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## Synergistic interactions of indole-2-carboxamides and $\beta$ -lactam antibiotics against *Mycobacterium abscessus*

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30 **ABSTRACT**

31

32 New drugs or therapeutic combinations are urgently needed against *Mycobacterium abscessus*.  
33 Previously, we demonstrated the potent activity of indole-2-carboxamides **6** and **12** against *M.*  
34 *abscessus*. We show here that these compounds act synergistically with imipenem and ceftazidime  
35 *in vitro* and increase the bactericidal activity of the  $\beta$ -lactams against *M. abscessus*. In addition,  
36 compound **12** also displays synergism with imipenem and ceftazidime within infected  
37 macrophages. The clinical potential of these new drug combinations requires further  
38 evaluation.

39 *Mycobacterium abscessus*, is a fast-growing mycobacterial species particularly frequent in  
40 patients with cystic fibrosis (CF), bronchiectasis and chronic obstructive pulmonary diseases  
41 (COPD) (1, 2). In the context of CF and COPD, *M. abscessus* has emerged as an important  
42 opportunistic pathogen responsible for significant mortality (3). However, treatment of *M.*  
43 *abscessus* lung disease remains particularly challenging, largely due to intrinsic resistance of *M.*  
44 *abscessus* to most antibiotic classes (1, 2). The typical treatment regimen includes a  
45 combination of macrolides, aminoglycosides and intravenous  $\beta$ -lactams (cefoxitin or imipenem)  
46 for at least 12 months (2). There is no reliable therapeutic strategy for the treatment of *M.*  
47 *abscessus* pulmonary infections and the lengthy treatment duration and drug toxicity effects  
48 are often accompanied with severe undesirable outcomes. Thus, there is an unmet clinical need  
49 for new drug regimens with improved efficacy to treat these infections. Along with the  
50 development of repurposed drugs, the drug pipeline has recently been fueled with chemical  
51 entities acting on new targets in *M. abscessus*, such as the mycolic acid transporter MmpL3  
52 inhibited by a wide range of structurally-unrelated small molecules (4). Chemical inhibition of  
53 MmpL3 abolishes the export of trehalose monomycolate to the outer membrane, leading to  
54 significant bacterial growth inhibition. In *M. abscessus*, these chemotypes include a piperidinol-  
55 based compound (PIPD1) (5), benzimidazoles (6) as well as indole-2-carboxamide derivatives (7,  
56 8). They exhibit high activity against clinical isolates *in vitro*, in macrophages, zebrafish or in an  
57 acute murine model of *M. abscessus* infection (5–7, 9). Due to their pronounced role in  
58 modulating the cell wall architecture and composition, it may be speculated that chemical  
59 inhibition of MmpL3 would increase the efficacy of other drugs. Although this has been  
60 reported in *M. tuberculosis* whereby the indolecarboxamides and adamantyl-ureas act  
61 synergistically with rifampin, bedaquiline, clofazimine and  $\beta$ -lactams (10), to date this has not  
62 been investigated in *M. abscessus*.

63 Indolecarboxamides **6** and **12** (**Figure 1**) present favorable absorption, distribution,  
64 metabolism, and excretion (ADME) properties (7, 11) and the ease to obtain them in high yields  
65 prompted us to investigate their interaction profiles with different classes of antibiotics active  
66 against *M. abscessus* and/or used as part of clinical treatment regimens. These include  
67 sutezolid; an oxazolidinone inhibiting bacterial translation (12), clofazimine; affecting the  
68 energy metabolism (13), and particularly  $\beta$ -lactams (the cephalosporin cefoxitin (FOX) and the  
69 carbapenem imipenem (IPM)); inhibiting peptidoglycan biosynthesis and reported to act in  
70 synergy with different drugs against *M. abscessus* (14, 15) (**Figure 1**). The minimal inhibitory

71 concentrations (MIC) were determined according to the CLSI guidelines (16) in Cation-Adjusted  
72 Mueller-Hinton Broth (CaMHB; Sigma-Aldrich). Pair combinations between Cpd6 and Cpd12  
73 with other drugs were tested in CaMHB in a typical checkerboard assay (17) with resazurin  
74 reduction as a metabolic readout. This allowed us to establish the fractional inhibitory  
75 concentration index (FICI) of each drug combination, where FICI was determined using formula:  
76  $MIC_A$  with B/ $MIC_A$  alone +  $MIC_B$  with A/ $MIC_B$  alone and values  $\leq 0.5$  were considered synergistic,  
77 between 0.5 and 4 as indifferent and  $\geq 4$  as antagonist (10). While Cpd12 showed a FICI value  $\leq$   
78 0.5 with IPM or FOX, indicative of synergistic interactions, no interaction (indifference) was  
79 recorded with clofazimine or sutezolid. A similar interaction profile was also observed when  
80 combining these drugs with Cpd6 (**Table 1**).

