Supporting Information

for

Synthesis and biological profile of 2,3-dihydro[1,3]thiazolo[4,5-b]pyridines, a novel class of acyl-ACP thioesterase inhibitors

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General synthetic procedures, characterization of all target compounds, methods for biological and biochemical testing, and scans of $^1$H and $^{13}$C NMR spectra of the new 2,3-dihydro[1,3]thiazolo[4,5-b]pyridines
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1. Chemistry

1.1. General information

All reagent-grade solvents and chemicals were purchased from standard commercial suppliers and used without further purification. All nonaqueous reactions were carried out under anhydrous conditions using dry solvents. Reactions were monitored by LC–MS or TLC carried out on 0.25 mm silica gel plates (60F-254). TLC plates were visualized using UV light. Yields refer to spectroscopically pure compounds unless otherwise stated. Flash column chromatography was carried out with a Biotage Isolera™ using CHROMABOND® Flash SiOH (Macherey-Nagel) columns in sizes ranging from 15 g to 120 g. The 1H NMR, 13C NMR and 19F NMR spectroscopy data that are reported for the chemical examples described (400 and 600 MHz for 1H NMR and 150 MHz for 13C NMR and 375 MHz for 19F NMR, solvent: CDCl3, CD3OD or d6-DMSO, internal standard: tetramethylsilane δ = 0.00 ppm), were recorded using a Bruker AVII spectrometer with a Bruker TBI-probe, and the signals listed have the following meanings: br = broad; s = singlet, d = doublet, t = triplet, dd = doublet of doublets, ddd = doublet of a doublet of doublets, m = multiplet, q = quartet, quint = quintet, sext = sextet, sept = septet, dq = doublet of quartets, dt = doublet of triplets. The abbreviations used for chemical groups are defined as follows: Me = CH3, Et = CH2CH3, r-Hex = C(CH3)2CH(CH3)2, r-Bu = C(CH3)3, n-Bu = unbranched butyl, n-Pr = unbranched propyl, c-Hex = cyclohexyl, ArH = aromatic hydrogen, HerH = heteroaromatic hydrogen. High resolution mass spectrometry (HRMS) was conducted using a Waters spectrometer with Q-ToF Premiers using electron spray ionization (ESI).

1.2. General synthetic procedures and characterization

1.2.1. General procedure for the synthesis of 6-bromo-5-2,3-dihydro[1,3]thiazolo[4,5-b]pyridines 13a–c

To a stirred mixture of 6-bromopyridin-2-amine (8, 1.00 equiv), a phenylboronic acid (1.16 equiv), and Na2CO3 (2.00 equiv) in a mixture of 1,4-dioxane and water (1:1), Pd(dppf)Cl2 (0.04 equiv) were added and stirred together at room temperature, before the mixture was stirred at 80 °C for 3 h. Thereafter, the reaction mixture was cooled to room temperature, diluted with water and extracted thoroughly with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The remaining residue was purified via column chromatography (gradient ethyl acetate/heptane) to afford the desired 6-phenylpyridin-2-amine 9. The corresponding 6-phenylpyridin-2-amine 9 (1.00 equiv) was dissolved in acetonitrile and cooled to 0 °C. Thereafter, N-bromosuccinimide (2.2 equiv) was added carefully. The reaction mixture was warmed to room temperature and stirred for 4 h. Subsequently, the reaction mixture was diluted with water and the resulting solid was filtered off. The solid was washed thoroughly with water and dried to afford the corresponding 3,5-dibromo-6-phenylpyridin-2-amine 10. To a stirred solution of a 3,5-dibromo-6-phenylpyridin-2-amine 10 (1.00 equiv) in N,N-dimethylformamide was added potassium O-ethyl dithiocarbonate (2.2 equiv) under an argon atmosphere at room temperature. The resulting mixture was heated under reflux conditions for 7 h. Thereafter, the reaction mixture was cooled to room temperature, poured onto ice water and acidified carefully with 2 N HCl. The obtained precipitate was filtered off, washed with water, collected and dried under reduced pressure to afford the corresponding 6-bromo-5-phenyl[1,3]thiazolo[4,5-b]pyridine-2-thiol and 6-bromo-5-(2-fluorophenyl)[1,3]thiazolo[4,5-b]pyridine-2(3H)thione thiol–thione tautomer 11. As IUPAC naming tools have afforded thiols as preferred names we have opted to use the thiol naming throughout the Supporting Information. However, several spectra indicate that the thiones are the preferred tautomer in the CDCl3 solution when
measuring the NMR spectra. In the next step, the corresponding 6-bromo-5-phenyl[1,3]thiazolo[4,5-
6-bromo-5-phenyl[1,3]thiazolo[4,5-b]pyridine 11 (1.0 equiv) was dissolved in acetic acid, and iron powder (15 equiv) was added carefully in portions. The resulting reaction mixture was stirred at a temperature of 100 °C for 10 h. After full conversion (indicated by LC–MS), the reaction mixture was cooled to 60 °C, and the iron powder was filtered off. The remaining solution was diluted with water, and the resulting precipitate was filtered, washed with water and dried under reduced pressure. The remaining crude residue was redissolved in dichloromethane, then water was added, followed by thorough extraction using dichloromethane. The combined organic layers were dried over anhydrous MgSO₄, filtered and dried under reduced pressure. The remaining residue was purified via column chromatography (gradient ethyl acetate/heptane) to afford the corresponding 6-bromo-5-phenyl[1,3]thiazolo[4,5-b]pyridine 12. The 6-bromo-5-phenyl[1,3]thiazolo[4,5-b]pyridine 12 (3.07 mmol, 1.0 equiv) was dissolved in absolute toluene (10 mL) in a flame-dried round-bottom flask under argon. Ammonia borane (285 mg, 9.20 mmol, 3.0 equiv) and B(C₆F₅)₃ (79 mg, 0.15 mmol, 0.05 equiv) were added to the solution and the resulting reaction mixture was stirred at a temperature of 45 °C for 5 h and concentrated thereafter under reduced pressure. The remaining residue was redissolved in acetonitrile, formic acid was added, and the reaction mixture was stirred at room temperature for 2 h. The phases were separated via phase separator and the organic layer was concentrated under reduced pressure. The remaining crude product was purified via column chromatography (gradient ethyl acetate/hexane) to afford the desired substituted 6-bromo-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine 13a–c.

