1H, H₃´), 8.45 (d, J = 2.6 Hz, 1H, H₅´), 8.35 (dd, J = 2.4, 1.5 Hz, 1H, H₆´), 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 C₁₈H₁₁Cl₂N₃O₂ (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, CDCl₃) δ 9.70 (d, J = 1.6 Hz, 1H, H₃´), 8.80 (bs, 1H, NH), 8.40 (d, J = 2.6 Hz, 1H, H₅´), 8.28–8.25 (m, 1H, H₆´), 8.21 (s, 1H, H₂), 8.13 (d, J = 7.8 Hz, 1H, ArH), 7.85 (d, J = 7.7 Hz, 1H, ArH). 13C NMR (126 MHz, CDCl₃) 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 C₁₂H₈F₃N₃O (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, DMSO-d₆) δ 11.31 (s, 1H, NH), 9.26 (d, J = 1.4 Hz, 1H, H₃´), 8.62 (d, J = 1.5 Hz, 1H, H₆´), 8.08–8.01 (m, 2H, H₂, H₆), 7.66–7.59 (m, 1H, H₄), 7.56–7.50 (m, 2H, H₃, H₅). 13C NMR (126 MHz, DMSO-d₆) δ 166.37, 148.26, 142.26, 141.96, 136.46, 133.32, 132.58, 128.61, 128.46. IR (ATR-Ge, cm⁻¹): 3342; 1655 (C=O, amide); 1541; 1511; 1484; 1340; 1249; 1131; 1103; 1020; 710; 690; 661. Anal. calcd. for C₁₁H₈ClN₃O (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, DMSO-d₆) δ 11.78 (s, 1H, OH), 11.12 (s, 1H, NH), 9.31 (d, J = 1.6 Hz, 1H, H₃´), 8.56 (d, J = 1.4 Hz, 1H, H₆´), 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). 13C NMR (126 MHz, DMSO-d₆) 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.
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-d6) 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-d6) δ 169.02, 165.09, 148.41, 147.90, 142.45, 142.11, 135.98, 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; 1513; 1434; 1119; 1159; 1136; 1017; 916; 748; 682. Anal. calcd. for C13H10ClN3O3 (MW 291.69): C, 53.53; H, 3.46; N, 14.41. Found: C, 53.38; 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-d6) 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-d6) 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 C13H10ClN3O3 (MW 291.69): C, 53.53; H, 3.46; N, 14.41. Found: C, 53.38; H, 3.38; N, 14.39.

3-((5-chloropyrazin-2-yl)carbamoyl)phenylacetate (2c-Ac). White solid. Yield: 48%. mp 184.1–184.6 °C. 1H NMR (500 MHz, DMSO-d6) 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-d6) 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, 14.45.

N-((5-chloropyrazin-2-yl)-4-hydroxybenzamide (2d). White solid. Yield: 36%. mp 210.9–212.6 °C. 1H NMR (500 MHz, DMSO-d6) 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-d6) 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 C13H10ClN3O3 (MW 291.69): C, 53.02; H, 3.44; N, 16.77.

4-((5-chloropyrazin-2-yl)carbamoyl)phenylacetate (2d-Ac). White solid. Yield: 55%. mp 170.9–171.8 °C. 1H NMR (500 MHz, DMSO-d6) 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-d6) 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-d6) 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-d6) δ 164.47, 157.26, 147.55,
N-(5-chloropyrazin-2-yl)-3-methoxybenzamide (2f). White solid. Yield: 79%. mp 124.2–125.7 °C. 1H NMR (500 MHz, DMSO-d6) 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 (d, J = 8.2, 2.6 Hz, 1H, ArH), 3.84 (s, 3H, OCH3). 13C NMR (126 MHz, DMSO-d6) 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 C12H10ClN3O2 (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)-4-methoxybenzamide (2g). White solid. Yield: 83%. mp 186.4–187.1 °C. 1H NMR (300 MHz, DMSO-d6) 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, CH3). 13C NMR (75 MHz, DMSO-d6) δ 166.17, 148.35, 142.83, 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 C12H10ClN3O (MW 247.68): C, 58.19; H, 4.07; N, 16.97. Found: C, 58.23; H, 4.13; N, 16.67.