81 To determine the optimal concentration of Cpd12 showing no or little activity against *M.*  
82 *abscessus* CIP104536<sup>T</sup> (S variant), cultures were exposed to concentrations ranging from 0.03 to  
83 0.125  $\mu\text{g/ml}$  Cpd12 prior to colony-forming units (CFU) determination at 5 days post-exposure.  
84 While at the MIC (0.125  $\mu\text{g/ml}$ ) there was a  $\sim 6$  to 7 Log drop in the CFU counts, no decrease  
85 was observed at 0.03  $\mu\text{g/ml}$  (**Figure 2A**). This concentration was thus chosen to investigate the  
86 potential synergistic activity of Cpd12 with  $\beta$ -lactams. IPM was used at 4, 8 and 16  $\mu\text{g/ml}$  and  
87 FOX was used at 16 and 32  $\mu\text{g/ml}$ , corresponding to concentrations 4- and 2-fold lower than  
88 their MIC, respectively (**Table 1**). At these sub-MIC, Cpd12 plus IPM decreased CFU counts by  $\sim 4$   
89 to 6 Log, as compared to Cpd12 or IPM alone. Similarly, FOX alone at 16 and 32  $\mu\text{g/ml}$  was  
90 accompanied by a reduction in the CFU counts, while the addition of 0.03  $\mu\text{g/ml}$  Cpd12 further  
91 reduced the CFU by  $\sim 2$  to 3 Log (**Figure 2A**). Comparable results were obtained when assessing  
92 the synergistic activity of Cpd6 with IPM or FOX (**Figure 2B**). At 0.06  $\mu\text{g/ml}$  and 0.125  $\mu\text{g/ml}$  of  
93 Cpd6, the CFU were reduced by  $\sim 1$  and 5 Log, respectively, and no further decrease in the CFU  
94 was observed at 0.25  $\mu\text{g/ml}$ . The addition of 4  $\mu\text{g/ml}$  or 8  $\mu\text{g/ml}$  IPM to 0.06  $\mu\text{g/ml}$  Cpd6  
95 resulted in a  $\sim 3$  Log decrease in the CFU as compared to IPM alone. Similarly, the simultaneous  
96 addition of 0.06  $\mu\text{g/ml}$  Cpd6 to FOX (at 16 or 32  $\mu\text{g/ml}$ ) exacerbated the effect of FOX, leading  
97 to a  $\sim 4$  Log decrease in the CFU as compared to FOX alone (**Figure 2B**). To assess whether these  
98 interactions are due to the chemical inhibition of MmpL3, the CFU killing assay was repeated  
99 using a strain highly resistant to both Cpd12 and Cpd6 due to the presence of an A309P  
100 missense mutation in MmpL3 ( $MIC_{Cpd12/Cpd6}$  of 32  $\mu\text{g/ml}$ , (7)). **Figure 2C** shows the Cpd12 plus  
101 IPM or Cpd12 plus FOX synergistic interactions were abolished, indicating that inhibition of  
102 MmpL3 is necessary to establish drug synergism with the  $\beta$ -lactams. This confirms a previous

103 study demonstrating that synergistic interactions between the indolecarboxamides NITD-304  
104 and NITD-349 with other clinically-relevant drugs is diminished in an MmpL3 mutant of *M.*  
105 *tuberculosis* resistant to indolecarboxamides (10).