1.2.2. Characterization of 6-bromo-5-2,3-dihydro[1,3]thiazolo[4,5-b]pyridines 13a–c

6-Bromo-5-phenyl-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine (13a, 700 mg, 70%). ¹H NMR (600 MHz, CDCl₃): δ 4.56 (s, 2H), 6.47-6.53 (m, 1H), 7.32-7.34 (m, 1H), 7.35-7.39 (m, 1H), 7.40-7.44 (m, 2H), 7.52-7.55 (m, 2H) ppm; ¹³C NMR (CDCl₃, 151 MHz): δ 49.0 (CH₂), 106.5 (C), 122.3 (C), 128.0 (CH), 128.2 (CH), 129.2 (CH), 131.8 (CH), 139.7 (C), 150.9 (C), 159.9 (C) ppm; HRMS (ESI, m/z): calcd. for C₁₂H₁₀N₂SBr, 292.9735 [M+H]+; found 292.9748.
Representative procedure for the synthesis of 6-bromo-5-(2-fluorophenyl)-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine (13b): To a stirred mixture of 6-bromopyridin-2-amine (8, 10.00 g, 56.88 mmol, 1.00 equiv), 2-fluorophenylboronic acid (9.52 g, 65.98 mmol, 1.16 equiv), and Na$_2$CO$_3$ (12.06 g, 113.8 mmol, 2.00 equiv) in a mixture of 1,4-dioxane (80 mL) and water (80 mL) at room temperature was added Pd(dppf)Cl$_2$ (1.67 g, 2.28 mmol, 0.04 equiv), and the mixture was stirred at 80 °C for 3 h. Thereafter, the reaction mixture was cooled to room temperature, diluted with water, and extracted thoroughly with ethyl acetate. The combined organic layer was washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The remaining residue was purified via column chromatography (gradient ethyl acetate/hexane) to afford 6-(2-fluorophenyl)pyridin-2-ylamine (9b, 9.98 g, 93%). $^1$H NMR (400 MHz, CDCl$_3$): δ$_H$ 7.92-7.88 (m, 1H), 7.53-7.49 (m, 1H), 7.36-7.31 (m, 1H), 7.25-7.20 (m, 1H), 7.16-7.10 (m, 1H), 6.50-6.48 (d, 1H), 4.53-4.44 (br. s, 2H, NH$_2$).

6-(2-Fluorophenyl)pyridin-2-ylamine (9b, 9.98 g, 52.49 mmol, 1.0 equiv) was dissolved in acetonitrile (140 mL) and cooled to 0 °C. Thereafter, N-bromosuccinimide (20.56 g, 115.49 mmol, 2.2 equiv) was added carefully. The reaction mixture was warmed to room temperature and stirred for 4 h. Subsequently, the reaction mixture was diluted with water, and the resulting solid was filtered off. The
solid was washed thoroughly with water and dried to afford 3,5-dibromo-6-(2-fluorophenyl)pyridin-2-ylamine (10b, 17.62 g, 97%) as an orange solid. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$H 7.92 (s, 1H), 7.44-7.33 (m, 2H), 7.25-7.20 (m, 1H), 7.17-7.12 (m, 1H), 5.07-4.98 (br. s, 2H, NH$_2$).

To a stirred solution of 3,5-dibromo-6-(2-fluorophenyl)pyridin-2-ylamine (10b, 17.62 g, 50.93 mmol, 1.0 equiv) in DMF (120 mL) at room temperature was added potassium O-ethyl dithiocarbonate (18.52 g, 112.04 mmol, 2.2 equiv). The resulting mixture was heated at reflux for 7 h. Thereafter, the reaction mixture was cooled to room temperature, poured onto ice water, and acidified with 2 N HCl. The obtained precipitate was filtered, washed with water, collected, and dried under reduced pressure to afford 11b as thiol–thione tautomer consisting of 6-bromo-5-(2-fluorophenyl)[1,3]thiazolo[4,5-b]pyridine-2-thiol and 6-bromo-5-(2-fluorophenyl)[1,3]thiazolo[4,5-b]pyridine-2(3H)thione (17.20 g, 97%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$H 9.96 (br. s, 1H), 8.01 (s, 1H), 7.50-7.44 (m, 1H), 7.41-7.38 (m, 1H), 7.29-7.25 (m, 1H), 7.19-7.15 (m, 1H).
The thiol–thione tautomer 11b (14.55 g, 42.64 mmol, 1.0 equiv) was dissolved in acetic acid (200 mL), and iron powder (35.71 g, 639.61 mmol, 15 equiv) was carefully added in portions. The resulting reaction mixture was stirred at 100 °C for 10 h. After full conversion (indicated by LC–MS), the reaction mixture was cooled to 60 °C, and the iron powder was filtered off. The remaining solution was diluted with water, and the resulting precipitate was filtered, washed with water, and dried under reduced pressure. The remaining crude residue was redissolved in DCM, then water was added, followed by thorough extraction. The combined organic layer was dried over magnesium sulfate, filtered, and dried under reduced pressure. The remaining residue was purified via column chromatography (gradient ethyl acetate/hexane) to afford 6-bromo-5-(2-fluorophenyl)[1,3]thiazolo[4,5-b]pyridine (12b, 8.75 g, 63%).