N-(5-chloropyrazin-2-yl)-4-ethylbenzamide (2i). White solid. Yield: 85%. mp 150.0–150.8 °C. 1H NMR (500 MHz, DMSO-d6) δ 11.22 (s, 1H, NH), 9.26 (d, J = 1.5 Hz, 1H, H3´), 8.63 (d, J = 1.4 Hz, 1H, H6´), 7.62 (d, J = 8.1 Hz, 2H, H2, H6), 7.36 (d, J = 7.9 Hz, 2H, H3, H5), 2.68 (q, J = 7.6 Hz, 2H, CH2), 1.21 (t, J = 7.6 Hz, 3H, CH3). 13C NMR (126 MHz, DMSO-d6) δ 166.01, 148.44, 142.12, 141.66, 136.41, 130.50, 125.25, 113.87, 55.66. IR (ATR-Ge, cm−1): 3384; 3020; 2937; 2856; 1664 (C=O, amide); 1607; 1578; 1543; 1495; 1441; 1263; 1239; 1188; 1137; 1093; 1026; 1015; 867; 852; 760. Anal. calcd. for C12H10ClN3O (MW 261.71): C, 59.46; H, 4.61; N, 15.8.
4-chloro-N-(5-chloropyrazin-2-yl)benzamide (2l). White solid. Yield: 8%. mp 225.0–226.3 °C. ¹H NMR (500 MHz, DMSO-d₆) 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), 7.70 (d, J = 8.5 Hz, 2H, H3, H5). ¹³C NMR (126 MHz, DMSO-d₆) 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; 1297; 1249; 1136; 1097; 1016; 840; 746. Anal. calcd. for C₁₁H₇Cl₂N₃O (MW 268.1): C, 49.28; H, 2.63; N, 15.67. Found: C, 49.29; H, 2.71; N, 15.37.

4-bromo-N-(5-chloropyrazin-2-yl)benzamide (2m). White solid. Yield: 43%. mp 229.2–230.5 °C. ¹H NMR (500 MHz, DMSO-d₆) 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). ¹³C NMR (126 MHz, DMSO-d₆) 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; 868; 848; 658. Anal. calcd. for C₁₁H₇BrClN₃O (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, DMSO-d₆) 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). ¹³C NMR (126 MHz, DMSO-d₆) 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; 1105; 1073; 1018; 916; 791; 750; 720; 694; 669. Anal. calcd. for C₁₂H₇ClF₃N₃O (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, DMSO-d₆) 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, ArH). ¹³C NMR (126 MHz, DMSO-d₆) 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; 3067; 1679 (C=O, amide); 1541; 1512; 1454; 1329;1237; 1181; 1131; 1073; 1018; 916; 791; 750; 720; 694; 669. Anal. calcd. for C₁₁H₈ClN₃O (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, DMSO-d₆) δ 10.86 (bs, 1H, NH), 9.44 (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, ArH). ¹³C NMR (126 MHz, DMSO-d₆) 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; 3066; 1686 (C=O, amide); 1542; 1398; 1292; 1267; 1164; 1008; 882. Anal. calcd. for C₁₂H₁₀ClN₃O₂ (MW 263.68): C, 56.55; H, 3.45; N, 17.98. Found: C, 56.59; H, 3.42; N, 17.85.

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, DMSO-d₆) 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). ³¹C NMR (126 MHz, DMSO-d₆) δ 166.07, 159.31, 148.62, 145.57, 138.67, 135.33,
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, DMSO-d6) 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, OCH3). 13C NMR (126 MHz, DMSO-d6) δ 165.64, 148.68, 144.52, 138.30, 165.02, 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; 736; 694. Anal. calcd. for C12H10ClN3O2 (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-methylbenzamide (3h). White to pale beige solid. Yield: 29%. mp 169.1-170.0 °C. 1H NMR (500 MHz, DMSO-d6) 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, CH3). 13C NMR (126 MHz, DMSO-d6) 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 C12H10ClN3O (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, DMSO-d6) 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, CH2), 1.21 (t, J = 7.6 Hz, 3H, CH3). 13C NMR (126 MHz, DMSO-d6) 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; 1271; 1152; 1008; 845; 726. Anal. calcd. for C13H12ClN3O (MW 261.71): C, 59.66; H, 4.62; N, 16.06. Found: C, 59.22; H, 4.49; N, 15.79.

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, DMSO-d6) 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, H6), 7.59-7.50 (m, 2H, ArH), 7.49-7.42 (m, 1H, ArH). 13C NMR (126 MHz, DMSO-d6) δ 166.07, 147.98, 145.76, 139.14, 134.56, 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 C11H7Cl2N3O (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, DMSO-d6) 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). 13C NMR (126 MHz, DMSO-d6) 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 C11H7Cl2N3O (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, DMSO-d6) 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). 13C NMR (126 MHz, DMSO-d6) 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 C₁₁H₇Cl₂N₃O (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, DMSO-d₆) 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). ¹³C NMR (126 MHz, DMSO-d₆) 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 C₁₂H₇ClF₃N₃O (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 µg·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; µg·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 µg·mL⁻¹ against M. avium, 3.13–12.5 µg·mL⁻¹ against M. kansasii, and 0.1–0.2 µg·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 µg·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, 15.625, 7.81, and 3.91 µg·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 µg·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 µg·mL⁻¹ for INH, 0.78–1.56 µg·mL⁻¹ for RIF, and 0.098–0.195 µg·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-methicillin 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% CO\(_2\) 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 1×10\(^4\) 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 CO\(_2\) 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 CO\(_2\) 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 IC\(_{50}\) 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 1×10\(^4\) 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% CO\(_2\) 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 $485\text{nm}_{\text{Ex}}/520\text{nm}_{\text{Em}}$. 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 IC$_{50}$ 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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