Supporting Information for

Structural characterization and antimycobacterial evaluation of a benzimidazole analogue of the anti-tuberculosis clinical drug candidate TBA-7371

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Contents

1. General

Compound **1** was synthesized as described in the literature (Manjunatha *et al.*, ACS Med. Chem. Lett. 2019, 10, 1480-1485) Starting materials were of reagent grade quality and used as received. Flash chromatography for compounds **A**, **B**, **C** and **1** was carried out with an InterChim PuriFlash 430 instrument (SiO_2, heptane/ethyl acetate gradient). Compound **D** was used as crude product without purification. NMR spectra were recorded on an Agilent Technologies VNMRS 400 or a Varian INOVA 500 NMR spectrometer. ^{1}H and ^{13}C chemical shifts are reported relative to the residual solvent signal of chloroform-*d* (δ_H = 7.26 ppm; δ_C = 77.0 ppm). The ^{19}F chemical shift for **1** is reported relative to the signal of $CFCl_3$ (δ_F = 0 ppm) as an external standard. Abbreviations: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, tt = triplet of triplets. The APCI mass spectrum for **C** was measured on an Advion Expressio S mass spectrometer, and the high-resolution ESI mass spectrum for **1** was recorded on a Bruker Daltonics Apex II FT-ICR mass spectrometer. HPLC analyses were performed using an Agilent 1260 HPLC instrument with UV diode array detection (50 mm Eclipse Plus C18 1.8 μm, 4.6 mm, methanol/water gradient, v = 1.0 mL min^{-1}, λ = 220 nm).

Analytical data

Methyl 1H-benzo[d]imidazole-4-carboxylate (**A**)

^{1}H NMR (400 MHz, chloroform-*d*) δ 10.53 (s, 1H), 8.15 (s, 1H), 8.05 (d, *J* = 8.1 Hz, 1H), 7.96 (d, *J* = 7.8 Hz, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 4.01 (s, 3H) ppm.

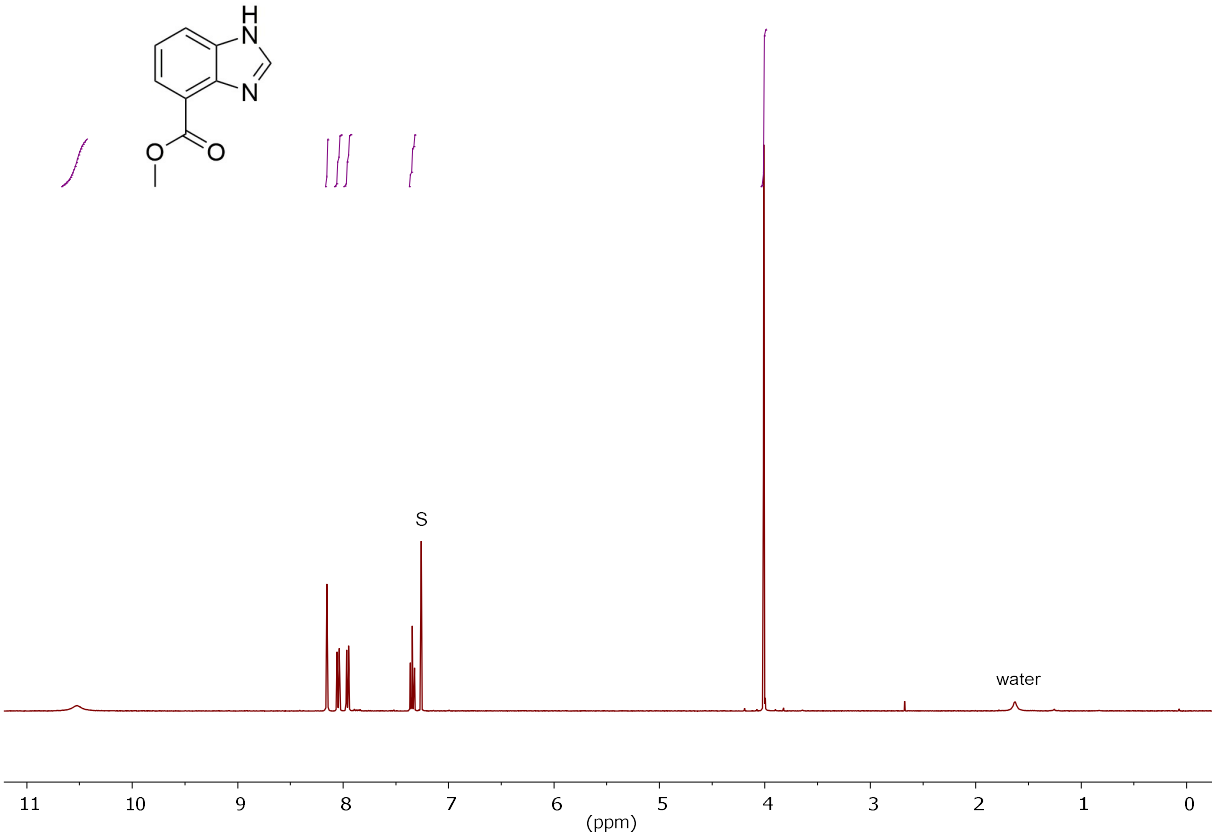


Figure S1 ^{1}H NMR spectrum of **A** in chloroform-*d* at room temperature. S denotes the residual solvent signal.