106 The *M. abscessus* complex comprises three subspecies, *M. abscessus* subsp. *abscessus*, *M.*  
107 *abscessus* subsp. *bolletii* and *M. abscessus* subsp. *massiliense* (18), displaying different drug  
108 susceptible profiles. We therefore tested the activity of the Cpd12/ $\beta$ -lactam combinations  
109 against a panel of *M. abscessus* complex clinical isolates (19, 20) by determining the CFU counts  
110 of two *M. abscessus* subsp. *abscessus* strains (1298 and 2587), two *M. abscessus* subsp. *bolletii*  
111 strains (97 and 116) and two *M. abscessus* subsp. *massiliense* strains (120 and 122). In general,  
112 the combination of Cpd12 plus IPM or Cpd12 plus FOX resulted in significantly reduced CFU  
113 counts as compared to the cultures exposed to Cpd12, IPM or FOX alone. However, the 6  
114 strains responded differently to each of these drug combinations (**Figure 3**). Overall, CFU  
115 determination *in vitro* was in direct agreement with the checkerboard results and indicate that  
116 low concentrations of Cpd6 and Cpd12 improve the activity of IPM or FOX against the *M.*  
117 *abscessus* complex *in vitro*.

118 The activity of IPM and FOX alone or in combination with Cpd12 was next evaluated using  
119 THP-1 macrophages infected with *M. abscessus* CIP104536<sup>T</sup> (S variant) carrying pTEC27, as  
120 previously described (6). Infected cells were either left untreated or exposed for 2 days to  
121 Cpd12, IPM or FOX alone or in combination, lysed and plated for subsequent intracellular  
122 bacterial load determination. While IPM and FOX displayed only minor effects at the  
123 concentrations tested, the addition of 0.06  $\mu\text{g/ml}$  Cpd12 significantly reduced the bacterial  
124 burden by  $\sim 0.5$  Log (**Figure 4A**). This effect was further exacerbated (1 Log reduction) when  
125 0.25  $\mu\text{g/ml}$  of Cpd12 was used. A microscopy-based infectivity assay reported earlier (6, 21) was  
126 subsequently used to quantify the impact of drug treatment on the percentage of infected THP-  
127 1 cells. The results confirm the pronounced reduction in the number of infected macrophages  
128 treated with Cpd12 plus IPM or Cpd12 plus FOX ( $\sim 50\%$  decrease with 0.06  $\mu\text{g/ml}$  Cpd12), as  
129 compared to cells treated with the drugs alone at day 2 post-infection (**Figure 4B and 4C**).  
130 Collectively, these findings suggest that the Cpd12/IPM and Cpd12/FOX combinations are  
131 effective on intracellular *M. abscessus*.

132 IPM use is usually associated with improved outcome for the treatment of *M. abscessus*  
133 pulmonary disease (22) and IPM combined with other antibiotics exerts a synergistic or  
134 additive effect contributing to its success (14, 15). However, resistance to IPM is also

135 emerging, highlighting the limiting application of IPM in the treatment of *M. abscessus*  
136 infections (23, 24). The present results highlight the therapeutic potential of the Cpd**12**/IPM  
137 combination against a panel of distinct clinical *M. abscessus* complex isolates. This  
138 combination may help lowering the effective dose of IPM, contributing to reductions in the  
139 emergence of IPM-resistance strains. Similarly, the use of indolecarboxamides as companion  
140 drugs further reduces the effective concentrations of FOX, restricting the eventual emergence  
141 of *M. abscessus*-resistant mutants. A plausible hypothesis explaining this synergistic activity  
142 may rely on the fact that the indolecarboxamides, through inhibition of mycolic acid transport  
143 at the bacterial surface, disorganise and disrupt the mycomembrane, which accelerates the  
144 penetration of the  $\beta$ -lactam drugs to reach their targets (the L,D-transpeptidase for IPM and  
145 the D,D-transpeptidase for FOX), leading to inhibition of peptidoglycan synthesis. Conversely,  
146 inhibition of the peptidoglycan transpeptide linkages by the  $\beta$ -lactams may also facilitate the  
147 access of Cpd**6**/Cpd**12** to their inner membrane target. However, other underlying  
148 mechanisms may be responsible to the observed synergistic effects and further research is  
149 required.

150 In summary, indole-2-carboxamides represent a promising chemotype improving the activity  
151 of FOX and IPM; two recommended drugs for the treatment of *M. abscessus* pulmonary  
152 infections (2). Future studies should evaluate whether  $\beta$ -lactamase inhibitors (33,34) would  
153 further improve the observed synergistic interactions. Our results indicate that the Cpd**12**/ $\beta$ -  
154 lactam combinations are highly effective within macrophages by reducing the intracellular  
155 bacterial burden and the percentage of infected cells, emphasizing the need for further  
156 evaluation in pre-clinical animal models.