$^1$H NMR (400 MHz, CDCl$_3$): δH 9.32 (s, 1H), 8.64 (s, 1H), 7.54-7.45 (m, 2H), 7.31-7.27 (m, 1H), 7.21-7.16 (m, 1H).
6-Bromo-5-(2-fluorophenyl)[1,3]thiazolo[4,5-b]pyridine (12b, 1,000 mg, 3.07 mmol, 1.0 equiv) was dissolved in absolute toluene (10 mL) in an oven-dried round-bottom flask under argon, to which ammonia borane (285 mg, 9.20 mmol, 3.0 equiv) and B(C₆F₅)₃ (79 mg, 0.15 mmol, 0.05 equiv) were added. The resulting reaction mixture was stirred at a temperature of 45 °C for 5 h and then concentrated under reduced pressure. The remaining residue was redissolved in acetonitrile, formic acid was added, and the reaction mixture was stirred at room temperature for 2 h. The phases were separated via phase separator, and the organic layer was concentrated under reduced pressure. The remaining crude product was purified via column chromatography (gradient ethyl acetate/hexane) to afford 6-bromo-5-(2-fluorophenyl)-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine (13b, 596 mg, 59%). ¹H NMR (600 MHz, CDCl₃): δ 4.56 (s, 2H), 6.88 (brs, 1H), 7.11–7.16 (m, 1H), 7.21 (td, J = 7.5, 1.1 Hz, 1H), 7.31 (s, 1H), 7.34 (td, J = 7.4, 1.9 Hz, 1H), 7.36–7.41 (m, 1H) ppm; ¹³C NMR (CDCl₃, 151 MHz): δ 49.0 (CH₂), 108.1 (C), 115.7 (CH), 115.9 (CH), 123.5 (C), 124.0 (CH), 127.7 (d, C), 130.4 (CH), 131.1 (CH), 146.2 (C), 158.7 (C), 160.3 (d, C) ppm; HRMS (ESI, m/z): calcd. C₁₂H₇BrFN₂S, 310.9671 [M+H]+; found 310.9654.
$^1$H NMR (400 MHz, CDCl$_3$) and LC–MS spectra of a second batch within the preparation of 13b:
Representative procedure for the synthesis of 6-bromo-5-(2-methylphenyl)-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine (13c): To a stirred mixture of 6-bromopyridin-2-amine (8, 15.00 g, 86.69 mmol, 1.00 equiv), 2-methylphenylboronic acid (23.58 g, 173.39 mmol, 2.00 equiv) and K₂CO₃ (23.96 g, 173.39 mmol, 2.00 equiv) in a mixture of 1,4-dioxane (160 mL) and water (160 mL) at room temperature was added Pd(dppf)Cl₂ (3.17 g, 4.34 mmol, 0.05 equiv) and the mixture was stirred at 80 °C for 3 h. Thereafter, the reaction mixture was cooled to room temperature, diluted with water and extracted thoroughly with ethyl acetate. The combined organic layer was washed with brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The remaining residue was purified via column chromatography (gradient ethyl acetate/hexane) to afford 6-(2-methylphenyl)pyridin-2-amine (9c, 9.40 g, 59%). ¹H NMR (400 MHz, CDCl₃): δH 7.51-7.49 (m, 1H), 7.37-7.34 (m, 1H), 7.30-7.23 (m, 2H), 6.74-6.72 (m, 1H), 6.48-6.44 (m, 1H), 4.52-4.46 (br. s, 2H, NH₂), 2.35 (s, 3H).

6-(2-Methylphenyl)pyridin-2-amine (9c, 8.97 g, 48.20 mmol, 1.0 equiv) was dissolved in acetonitrile (140 mL) and cooled to 0 °C. Thereafter, N-bromosuccinimide (18.87 g, 106.04 mmol, 2.2 equiv) was added carefully. The reaction mixture was warmed to room temperature and stirred for 2 h.
Subsequently, the reaction mixture was diluted with water and the resulting solid was filtered off. The solid was washed thoroughly with water and dried to afford compound 3,5-dibromo-6-(2-methylphenyl)pyridin-2-ylamine (10c, 13.10 g, 79%) as an orange solid. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$H 7.91 (s, 1H), 7.33-7.27 (m, 3H), 7.20-7.17 (m, 1H), 5.04-4.96 (br. s, 2H, NH$_2$), 2.18 (s, 3H). The reaction was repeated on the same scale to afford 26.17 g of 10c in total.

To a stirred solution of 3,5-dibromo-6-(2-methylphenyl)pyridin-2-ylamine (10c, 26.17 g, 76.50 mmol, 1.0 equiv) in N,N-dimethylformamide (120 mL) at room temperature was added potassium O-ethyl dithiocarbonate (27.81 g, 168.31 mmol, 2.2 equiv). The resulting mixture was heated under reflux conditions for 6.5 h. Thereafter, the reaction mixture was cooled to room temperature, poured onto ice water and acidified with 2 N HCl. The obtained precipitate was filtered, washed with water, collected and dried under reduced pressure to afford the 6-bromo-5-(2-methylphenyl)[1,3]thiazolo[4,5-b]pyridine-2-thiol and 6-bromo-5-(2-methylphenyl)[1,3]thiazolo[4,5-b]pyridine-2(3H)-thione thiol–thione tautomer 11c (25.00 g, 97%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$H 9.72 (br. s, 1H), 8.01 (s, 1H), 7.37-7.35 (m, 1H), 7.33-7.29 (m, 2H), 7.21-7.19 (m, 1H), 2.16 (s, 3H).
6-Bromo-5-(2-methylphenyl)[1,3]thiazolo[4,5-b]pyridine-2-thiol (11c, 26.81 g, 79 mmol, 1.0 equiv) was dissolved in acetic acid (350 mL), and iron powder (55.49 g, 994 mmol, 15 equiv) was added carefully in portions. The resulting reaction mixture was stirred at a temperature of 100 °C for 8 h. After full conversion (indicated by LC–MS), the reaction mixture was cooled to 60 °C, and the iron powder was filtered off. The remaining solution was diluted with water, and the resulting precipitate was filtered, washed with water and dried under reduced pressure. The remaining crude residue was redissolved in dichloromethane, then water was added, followed by thorough extraction. The combined organic layer was dried over magnesium sulfate, filtered and dried under reduced pressure. The remaining residue was purified via column chromatography (gradient ethyl acetate/hexane) to afford 6-bromo-5-(2-methylphenyl)[1,3]thiazolo[4,5-b]pyridine (12c, 8.75 g, 65%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta_{H}$ 9.32 (s, 1H), 8.63 (s, 1H), 7.38–7.35 (m, 1H), 7.32–7.27 (m, 3H), 2.18 (s, 3H).
6-Bromo-5-(2-methylphenyl)[1,3]thiazolo[4,5-b]pyridine (12c, 1,000 mg, 3.07 mmol, 1.0 equiv) was dissolved in absolute toluene (10 mL) in a oven-dried round-bottom flask under argon, and ammonia borane (285 mg, 9.20 mmol, 3.0 equiv) and B(C$_6$F$_5$)$_3$ (79 mg, 0.15 mmol, 0.05 equiv) were added. The resulting reaction mixture was stirred at a temperature of 45 °C for 5 h and concentrated thereafter under reduced pressure. The remaining residue was redissolved in acetonitrile, formic acid was added, and the reaction mixture was stirred at room temperature for 2 h. The phases were separated via phase separator and the organic layer was concentrated under reduced pressure. The remaining crude product was purified via column chromatography (gradient ethyl acetate/hexane) to afford 6-bromo-5-(2-methylphenyl)-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine (13c, yield after formic acid-mediated borane cleavage of intermediates 17d and 17e: 109 mg, 54%).$^1$H NMR (600 MHz, CDCl$_3$): δ 2.18 (s, 3H), 4.34 (s, 2H), 7.15–7.18 (m, 1H), 7.22–7.26 (m, 2H), 7.26–7.30 (m, 2H), 7.35–7.40 (m, 1H) ppm; $^{13}$C NMR (CDCl$_3$, 151 MHz): δ 19.4 (CH$_3$), 48.9 (CH$_2$), 107.0 (C), 122.6 (C), 125.6 (CH), 128.4 (CH), 128.9 (CH), 130.0 (CH), 130.9 (CH), 136.1 (C), 139.7 (C), 151.2 (C), 160.0 (C) ppm; HRMS (ESI, m/z): calcd. for C$_{13}$H$_{12}$BrN$_2$S, 306.9919 [M+H]$^+$; found 306.9905.
$^1$H NMR (400 MHz, DMSO-d$_6$):