Methyl 1-((6-methoxy-5-methylpyrimidin-4-yl)methyl)-1H-benzo[d]imidazole-4-carboxylate (**B**)

^{1}H NMR (400 MHz, chloroform-*d*) δ 8.56 (s, 1H), 8.17 (s, 1H), 8.00 (d, *J* = 7.9, 1H), 7.62 (d, *J* = 7.9, 1H), 7.32 (t, *J* = 7.9 Hz, 1H), 5.42 (s, 2H), 4.06 (s, 3H), 3.99 (s, 3H), 2.20 (s, 3H) ppm.

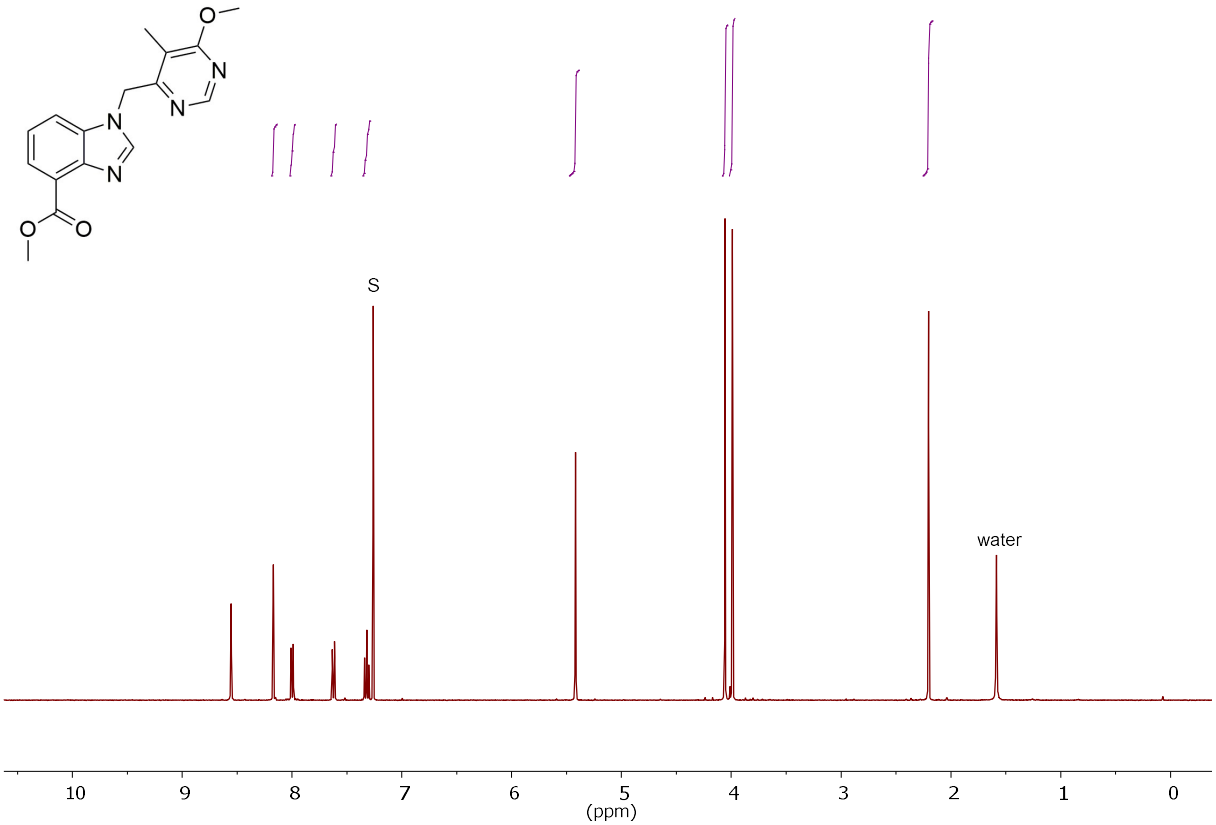


Figure S2 ^{1}H NMR spectrum of **B** in chloroform-*d* at room temperature. S denotes the residual solvent signal.

Methyl 1-((6-methoxy-5-methylpyrimidin-4-yl)methyl)-1H-benzo[d]imidazole-7-carboxylate (**C**)

^{1}H NMR (400 MHz, chloroform-*d*) δ 8.33 (s, 1H), 7.98 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.95 (s, 1H), 7.78 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.26 (t, *J* = 7.9 Hz, 1H), 5.82 (s, 2H), 3.95 (s, 3H), 3.73 (s, 3H), 2.21 (s, 3H) ppm.