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161

162 The authors have no conflict of interest to declare.

163

164 All data will be made publicly available upon publication and upon request for peer review.



165 **REFERENCES**

- 166
- 167 1. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R,  
168 Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ, Winthrop K, ATS  
169 Mycobacterial Diseases Subcommittee, American Thoracic Society, Infectious Disease Society of  
170 America. 2007. An official ATS/IDSA statement: diagnosis, treatment, and prevention of  
171 nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 175:367–416.
- 172 2. Floto RA, Olivier KN, Saiman L, Daley CL, Herrmann J-L, Nick JA, Noone PG, Bilton D, Corris P,  
173 Gibson RL, Hempstead SE, Koetz K, Sadosky KA, Sermet-Gaudelus I, Smyth AR, van Ingen J,  
174 Wallace RJ, Winthrop KL, Marshall BC, Haworth CS. 2016. US Cystic Fibrosis Foundation and  
175 European Cystic Fibrosis Society consensus recommendations for the management of non-  
176 tuberculous mycobacteria in individuals with cystic fibrosis: executive summary. *Thorax* 71:88–90.
- 177 3. Brode SK, Daley CL, Marras TK. 2014. The epidemiologic relationship between tuberculosis and  
178 non-tuberculous mycobacterial disease: a systematic review. *Int J Tuberc Lung Dis Off J Int Union  
179 Tuberc Lung Dis* 18:1370–1377.
- 180 4. Li W, Yazidi A, Pandya AN, Hegde P, Tong W, Calado Nogueira de Moura V, North EJ, Sygusch J,  
181 Jackson M. 2018. MmpL3 as a target for the treatment of drug-resistant nontuberculous  
182 mycobacterial infections. *Front Microbiol* 9:1547.
- 183 5. Dupont C, Viljoen A, Dubar F, Blaise M, Bernut A, Pawlik A, Bouchier C, Brosch R, Guérardel Y,  
184 Lelièvre J, Ballell L, Herrmann J-L, Biot C, Kremer L. 2016. A new piperidinol derivative targeting  
185 mycolic acid transport in *Mycobacterium abscessus*. *Mol Microbiol* 101:515–529.
- 186 6. Raynaud C, Daher W, Johansen MD, Roquet-Baneres F, Blaise M, Onajole OK, Kozikowski AP,  
187 Herrmann JL, Dziadek J, Gobis K, Kremer L. 2020. Active benzimidazole derivatives targeting the  
188 MmpL3 transporter in *Mycobacterium abscessus*. *ACS Infect Dis* doi: 10.1021/acinfecdis.9b00389.
- 189 7. Kozikowski AP, Onajole OK, Stec J, Dupont C, Viljoen A, Richard M, Chaira T, Lun S, Bishai W, Raj VS,  
190 Ordway D, Kremer L. 2017. Targeting mycolic acid transport by indole-2-carboxamides for the  
191 treatment of *Mycobacterium abscessus* infections. *J Med Chem* 60:5876–5888.
- 192 8. Franz ND, Belardinelli JM, Kaminski MA, Dunn LC, Calado Nogueira de Moura V, Blaha MA, Truong  
193 DD, Li W, Jackson M, North EJ. 2017. Design, synthesis and evaluation of indole-2-carboxamides  
194 with pan anti-mycobacterial activity. *Bioorg Med Chem* 25:3746–3755.