LC–MS:

3: UV Detector: TMC: Wavelength Range: (210 - 450)

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$^{310}C$ 310.2

m/z

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$^{308}C$ 308.1

m/z
1.2.3. General procedure for the synthesis of substituted 6-methyl-2,3-dihydro[1,3]thiazolo[4,5-b]pyridines 7a–c

The corresponding 6-bromo-5-phenyl[1,3]thiazolo[4,5-b]pyridine 13a–c (4.56 mmol, 1.0 equiv), methylboronic acid (1.13 g, 18.24 mmol, 4.0 equiv), potassium phosphate (1.94 g, 9.12 mmol, 2.0 equiv), palladium(II) acetate (103 mg, 0.46 mmol, 0.1 equiv) and 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (579 mg, 1.37 mmol, 0.3 equiv) were consecutively dissolved in absolute toluene (40 mL) in a flame-dried round-bottom flask under argon. The resulting reaction mixture was stirred under reflux conditions for 3.5 h. After cooling to room temperature, water (80 mL) and toluene (20 mL) were added, followed by addition of ammonium pyrrolidine dithiocarbamate (328 mg, 2.00 mmol, 0.44 equiv). The reaction mixture was stirred for 1 h at room temperature, and the organic layer was washed with saturated aq NaHCO₃ solution, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The resulting crude residue was purified via column chromatography (gradient ethyl acetate/heptane) to afford the desired 5-phenyl-6-methyl[1,3]thiazolo[4,5-b]pyridine 5, 15a or 15c as an intermediate. The corresponding 5-phenyl-6-methyl[1,3]thiazolo[4,5-b]pyridine 5, 15a or 15c (1.23 mmol, 1.0 equiv) was dissolved in absolute toluene (10 mL) in a flame-dried round-bottom flask under argon. Ammonia borane (114 mg, 3.69 mmol, 3.0 equiv) and B(C₆F₅)₃ (32 mg, 0.06 mmol, 0.05 equiv) were added and the resulting reaction mixture was stirred at a temperature of 45 °C for 5 h and concentrated thereafter under reduced pressure. The remaining residue was redissolved in acetonitrile, formic acid was added, and the reaction mixture was stirred at room temperature for further 45 min. The phases were separated, and the organic layer was concentrated under reduced pressure. The resulting crude product was purified via column chromatography (gradient ethyl acetate/heptane) to afford the desired 5-phenyl-6-methyl-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine 7a–c.

1.2.4. Characterization of substituted 6-methyl-2,3-dihydro[1,3]thiazolo[4,5-b]pyridines 7a–c

6-Methyl-5-phenyl-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine (7a, 610 mg, 55%). ¹H NMR (600 MHz, CDCl₃): δ 2.08 (s, 3H), 4.30 (s, 2H), 7.03 (s, 1H), 7.31–7.35 (m, 2H), 7.40–7.41 (m, 4H) ppm; ¹³C NMR (CDCl₃, 151 MHz): δ 18.8 (CH₃), 48.6 (CH₂), 119.6 (C), 120.2 (C), 127.4 (CH), 128.1 (CH), 129.0 (CH), 130.5 (CH), 140.7 (C), 150.8 (C), 159.4 (C) ppm; HRMS (ESI, m/z): calcd. for C₁₃H₁₃N₂S, 229.0799 [M+H]⁺; found 229.0790.
5-(2-Fluorophenyl)-6-methyl-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine (7b, 630 mg, 62%). \(^1\)H NMR (600 MHz, CDCl\(_3\)):\(\delta\) 2.00 (s, 3H), 4.55 (s, 2H), 6.61 (brs, 1H), 7.07 (s, 1H), 7.09–7.12 (m, 1H), 7.19–7.21 (m, 1H), 7.32–7.36 (m, 2H) ppm; \(^1\)^C NMR (CDCl\(_3\), 151 MHz): \(\delta\) 17.96 (CH\(_3\)), 17.99 (CH\(_3\)), 48.8 (CH\(_2\)), 115.6 (CH), 115.7 (CH), 121.0 (C), 121.8 (C), 124.15 (CH), 124.18 (CH), 129.59 (CH), 129.64 (CH), 130.2 (CH), 131.40 (CH), 131.43 (CH), 145.6 (C), 158.9 (C), 159.2 (C), 160.5 (C) ppm; HRMS (ESI, m/z): calcd. for C\(_{13}\)H\(_{12}\)FN\(_2\)S, 247.0705 [M+H]\(^+\); found 247.0724.
6-Methyl-5-(2-methylphenyl)-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine (7c, 212 mg, 52%). \(^1\)H NMR (600 MHz, CDCl\(_3\)): \(\delta\) 1.85 (s, 3H), 2.12 (s, 3H), 4.22 (s, 2H), 7.02 (s, 1H), 7.13 (d, \(J = 7.2\) Hz, 1H), 7.19–7.26 (m, 3H), 7.69 (brs, 1H) ppm; \(^1\)C NMR (CDCl\(_3\), 151 MHz): \(\delta\) 17.8 (CH\(_3\)), 19.4 (CH\(_3\)), 48.5 (CH\(_2\)), 119.7 (C), 120.0 (C), 125.6 (CH), 127.6 (CH), 129.0 (CH), 129.9 (CH), 130.0 (CH), 136.1 (C), 140.2 (C), 151.0 (C), 159.3 (C) ppm; HRMS (ESI, m/z): calcd. for C\(_{14}\)H\(_{15}\)N\(_2\)S, 243.0956 [M+H]\(^+\); found 243.0963.
1.2.5. General procedure for the synthesis of N-acylated 6-bromo-2,3-dihydro[1,3]thiazolo[4,5-b]pyridines 14a–c

The corresponding acid chloride (0.36 mmol, 1.1 equiv) and triethylamine (0.10 mL, 0.72 mmol, 2.2 equiv) were added to a stirred solution of the corresponding 6-bromo-5-phenyl-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine 13 (0.33 mmol, 1.00 equiv) in absolute dichloromethane (5 mL). The resulting reaction mixture was stirred at room temperature for 2 h, followed by dilution with dichloromethane and water, and subsequent extraction and phase separation. The aqueous layer was thoroughly extracted with dichloromethane, and the combined organic layers were dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The resulting crude product was purified via column chromatography (gradient ethyl acetate/heptane) to afford the desired target compounds 14a–c.