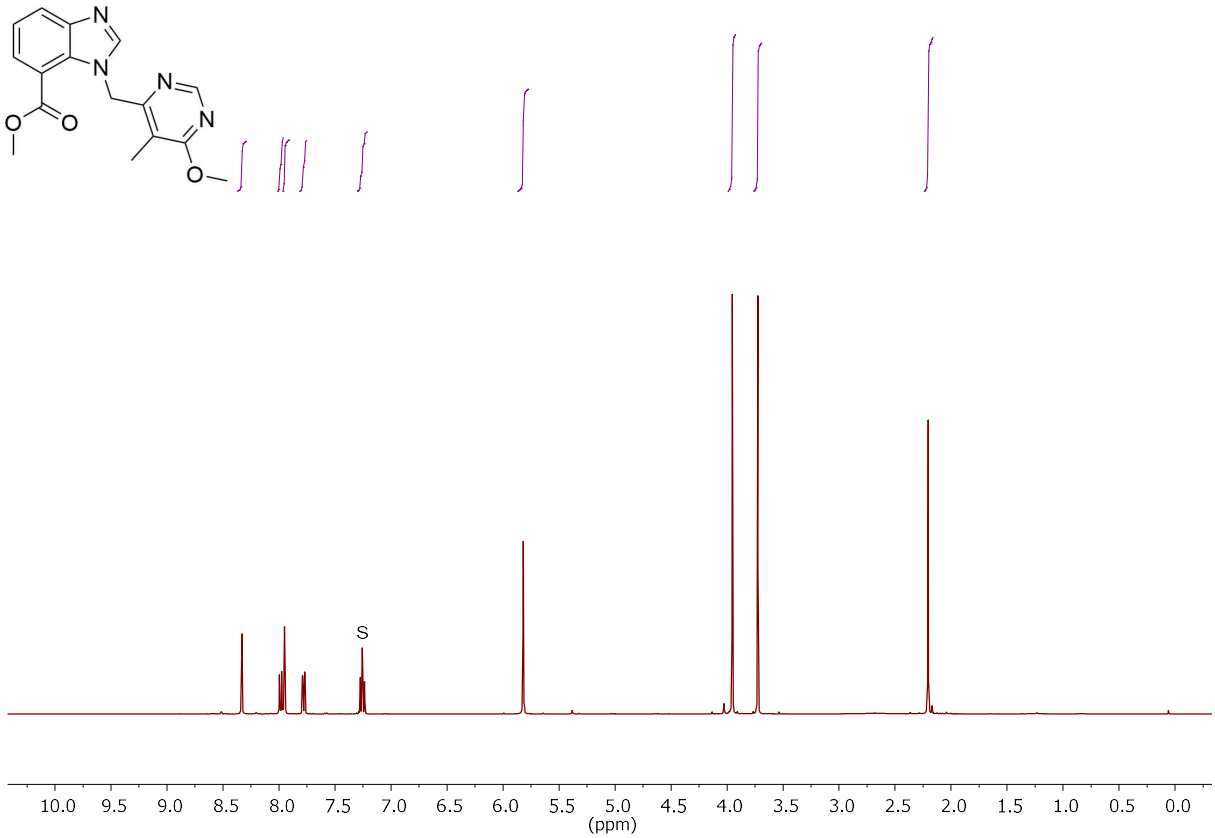


Figure S3 ^{1}H NMR spectrum of **C** in chloroform-*d* at room temperature. S denotes the residual solvent signal.

^{13}C NMR (101 MHz, chloroform-*d*) δ 167.8, 166.8, 161.3, 155.3, 147.0, 145.9, 132.8, 126.4, 125.4, 121.4, 117.0, 114.5, 54.2, 52.2, 49.7, 9.7 ppm.