- 195 9. Pandya AN, Prathipati PK, Hegde P, Li W, Graham KF, Mandal S, Drescher KM, Destache CJ, Ordway  
196 D, Jackson M, North EJ. 2019. Indole-2-carboxamides are active against *Mycobacterium abscessus*  
197 in a mouse model of acute infection. *Antimicrob Agents Chemother* 63:e02245-18.
- 198 10. Li W, Sanchez-Hidalgo A, Jones V, de Moura VCN, North EJ, Jackson M. 2017. Synergistic  
199 interactions of MmpL3 inhibitors with antitubercular compounds *in vitro*. *Antimicrob Agents*  
200 *Chemother* 61:e02399-16.
- 201 11. Stec J, Onajole OK, Lun S, Guo H, Merenbloom B, Vistoli G, Bishai WR, Kozikowski AP. 2016. Indole-  
202 2-carboxamide-based MmpL3 inhibitors show exceptional antitubercular activity in an animal  
203 model of tuberculosis infection. *J Med Chem* 59:6232–6247.
- 204 12. Shaw KJ, Barbachyn MR. 2011. The oxazolidinones: past, present, and future. *Ann N Y Acad Sci*  
205 1241:48–70.
- 206 13. Richard M, Gutiérrez AV, Viljoen A, Rodriguez-Rincon D, Roquet-Baneres F, Blaise M, Everall I,  
207 Parkhill J, Floto RA, Kremer L. 2019. Mutations in the MAB\_2299c TetR regulator confer cross-  
208 resistance to clofazimine and bedaquiline in *Mycobacterium abscessus*. *Antimicrob Agents*  
209 *Chemother* 63:e01316-18.
- 210 14. Miyasaka T, Kunishima H, Komatsu M, Tamai K, Mitsutake K, Kanemitsu K, Ohisa Y, Yanagisawa H,  
211 Kaku M. 2007. In vitro efficacy of imipenem in combination with six antimicrobial agents against  
212 *Mycobacterium abscessus*. *Int J Antimicrob Agents* 30:255–258.
- 213 15. Le Run E, Arthur M, Mainardi J-L. 2018. *In vitro* and intracellular activity of imipenem combined  
214 with rifabutin and avibactam against *Mycobacterium abscessus*. *Antimicrob Agents Chemother* 62:  
215 e00623-18.
- 216 16. Woods, GL, Brown-Elliott, BA, Conville, PS, Desmond, EP, Hall, GS, Lin G, Pfyffer GE, Ridderhof, JC,  
217 Siddiqi, SH, Wallace, RJ. 2011. Susceptibility testing of mycobacteria, nocardiae and other aerobic  
218 actinomycetes: approved standard Second Edition. M24-A2. Clinical and Laboratory Standards  
219 Institute, Wayne, PA 2011.
- 220 17. Odds FC. 2003. Synergy, antagonism, and what the checkerboard puts between them. *J*  
221 *Antimicrob Chemother* 52:1.

- 222 18. Adekambi T, Sassi M, van Ingen J, Drancourt M. 2017. Reinstating *Mycobacterium massiliense* and  
223 *Mycobacterium bolletii* as species of the *Mycobacterium abscessus* complex. Int J Syst Evol  
224 Microbiol 67:2726–2730.
- 225 19. Singh S, Bouzinbi N, Chaturvedi V, Godreuil S, Kremer L. 2014. *In vitro* evaluation of a new drug  
226 combination against clinical isolates belonging to the *Mycobacterium abscessus* complex. Clin  
227 Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 20:O1124-1127.
- 228 20. Halloum I, Viljoen A, Khanna V, Craig D, Bouchier C, Brosch R, Coxon G, Kremer L. 2017. Resistance  
229 to thiacetazone derivatives active against *Mycobacterium abscessus* involves mutations in the  
230 MmpL5 transcriptional repressor MAB\_4384. Antimicrob Agents Chemother 61: e02509-16.
- 231 21. Viljoen A, Raynaud C, Johansen MD, Roquet-Banères F, Herrmann J-L, Daher W, Kremer L. 2019.  
232 Improved activity of bedaquiline by verapamil against *Mycobacterium abscessus in vitro* and in  
233 macrophages. Antimicrob Agents Chemother. 63:e00705-19.
- 234 22. Kwak N, Dalcolmo MP, Daley CL, Eather G, Gayoso R, Hasegawa N, Jhun BW, Koh W-J, Namkoong  
235 H, Park J, Thomson R, van Ingen J, Zweijpfenning SMH, Yim J-J. 2019. *Mycobacterium abscessus*  
236 pulmonary disease: individual patient data meta-analysis. Eur Respir J 54: 1801991.
- 237 23. Lee M-C, Sun P-L, Wu T-L, Wang L-H, Yang C-H, Chung W-H, Kuo A-J, Liu T-P, Lu J-J, Chiu C-H, Lai H-  
238 C, Chen N-Y, Yang J-H, Wu T-S. 2017. Antimicrobial resistance in *Mycobacterium abscessus*  
239 complex isolated from patients with skin and soft tissue infections at a tertiary teaching hospital in  
240 Taiwan. J Antimicrob Chemother 72:2782–2786.
- 241 24. Li B, Yang S, Chu H, Zhang Z, Liu W, Luo L, Ma W, Xu X. 2017. Relationship between antibiotic  
242 susceptibility and genotype in *Mycobacterium abscessus* clinical isolates. Front Microbiol 8:1739.
- 243 25. Dubée V, Bernut A, Cortes M, Lesne T, Dorchene D, Lefebvre A-L, Hugonnet J-E, Gutmann L,  
244 Mainardi J-L, Herrmann J-L, Gaillard J-L, Kremer L, Arthur M. 2015.  $\beta$ -Lactamase inhibition by  
245 avibactam in *Mycobacterium abscessus*. J Antimicrob Chemother 70:1051–1058.
- 246 26. Kaushik A, Ammerman NC, Lee J, Martins O, Kreiswirth BN, Lamichhane G, Parrish NM,  
247 Nueremberger EL. 2019. *In vitro* activity of the new  $\beta$ -lactamase inhibitors relebactam and  
248 vaborbactam in combination with  $\beta$ -lactams against *Mycobacterium abscessus* complex clinical  
249 isolates. Antimicrob Agents Chemother 63:e02623-18.