1.2.6. Characterization of N-acylated 6-bromo-2,3-dihydro[1,3]thiazolo[4,5-b]pyridines 14a–c

14a

1-(6-Bromo-5-phenyl[1,3]thiazolo[4,5-b]pyridin-3(2H)-yl)ethanone (14a, 92 mg, 72%). ¹H NMR (600 MHz, CDCl₃): δ 2.65 (s, 3H), 5.35 (s, 2H), 7.39–7.47 (m, 3H), 7.65–7.66 (m, 1H), 7.66–7.70 (m, 2H) ppm; ¹³C NMR (CDCl₃, 151 MHz): δ 25.0 (CH₃), 49.9 (CH₂), 112.8 (C), 126.3 (C), 127.9 (CH), 128.7 (CH), 129.7 (CH), 134.6 (CH), 138.8 (C), 150.7 (C), 151.2 (C), 170.4 (C) ppm; HRMS (ESI, m/z): calcd. for C₁₄H₁₂BrN₂O₃S, 334.9854 [M+H]⁺; found 334.9854.

[UV detector spectrum and mass spectra provided here]
1-[6-Bromo-5-(2-fluorophenyl)[1,3]thiazolo[4,5-b]pyridin-3(2H)-yl]-2-methylpropan-1-one (14b, 22 mg, 17%). $^1$H NMR (600 MHz, CDCl$_3$): δ 1.14 (d, $J = 6.9$ Hz, 6H), 3.95–4.00 (m, 1H), 5.35 (s, 2H), 7.15–7.25 (m, 2H), 7.38–7.45 (m, 2H), 7.64–7.66 (m, 1H) ppm; $^{13}$C NMR (CDCl$_3$, 151 MHz): δ 19.0 (CH$_3$), 32.8 (CH), 50.3 (CH$_2$), 114.8 (C), 115.8 (CH), 123.8 (CH), 127.3 (C), 127.6 (C), 130.6 (CH), 131.2 (CH), 133.9 (CH), 147.3 (C), 150.4 (C), 159.5 (d, C), 177.9 (C) ppm; HRMS (ESI, m/z): calcd. for C$_{16}$H$_{15}$BrFN$_2$OS, 381.0065 [M+H]$^+$; found 381.0072.

![UV Detector](image-url)
1-[6-Bromo-5-(2-methylphenyl)[1,3]thiazolo[4,5-b]pyridin-3(2H)-yl]ethanone (14c, 51 mg, 73%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 2.20–2.23 (m, 3H), 2.57 (s, 3H), 5.36–5.43 (m, 2H), 7.21–7.23 (m, 1H), 7.28–7.32 (m, 2H), 7.34–7.37 (m, 1H), 7.67–7.69 (m, 1H) ppm; $^{13}$C NMR (CDCl$_3$, 151 MHz): $\delta$ 19.6 (CH$_3$), 24.8 (CH$_3$), 49.8 (CH$_3$), 114.6 (C), 125.5 (CH), 126.5 (C), 128.6 (CH), 129.1 (CH), 130.1 (CH), 133.8 (CH), 135.9 (C), 139.1 (C), 150.4 (C), 152.7 (C), 170.5 (C) ppm; HRMS (ESI, m/z): calcd. for C$_{15}$H$_{14}$BrN$_2$OS, 349.0025 [M+H]$^+$; found 349.0010.
1.2.7. General procedure for the synthesis of N-acylated 6-methyl-2,3-dihydro[1,3]thiazolo[4,5-b]pyridines 16a–f

The corresponding acyl chloride (0.31 mmol, 1.1 equiv) and triethylamine (0.09 mL, 0.63 mmol, 2.2 equiv) were added to a stirred solution of the corresponding 6-methyl-5-phenyl-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine 7a–c (0.28 mmol, 1.00 equiv) in absolute DCM (5 mL). The resulting reaction mixture was stirred at room temperature for 30–120 min, followed by dilution with DCM and water, and subsequent extraction and phase separation. The aqueous layer was thoroughly extracted with DCM, and the combined organic layer was dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The remaining crude product was purified via column chromatography (gradient ethyl acetate/heptane) to afford the corresponding desired target compound 16a–f.

1.2.8. Characterization of N-acylated 6-methyl-2,3-dihydro[1,3]thiazolo[4,5-b]pyridines 16a–f

![Chemical structure of 16a](image)

1-(6-Methyl-5-phenyl[1,3]thiazolo[4,5-b]pyridin-3(2H)-yl)ethanone (16a, 150 mg, 94%). ¹H NMR (600 MHz, CDCl₃): δ 2.32 (s, 3H), 2.68 (s, 3H), 5.32 (s, 2H), 7.33 (s, 1H), 7.36–7.39 (m, 1H), 7.42–7.45 (m, 2H), 7.51–7.53 (m, 2H) ppm; ¹³C NMR (CDCl₃, 151 MHz): δ 19.7 (CH₃), 24.8 (CH₃), 49.2 (CH₂), 124.1 (C), 125.9 (C), 127.9 (CH), 128.1 (CH), 129.0 (CH), 139.9 (C), 149.6 (C), 151.8 (C), 170.5 (C) ppm; HRMS (ESI, m/z): calcd. for C₁₅H₁₅N₂O₂S, 271.0906 [M+H]⁺; found 271.0905.
2-Methyl-1-(6-methyl-5-phenyl[1,3]thiazolo[4,5-b]pyridin-3(2H)-yl)propan-1-one (16b, 130 mg, 72%). ¹H NMR (600 MHz, CDCl₃): δ 1.20 (d, J = 6.7 Hz, 6H), 2.32 (s, 2H), 4.15–4.19 (m, 1H), 5.32 (s, 2H), 7.33 (s, 1H), 7.37–7.39 (m, 1H), 7.43–7.45 (m, 2H), 7.51–7.53 (m, 2H) ppm; ¹³C NMR (CDCl₃, 151 MHz): δ 19.2 (2 × CH₃), 19.7 (CH₃), 32.6 (CH), 49.6 (CH₂), 124.3 (C), 125.8 (C), 127.8 (CH), 128.0 (CH), 129.0 (CH), 132.9 (CH), 139.9 (C), 149.4 (C), 151.8 (C), 177.9 (C) ppm; HRMS (ESI, m/z): calcd. for C₁₁H₁₀N₂O₃ 299.1218 [M+H]⁺; found 299.1218.
16c