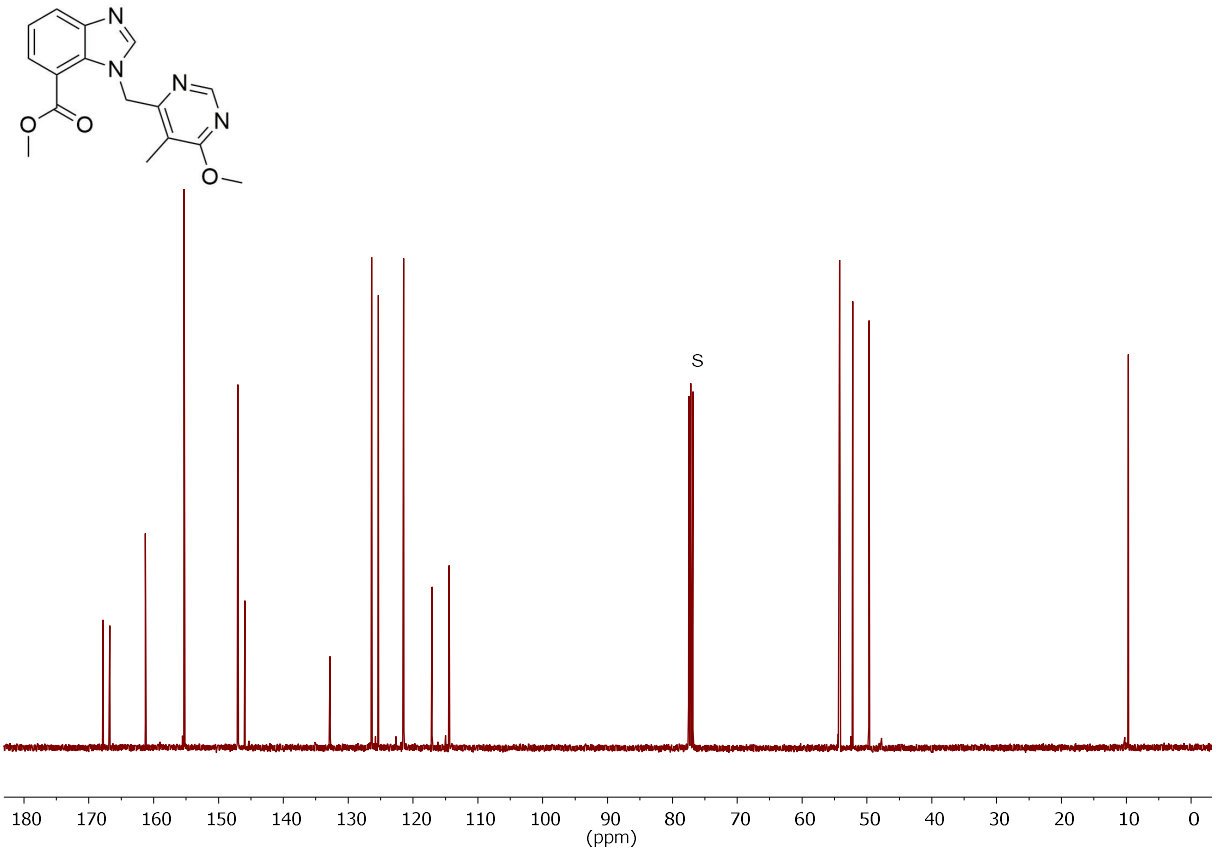


Figure S4 ^{13}C NMR spectrum of **C** in chloroform-*d* at room temperature. S denotes the residual solvent signal.

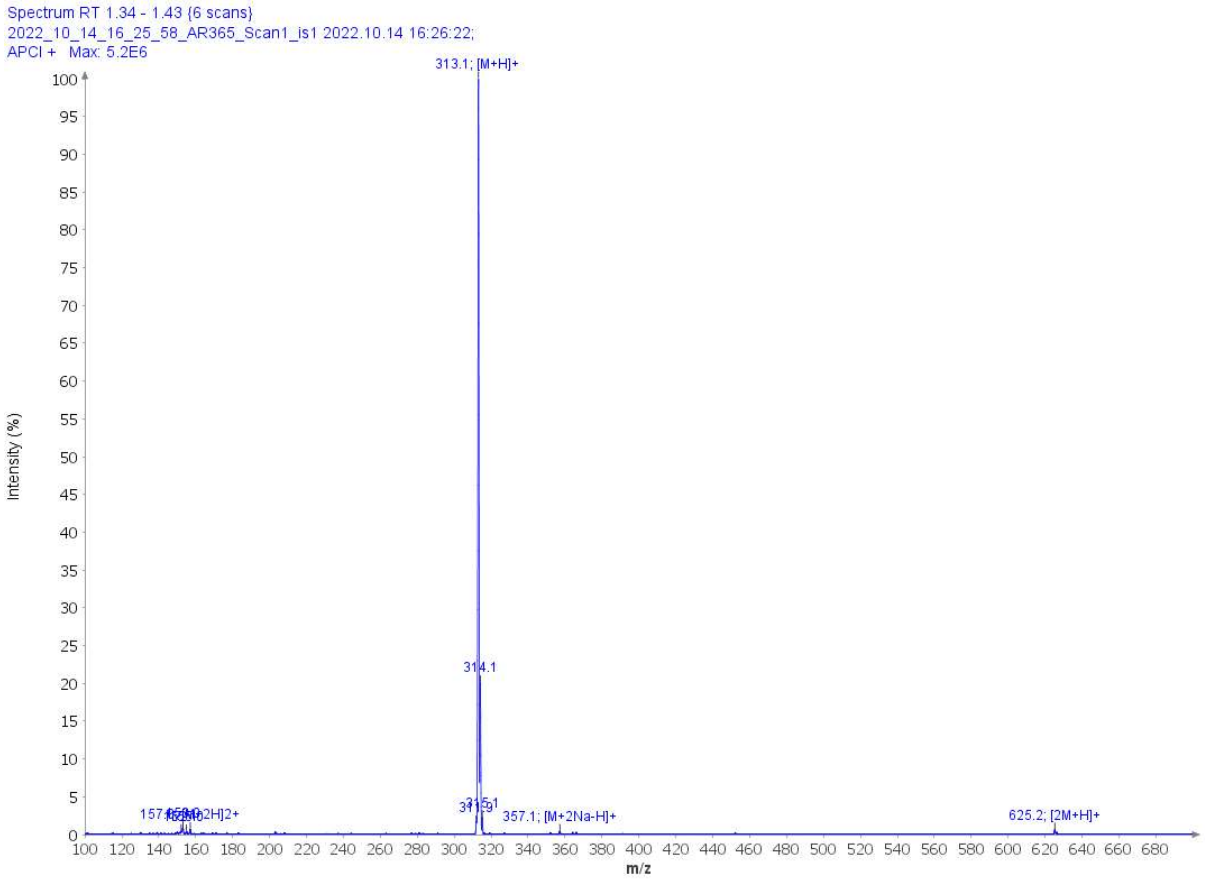


Figure S4 APCI$^+$ mass spectrum of **C** in methanol.

N-(2-fluoroethyl)-1-((6-methoxy-5-methylpyrimidin-4-yl)methyl)-1H-benzo[d]imidazole-4-carboxamide (**1**)

^{1}H NMR (400 MHz, chloroform-*d*) δ 10.13 – 10.03 (m, 1H), 8.54 (s, 1H), 8.15 (dd, *J* = 7.6, 1.0 Hz, 1H), 8.10 (s, 1H), 7.52 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.36 (t, *J* = 7.8 Hz, 1H), 5.41 (s, 2H), 4.72 (t, *J* = 5.0 Hz, 1H), 4.60 (t, *J* = 5.0 Hz, 1H), 3.99 (s, 3H), 3.93 (q, *J* = 5.2 Hz, 1H), 3.86 (q, *J* = 5.2 Hz, 1H), 2.24 (s, 3H) ppm.