250

251 **Table 1.** Interaction of Cpd6 and Cpd12 with other antibiotics against *M. abscessus* CIP104536<sup>T</sup>  
 252 (smooth strain) assessed by checkerboards REMA in CaMH. Results are the mean of the FICI ±  
 253 SD of 3 independent experiments. SUT, sutezolid; IPM, imipenem; FOX, ceftiofur; CFZ,  
 254 clofazimine.

Compound	MIC	Interaction with Cpd12			Interaction with Cpd6		
		FICI (mean)	SD	Outcome	FICI (mean)	SD	Outcome
Cpd12	0.125						
Cpd6	0.25						
SUT	16	0.84	±0.27	Indifferent	0.62	±0	Indifferent
IPM	16	0.5	±0.18	<b>Synergistic</b>	0.5	±0	<b>Synergistic</b>
FOX	64	0.45	±0.14	<b>Synergistic</b>	0.44	±0.16	<b>Synergistic</b>
CFZ	0.5	0.88	±0.18	Indifferent	0.88	±0.18	Indifferent

260 **FIGURE LEGENDS**

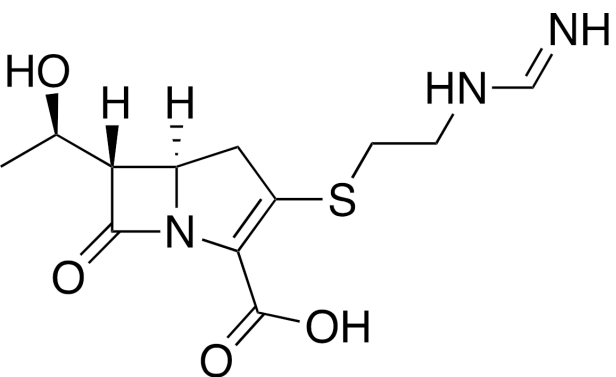
261  
262 **Figure 1.** Structures of imipenem, ceftioxin and the lead indolecarboxamides **6** and **12** used in  
263 this study.

264  
265 **Figure 2.** Synergistic activity of indole-2-carboxamide derivatives with IPM and FOX *in vitro*. CFU  
266 counts of Cpd**12** (**A**) or Cpd**6** (**B**) given alone or in combination with imipenem (IPM) or ceftioxin  
267 (FOX). *M. abscessus* cultures were incubated at 30 °C in CaMH for 5 days in the presence of the  
268 indicated compounds (µg/ml) and plated on LB agar prior to CFU enumeration. (**C**) CFU  
269 determination of *M. abscessus* mutant A309P (spontaneous resistant strain to Cpd**12** carrying  
270 the A309P mutation in MmpL3) was exposed to the indicated antibiotics (µg/ml) at 30 °C in  
271 CaMH for 5 days. Graphs represent the mean of three independent experiments completed in  
272 triplicate. Data are expressed as the mean ± SD. The statistical test used is a non-parametric  
273 Mann Whitney *t*-test in which the combinations were compared to the drugs alone. ns, non-  
274 significant. \*\**P* ≤ 0.01, \*\*\**P* ≤ 0.001.