1-[5-(2-Fluorophenyl)-6-methyl[1,3]thiazolo[4,5-b]pyridin-3(2H)-yl]ethanone (16c, 150 mg, 84%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 2.16 (d, $J = 2.2$ Hz, 3H), 2.61 (s, 2H), 5.32 (s, 2H), 7.12–7.15 (m, 1H), 7.21–7.24 (m, 1H), 7.34 (s, 1H), 7.37–7.49 (m, 2H) ppm; $^{13}$C NMR (CDCl$_3$, 151 MHz): $\delta$ 18.41 (CH$_3$), 18.44 (CH$_3$), 24.7 (CH$_3$), 49.3 (CH$_3$), 115.7 (CH), 115.8 (CH), 124.09 (CH), 124.12 (CH), 125.2 (C), 127.8 (C), 129.9 (CH), 130.0 (CH), 131.37 (CH), 131.40 (CH), 132.1 (CH), 147.0 (C), 149.6 (C), 158.9 (C), 160.6 (C), 170.6 (C) ppm; HRMS (ESI, m/z): calcd. for C$_{15}$H$_{14}$FN$_2$OS, 289.0811 [M+H]$^+$; found 289.0806.
16d

1-[5-(2-Fluorophenyl)-6-methyl[1,3]thiazolo[4,5-b]pyridin-3(2H)-yl]-2-methylpropan-1-one (16d, 109 mg, 75%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 1.15 (d, $J = 6.7$ Hz, 6H), 2.15 (d, $J = 1.9$ Hz, 3H), 4.08–4.11 (m, 1H), 5.32 (s, 2H), 7.13–7.16 (m, 1H), 7.22–7.25 (m, 1H), 7.34 (s, 1H), 7.36–7.41 (m, 2H) ppm; $^{13}$C NMR (CDCl$_3$, 151 MHz): $\delta$ 18.40 (CH$_3$), 18.43 (CH$_3$), 19.1 (2 × CH$_3$), 32.5 (CH), 49.7 (CH$_2$), 115.6 (CH), 115.8 (CH), 124.08 (CH), 124.10 (CH), 125.3 (C), 127.66 (C), 127.7 (C), 127.8 (C), 129.86 (CH), 129.9 (CH), 131.38 (CH), 131.40 (CH), 132.2 (CH), 147.1 (CH), 149.4 (CH), 159.0 (C), 160.6 (C), 177.9 (C) ppm; HRMS (ESI, m/z): calcd. for C$_{17}$H$_{18}$FN$_2$OS, 317.1124 [M+H]$^+$; found 317.1109.
16e

1-[6-Methyl-5-(2-methylphenyl)[1,3]thiazolo[4,5-b]pyridin-3(2H)-yl]ethanone (16e, 150 mg, 84%). ¹H NMR (600 MHz, CDCl₃): δ 2.02 (s, 3H), 2.14 (s, 3H), 2.57 (s, 3H), 5.33 (s, 2H), 7.13 (d, J = 7.2 Hz, 1H), 7.22–7.31 (m, 3H), 7.34 (s, 1H) ppm; ¹³C NMR (CDCl₃, 151 MHz): δ 18.5 (CH₃), 19.6 (CH₃), 24.6 (CH₃), 49.2 (CH₂), 124.2 (C), 125.6 (CH), 126.7 (C), 127.9 (CH), 129.0 (CH), 130.2 (CH), 132.2 (CH), 135.9 (C), 139.5 (C), 149.3 (C), 152.8 (C), 170.6 (C) ppm; HRMS (ESI, m/z): calcd. for C₁₆H₁₇N₂OS, 285.1062 [M+H]+; found 285.1066.

![UV detector and mass spectra graphs](image)
2-Methyl-1-[6-methyl-5-(2-methylphenyl)[1,3]thiazolo[4,5-b]pyridin-3(2H)-yl]propan-1-one (16f, 145 mg, 74%). \(^1\)H NMR (600 MHz, CDCl\(_3\)): \(\delta\) 1.10 (d, \(J = 6.7\) Hz, 6H), 2.01 (s, 3H), 2.13 (s, 3H), 5.33 (s, 2H), 4.08–4.12 (m, 1H), 5.33 (s, 2H), 7.12 (d, \(J = 7.2\) Hz, 1H), 7.23–7.31 (m, 3H), 7.34 (s, 1H) ppm; \(^{13}\)C NMR (CDCl\(_3\), 151 MHz): \(\delta\) 18.5 (2 × CH\(_3\)), 19.5 (CH\(_3\)), 32.3 (CH\(_3\)), 49.7 (CH\(_2\)), 124.4 (C), 125.6 (CH), 126.6 (C), 127.9 (CH), 129.0 (CH), 130.1 (CH), 132.2 (CH), 135.8 (C), 139.6 (C), 149.1 (C), 152.9 (C), 178.0 (C) ppm; HRMS (ESI, m/z): calcd. for C\(_{18}\)H\(_{21}\)N\(_2\)O\(_2\), 313.1375 [M+H]\(^{+}\); found 313.1363.

3: UV Detector: 1AC; Wavelength Range: (210 – 450) 1.92e+2

Peak Number | Time | Area % Total | RT LogP |
--- | --- | --- | --- |
1 | 1.85 | 99.312 | 1295 |

Peak ID | Time | Mass Found |
--- | --- | --- |
1 | 1.85 | 313 |

1: (Time: 1.85) Combine (250:256–228:241) 2:MS ES+ 7.3e+007

Peak ID | Time | Mass Found |
--- | --- | --- |
1 | 1.85 | 313.3 |

1: (Time: 1.85) Combine (249:256–227:241) 2:MS ES– 3.5e+004

Peak ID | Time | Mass Found |
--- | --- | --- |
1 | 1.85 | 145.0 |

Peak ID | Time | Mass Found |
--- | --- | --- |
1 | 1.85 | 145.0 |

1: (Time: 1.85) Combine (249:256–227:241) 2:MS ES– 3.5e+004

Peak ID | Time | Mass Found |
--- | --- | --- |
1 | 1.85 | 145.0 | 265.3 | 299.4 | 395.3 | 483.1 | 451.6 | 551.3 | 670.9 | 743.0 | 832.1 | 912.1 | 998.5 | 1000.0
1.2.9. Characterization of borylated compounds 17a–e and side product 17f

![Image of borylated compound 17a]

**17a**

In a dedicated additional synthesis complementing the optimization work outlined in Table 1 of the Main Manuscript, 6-methyl-5-phenyl[1,3]thiazolo[4,5-5]pyridine (15a, 3.07 mmol, 1.0 equiv) was dissolved in absolute toluene (10 mL) in a flame-dried round-bottom flask under argon. Ammonia borane (285 mg, 9.20 mmol, 3.0 equiv) and B(C₆F₅)₃ (79 mg, 0.15 mmol, 0.05 equiv) were added to the solution and the resulting reaction mixture was stirred at a temperature of 45 °C for 5 h and concentrated thereafter under reduced pressure. The remaining crude product was purified carefully via column chromatography (gradient ethyl acetate/hexane) to afford the borylated 2,3-dihydro[1,3]thiazolo[4,5-5]pyridine 17a.