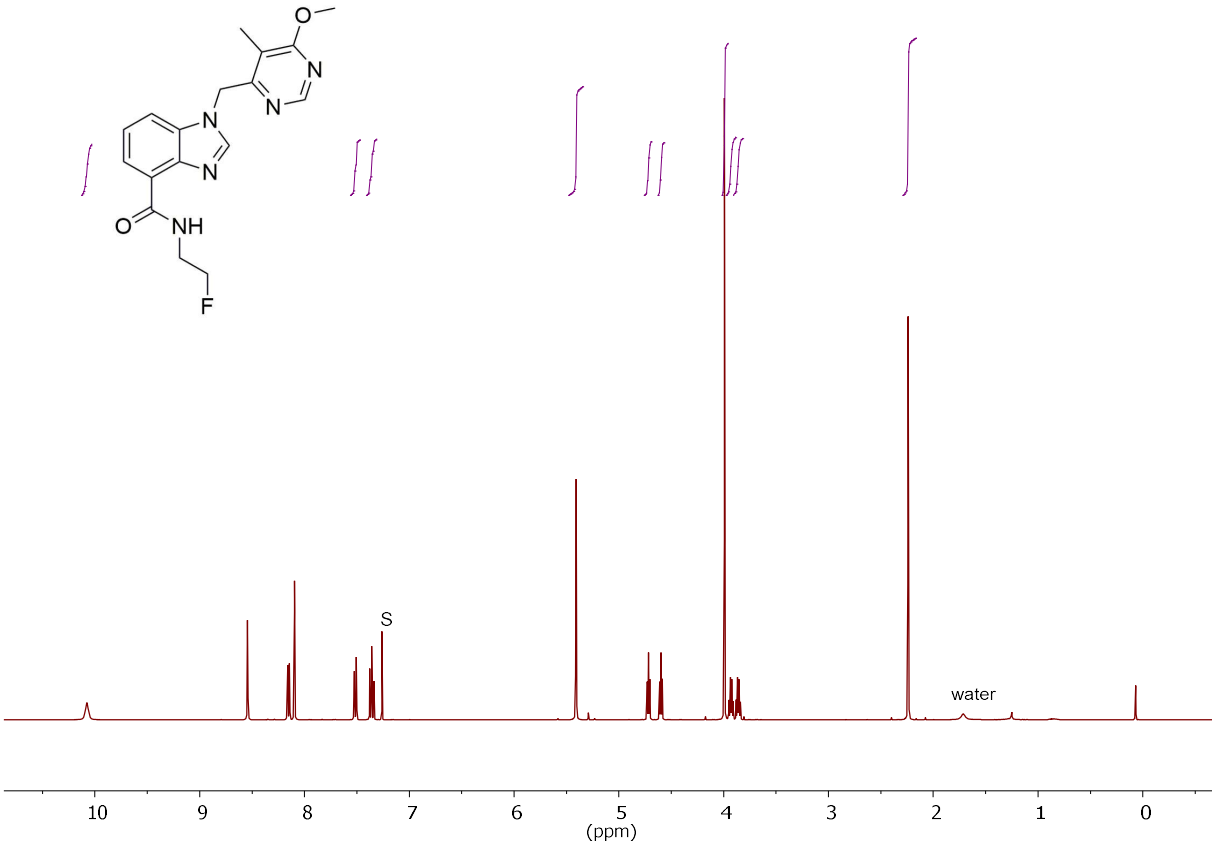


Figure S5 ^{1}H NMR spectrum of **1** in chloroform-*d* at room temperature. S denotes the residual solvent signal.

^{13}C NMR (126 MHz, chloroform-*d*) δ 168.4, 165.9, 159.0, 155.8, 143.7, 141.3, 134.3, 124.3, 123.6, 123.2, 116.1, 113.6, 83.1 (d, $^{1}J_{C,F}$ = 167 Hz), 54.5, 47.5, 40.2 (d, $^{2}J_{C,F}$ = 21 Hz), 10.3 ppm.