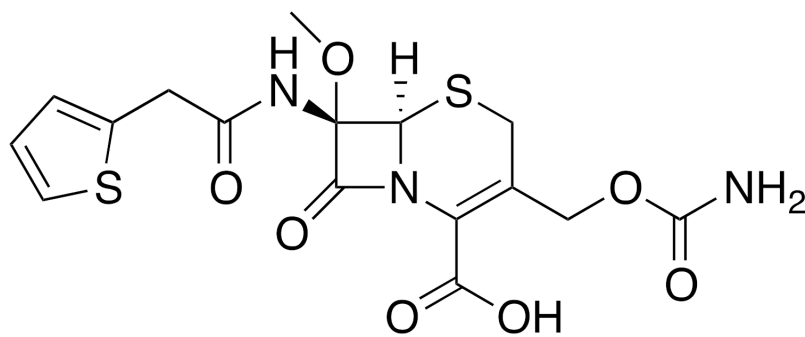
275  
276 **Figure 3.** CFU determination of clinical isolates exposed to Cpd**12** given alone or in combination  
277 with imipenem (IPM) or ceftioxin (FOX). *M. abscessus* cultures were incubated at 30 °C in CaMH  
278 for 5 days in the presence of the indicated compounds (µg/ml) and plated on LB agar prior to  
279 CFU enumeration. Data are expressed as the mean ± SD from three independent experiments  
280 completed in triplicate. The statistical test used is a non-parametric Mann Whitney *t*-test in  
281 which the combinations were compared to the drugs alone. \**P* ≤ 0.05, \*\**P* ≤ 0.01, \*\*\**P* ≤ 0.001.

282  
283 **Figure 4.** Impact of Cpd12 alone or in combination on intracellular-residing *M. abscessus*. THP-1  
284 macrophages were infected with *M. abscessus* S expressing TdTomato (MOI of 2:1) and treated  
285 with the indicated compounds (µg/ml). (**A**) CFUs were determined at day 0 and day 2 post-  
286 infection. Data represents the mean ± SD of three independent experiments completed in  
287 triplicate. For statistical analysis, non-parametric Mann Whitney's *t*-test was performed. \*\**p* ≤  
288 0.01, \*\*\**p* ≤ 0.001. (**B**) Percentage of infected THP-1 macrophages at day 0 and day 2 post-  
289 infection. Data shown are mean values ± SD for one representative experiment completed in  
290 triplicate. One-way ANOVA kruskal-wallis was used as a statistical test. \*\*\**p* ≤ 0.001. (**C**)  
291 Immunofluorescent fields were taken at day 2 post-infection at magnification x40 (using

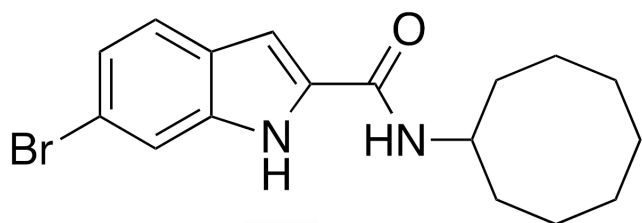
292 confocal microscopy) showing the nuclei of macrophages (DAPI in blue), infected with red-  
293 fluorescent *M. abscessus* in absence or in presence of the drugs used alone or in combination.  
294 Yellow arrows emphasize red-fluorescent *M. abscessus* (tdTomato) within macrophages. Only  
295 intracellular bacteria that were individually observed under the microscope were recorded.



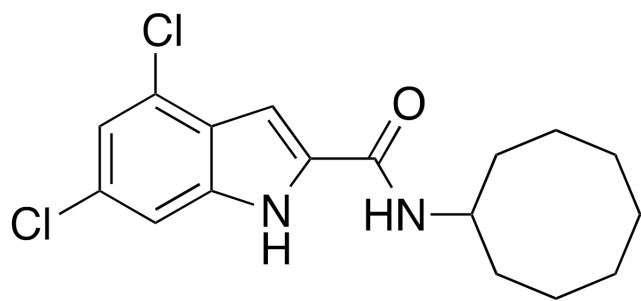
**Imipenem**



**Cefoxitin**



**Compound 6**



**Compound 12**