3-Boryl-6-methyl-5-phenyl-2,3-dihydro[1,3]thiazolo[4,5-5]pyridine (17a, 49 mg, 11%). ¹H NMR (400 MHz, CDCl₃): δ 1.63 (br s, 2H), 2.15 (s, 3H), 4.69–4.72 (m, 2H), 7.10–7.12 (m, 1H), 7.31–7.38 (m, 2H), 7.39–7.48 (m, 3H), 7.21–7.24 (m, 1H) ppm; HRMS (ESI, m/z): calcd. for C₁₄H₁₅BN₂S [M⁺] 254.1049; found 254.1042.
In a dedicated additional synthesis complementing the optimization work outlined in Table 1 of the Main Manuscript, 6-methyl-5-(2-fluorophenyl)-[1,3]thiazolo[4,5-b]pyridine (5, 3.07 mmol, 1.0 equiv) was dissolved in absolute toluene (10 mL) in a flame-dried round-bottom flask under argon. Ammonia borane (285 mg, 9.20 mmol, 3.0 equiv) and B(C$_6$F$_5$)$_3$ (79 mg, 0.15 mmol, 0.05 equiv) were added to the solution and the resulting reaction mixture was stirred at a temperature of 45 °C for 5 h and concentrated thereafter under reduced pressure. The remaining crude product was purified carefully via column chromatography (gradient ethyl acetate/hexane) to afford the borylated 2,3-dihydro[1,3]thiazolo[4,5-b]pyridine 17b together with inseparable impurities that may have emerged from further azaboretidine structures.

3-Boryl-6-methyl-5-(2-fluorophenyl)-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine (17b, 78 mg, 83% purity, 18%). $^1$H NMR (400 MHz, CDCl$_3$): δ 1.77 (s, 3H), 2.40-2.65 (br. d, 2H), 5.05-5.12 (m, 2H), 6.89-6.91 (m, 1H), 7.08-7.25 (m, 3H), 7.32–7.48 (m, 1H) ppm; HRMS (ESI, m/z): calcd. for C$_{13}$H$_{12}$BF$_2$N$_2$S [M]$^+$ 258.0798; found 258.0805.
In a dedicated additional synthesis complementing the optimization work outlined in Table 1 of the Main Manuscript, 6-methyl-5-(2-methylphenyl)[1,3]thiazolo[4,5-b]pyridine (15c, 3.07 mmol, 1.0 equiv) was dissolved in absolute toluene (10 mL) in a flame-dried round-bottom flask under argon. Ammonia borane (285 mg, 9.20 mmol, 3.0 equiv) and B(C₆F₅)₃ (79 mg, 0.15 mmol, 0.05 equiv) were added to the solution and the resulting reaction mixture was stirred at a temperature of 45 °C for 5 h and concentrated thereafter under reduced pressure. The remaining crude product was purified carefully via column chromatography (gradient ethyl acetate/hexane) to afford the borylated 2,3-dihydro[1,3]thiazolo[4,5-b]pyridine 17c.

3-Boryl-6-methyl-5-(2-methylphenyl)-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine (17c, 61 mg, 12%). ¹H NMR (400 MHz, CDCl₃): δ 2.03 (s, 3H), 2.31 (s, 3H), 2.51 (br s, 2H), 5.11–5.15 (m, 2H), 6.87–6.89 (m, 1H), 7.10–7.18 (m, 3H), 7.21–7.24 (m, 1H) ppm; HRMS (ESI, m/z): calcd. for C₁₄H₁₅BN₂S [M]+ 254.1049; found 254.1042.
6-Bromo-5-(2-methylphenyl)[1,3]thiazolo[4,5-b]pyridine (3.07 mmol, 1.0 equiv) was dissolved in absolute toluene (10 mL) in a flame-dried round-bottom flask under argon. Ammonia borane (285 mg, 9.20 mmol, 3.0 equiv) and B(C₆F₅)₃ (79 mg, 0.15 mmol, 0.05 equiv) were added to the solution and the resulting reaction mixture was stirred at a temperature of 45 °C for 5 h and concentrated thereafter under reduced pressure. The remaining residue was redissolved in acetonitrile, formic acid was added, and the reaction mixture was stirred at room temperature for 2 h. The phases were separated via phase separator and the organic layer was concentrated under reduced pressure. The remaining crude product was purified via column chromatography (gradient ethyl acetate/hexane) to afford the desired substituted 6-bromo-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine 13c. As this reduction was subject to a detailed optimization, borylated intermediates 17d, 17e and arylated side product 17f could be isolated in several cases depending on the reaction conditions.