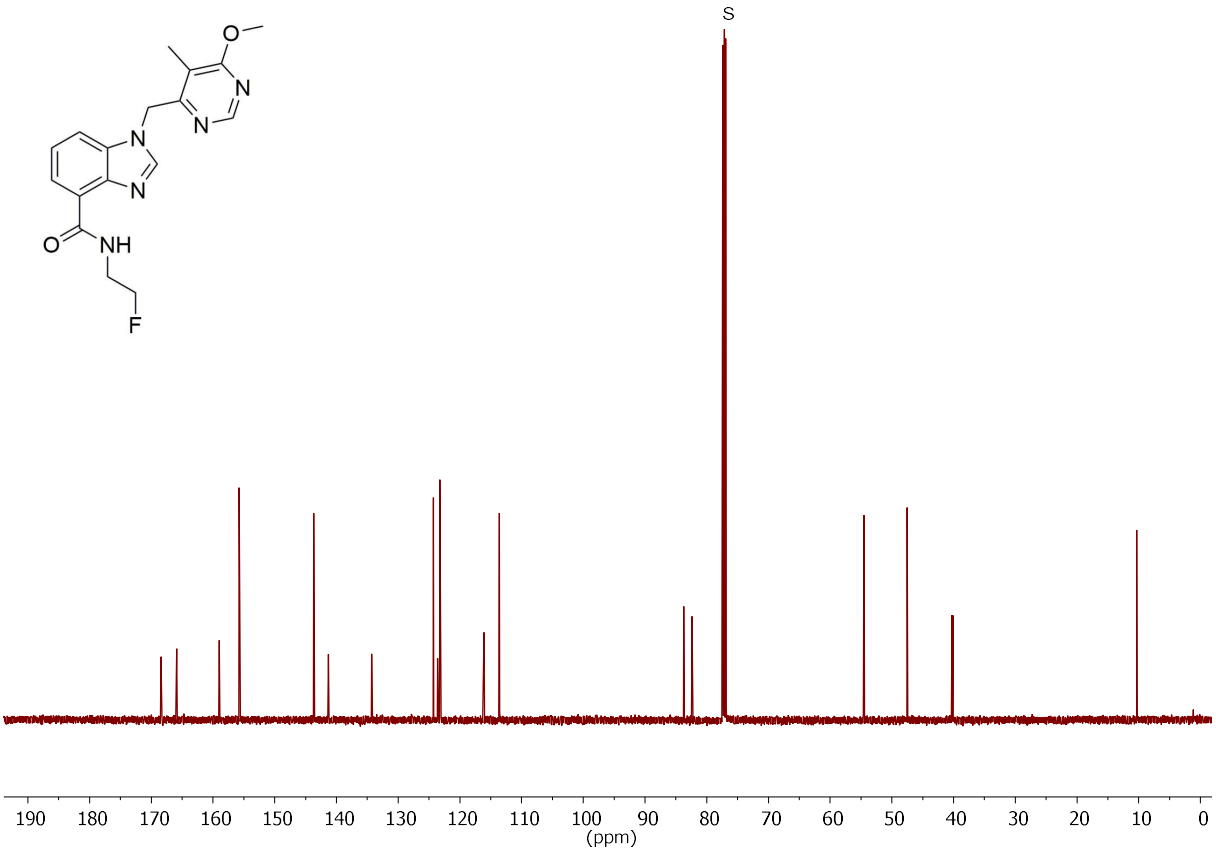


Figure S6 ^{13}C NMR spectrum of **1** in chloroform-*d* at room temperature. S denotes the residual solvent signal.

^{19}F NMR (376 MHz, Chloroform-*d*) δ -223.01 (tt, $J_{H,F}$ = 47.5, 27.2 Hz) ppm.

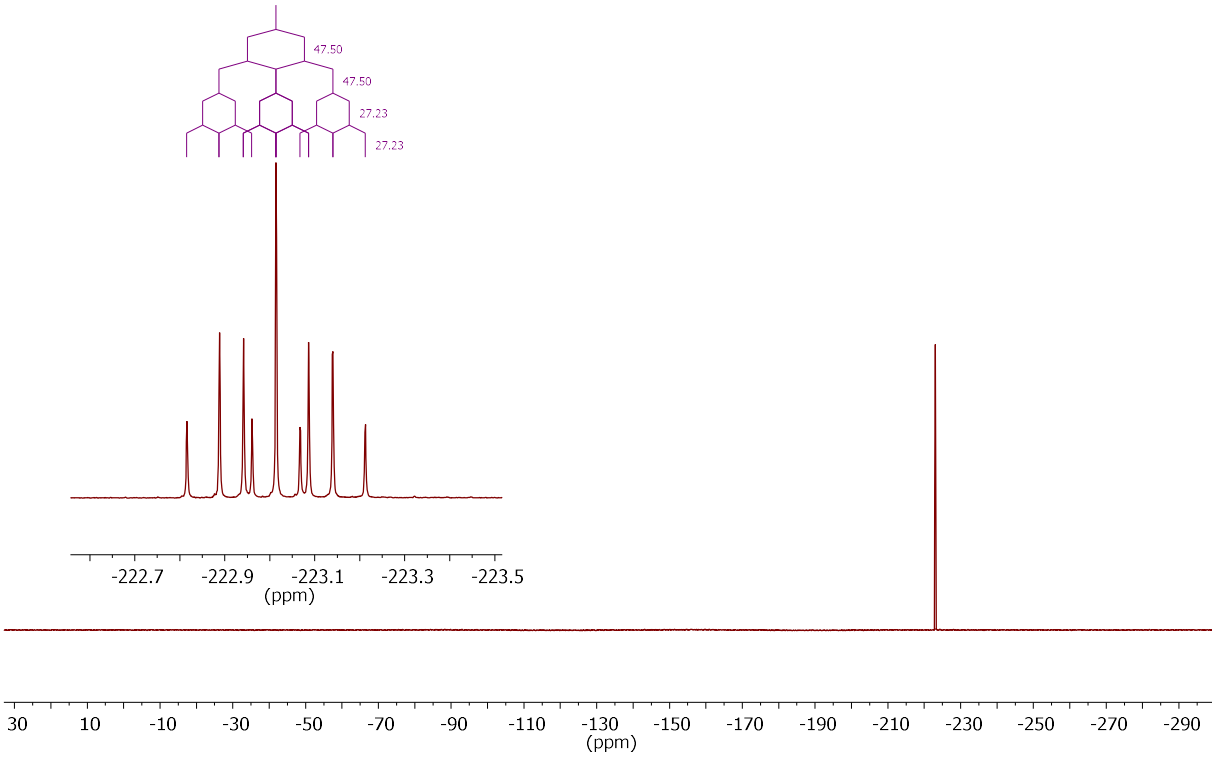


Figure S7 ^{19}F NMR spectrum of **1** in chloroform-*d* at room temperature.

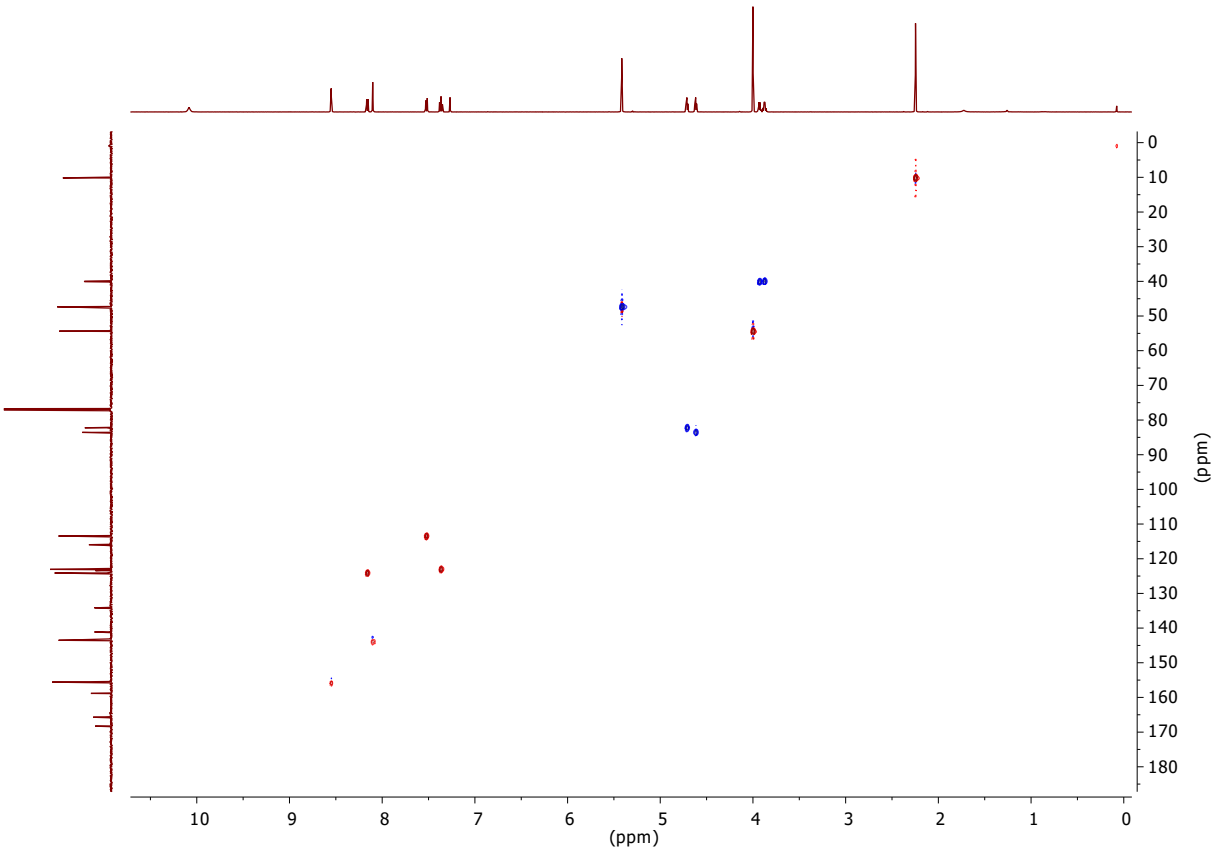


Figure S8 ^{1}H-^{13}C HSQC NMR spectrum of **1** in chloroform-*d* at room temperature.

HRMS(ESI): *m/z* calcd. for $C_{17}H_{19}FN_5O_2^+$ $[M+H]^+$, 344.1517; found, 344.1519, $C_{17}H_{18}FN_5NaO_2^+$ $[M+Na]^+$, 366.1317; found, 366.1341.