3-Boryl-6-bromo-5-(2-methylphenyl)-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine (17d, 179 mg, 29%). ¹H NMR (600 MHz, CDCl₃): δ 2.14 (s, 3H), 2.51 (brs, 2H), 5.11–5.14 (m, 2H), 7.01–7.05 (m, 1H), 7.10–7.12 (m, 1H), 7.22–7.25 (m, 2H), 7.30–7.34 (m, 1H) ppm; ¹³C NMR (CDCl₃, 151 MHz): δ 19.4 (CH₃), 56.8 (CH₂), 104.0 (C), 125.6 (CH), 128.3 (CH), 128.9 (CH), 129.0 (CH), 129.7 (CH), 131.0 (C), 136.3 (C), 136.4 (C), 144.3 (C), 157.5 (C) ppm; HRMS (ESI, m/z): calcd. for C₁₃H₁₂BBrN₂S [M]+ 317.9997; found 317.9982.
4-[(6-Bromo-5-(2-methylphenyl)[1,3]thiazolo[4,5-b]pyridin-3(2H)-yl]-1,3,2,4-diazadiboretidin-2-
amine (17e, 106 mg, 9%). \( ^1H \) NMR (600 MHz, CDCl\(_3\)): \( \delta \) 2.12 (s, 3H), 1.90-2.40 (br m, 2H), 2.62-2.78 (br d, 1H), 3.24-3.41 (br d, 1H), 5.17-5.19 (m, 1H), 5.31–5.35 (m, 1H), 6.99–7.03 (m, 1H), 7.22–7.28 (m, 2H), 7.31–7.36 (m, 1H), 8.16–8.17 (m, 1H) ppm; \( ^{13}C \) NMR (CDCl\(_3\), 151 MHz): \( \delta \) 19.4 (CH\(_3\)), 55.5 (CH\(_2\)), 104.3 (C), 125.8 (CH), 128.4 (CH), 128.9 (CH), 129.2 (CH), 129.7 (CH), 130.0 (C), 135.8 (C), 136.4 (C), 144.3 (C), 157.3 (C) ppm; HRMS (ESI, m/z): calcd. for C\(_{13}\)H\(_{14}\)BrN\(_5\)S [M]+ 373.0339; found 373.0352.
5-(2-Fluorophenyl)-6-pentafluorophenyl-2,3-dihydro[1,3]thiazolo[4,5-b]pyridine (17f, 24 mg, 2%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 2.20 (s, 3H), 4.55 (s, 2H), 6.58 (br. s, 1H, NH), 7.16-7.18 (m, 1H), 7.21–7.31 (m, 4H) ppm; HRMS (ESI, m/z): calcd. for $\text{C}_{19}\text{H}_{11}\text{F}_5\text{N}_2\text{S}$ [M$^+$] 394.0563; found 394.0552.
2. Biology and biochemistry

2.1. In vivo greenhouse screening

Seeds of mono- and dicotyledonous weed plants and crop plants were sown in plastic or organic planting pots in sandy loam and covered with soil (replicates: $n = 10$, i.e., 10 monocotyledonous weed seeds were grown per pot or $n = 5$, i.e., 5 dicotyledonous weed seeds were grown per pot). The 2,3-dihydro[1,3]thiazolo[4,5-b]pyridines described above (e.g., 7a–c, 13a–c, 14a–c, 16a–f, and 17a–b), formulated in the form of wettable powder (WP), were applied to the surface of the covering soil as aqueous suspension or emulsion, with the addition of 0.5% of an additive at an application rate of 600 L of water/ha (converted). Following treatment, the pots were placed in a greenhouse and kept under optimum growth conditions for the test plants. The test plants were placed in the greenhouse for ca. three weeks under optimum growth conditions, and then the effect of the preparations was assessed visually in comparison to untreated control plants (herbicidal effect: 100% = plants died off, 0% = as untreated control plants). Efficacy values were given based on a rating scale by final visual experts’ assessment of green mass, i.e., 5 ≥ 90% inhibition, 4 = 70–89% inhibition, 3 = 50–69% inhibition, 2 = 40–49% inhibition, 1 = 21–39% inhibition, and — < 20% inhibition. Advanced screening was carried out with or as emulsifiable concentrate formulations, three replicate pots, and a standardized number of seeds per pot depending on the plant species (10 seeds for corn and wheat spectrum). The harmful plants and crops used in greenhouse tests were the following species: Alopecurus myosuroides (ALOMY), resistant Alopecurus myosuroides (ALOMY_R, origin: Germany), Apera spica-venti (APESV), Brachiaria platyphylla (BRAPP), Bromus tectorum (BROTE), Echinochloa crus-galli (ECHCG), Digitaria sanguinalis (DIGSA), Eleusine indica (ELEIN), Glycine max (GLXMA), Lolium rigidum (LOLRI), Lolium sp. (LOLSS), resistant Lolium sp. (LOLSS_R, origin: France), Poa annua (POAAN), Setaria viridis (SETVI), Sorghum halepense (SORHA), Triticum aestivum (TRZAS), and Zea mays (ZEAMX).
2.2. Biochemistry

2.2.1. LpFAT A expression and purification

The fat a03 gene from *Lemna paucicostata*, in which the N-terminal amino acids representing the chloroplast transit peptide were replaced by an N-terminal 6xHis-tag, was cloned into a pET24 vector [1]. The LpFAT A protein was expressed in *E. coli* BL21Star(DE3) cells. 5 mL of an overnight culture of *E. coli* cells grown in LB medium with 100 µg/mL carbenicillin were used to inoculate 0.5 L of autoinduction medium containing 100 µg/mL carbenicillin [2]. The bacteria were grown at 37 °C and 120 rpm for about 4.5 h to reach OD_{600} = 0.6 and then further cultivated at 21 °C overnight. The bacteria were harvested by centrifugation (20 min, 6,000 g) and stored frozen at −80 °C. LpFAT A protein was purified using the Ni-NTA Fast Start Kit (Qiagen GmbH, Germany) according to the instructions of the manufacturer. Active fractions were pooled together, and the buffer was exchanged into 25 mM potassium phosphate buffer pH 7.3 containing 10% glycerol with PD10 columns (GE Healthcare). Aliquots of the protein solution were frozen in liquid nitrogen and stored at −80 °C.

2.2.2. LpFAT A fluorescence polarization assay

Fluorescence polarization (FP) competition assays were performed at room temperature in black 96-well microtiter plates (Greiner, Catalog No. 655900). The assay mixture contained 25 mM potassium phosphate buffer pH 7.3, 200 mM NaCl, 0.01% Triton X-100, 2 nM fluorescent tracer, 0.4 µg of purified LpFAT A protein and different amounts of the test compound in a total volume of 100 µL. FP was measured with a BMG CLARIOstar microtiter plate reader using the FP filter set for fluorescein (Ex 482-46, Em 530-40, LP 504). FP is the difference between wells containing LpFAT A and wells containing only tracer. The pI_{50} values were calculated from plots of inhibition values vs test compound concentration using Model 205 of the ID Business Solutions Ltd Xlfit software suite. The FAT A binding fluorescent tracer was synthesized from (2S,4S)-4-[(2,6-difluorophenyl)methoxymethyl]-4-ethyl-2-methyl-**N**-(prop-2-ynylcarbamoyl)-1,3-dioxolane-2-carboxamide [1] and FAM azide, 5-isomer (Broadpharm BP-22544, San Diego, CA) by click chemistry [3] and was purified by flash column chromatography on silica gel.

3. References