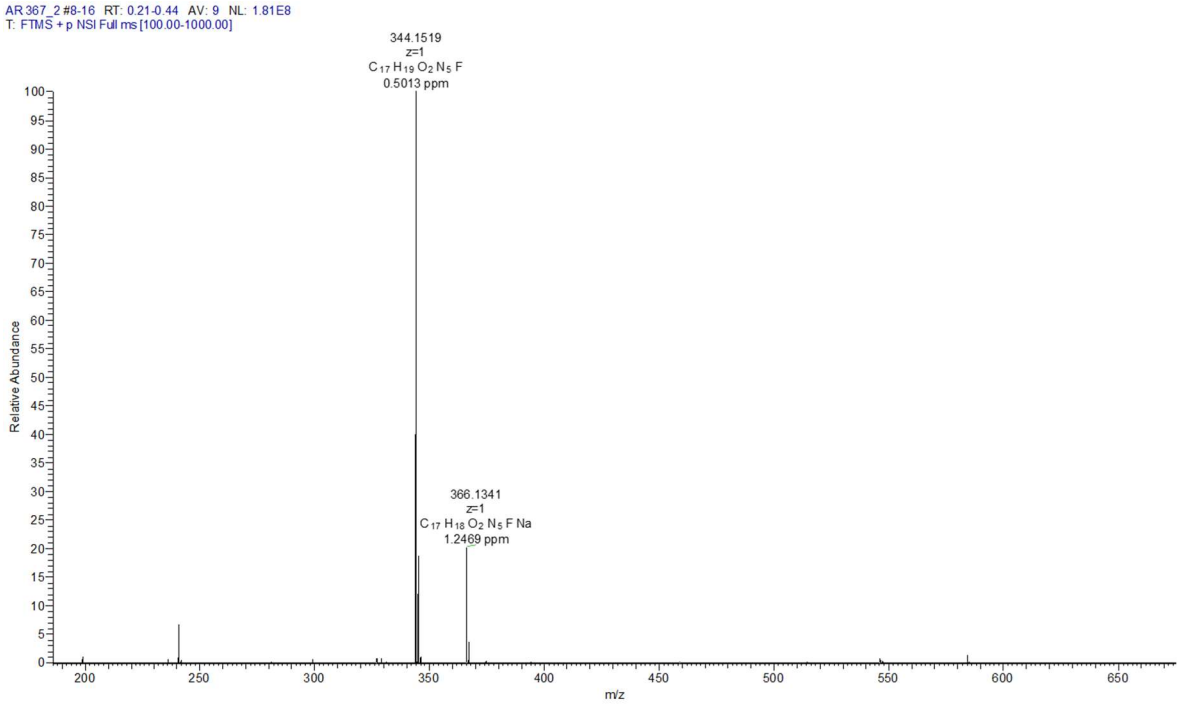
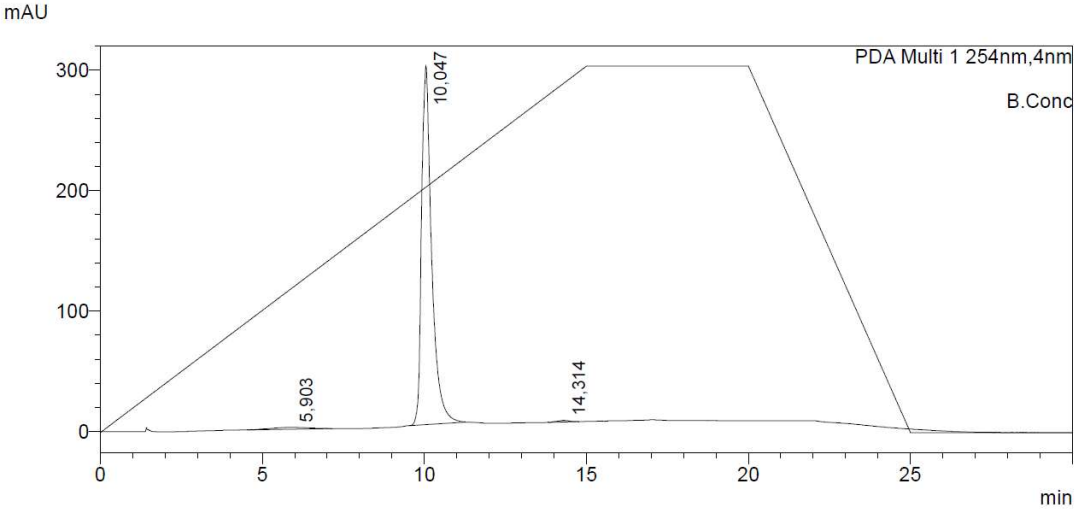


Figure S9 HRMS(ESI$^+$) spectrum of **1** in methanol.



PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	5,903	124569	1643	1,884
2	10,047	6454580	297459	97,595
3	14,314	34468	1349	0,521
Total		6613618	300451	100,000

Figure S10 HPLC analysis of **1**.

3. Antimicrobial susceptibility testing

MIC_{90} determination by optical density (OD) measurements

MICs were determined against *M. smegmatis* mc^2 155 pTEC27 and *M. abscessus* ATCC 19977 pTEC27 by the broth microdilution method in Middlebrook 7H9 medium + 10 % ADS + 0.05 % polysorbate 80 and in Mueller Hinton II Broth + 0.05 % polysorbate 80. A nine-point twofold serial dilution of each compound was prepared in 96-well flat clear bottom plates (Sarstedt, 3924500). Column 1 contained only medium as sterile control, column 2 positive controls (100 µM amikacin) and column 3 negative controls (1 % DMSO). The starting inoculum was diluted from a preculture at the mid-log phase (OD_{600} = 0.2 - 0.8). The concentration of the inoculum was standardized to approximately 5×10^5 cells/mL (OD_{600} = 0.1 was equivalent to 1×10^8 CFU/ml). The plates were sealed with Parafilm, placed in a container with moist tissue and incubated for 3 days at 37 °C. The assays were performed in duplicate. After incubation, the OD_{550} was measured with a BMG Labtech Fluostar Optima plate reader.

Calculation of % growth inhibition

The controls were used to monitor the assay quality through the determination of the *Z*-score and for normalizing the data on a plate basis. The *Z*-score was determined using the following equation:

$$Z' = 1 - \frac{3(SD_{amikacin} + SD_{DMSO})}{M_{amikacin} - M_{DMSO}}$$

(SD: standard deviation; M: mean)

% Growth inhibition was calculated as follows:

$$\% \text{ inhibition} = -100\ \% \times \frac{OD(sample) - OD(DMSO)}{OD(DMSO) - OD(amikacin)}$$

Mean values were calculated from both duplicates and the lowest concentration with a growth inhibition > 90 % was reported as MIC_{90}.