Synthesis and biological evaluation of optimized inhibitors of the mitotic kinesin Kif18A

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Content

S1 BTB-1 analogs synthesis:

General:

Melting point is uncorrected and was determined on a Gallenkamp melting point apparatus. Flash chromatography was performed on Merck silica gel 60. All reaction were monitored with TLC using pre-coated plastic sheets with fluorescence indicator Polygram Sil G/UV_{254} from Macherey-Nagel with a thickness of 0.2 mm. ^{1}H and ^{13}C NMR spectra were recorded on Avance III 400 or Avance III 600 NMR spectrometer from Bruker at room temperature and the chemical shifts are reported relative to the residual solvent peak. The elementar vario MICRO Cube was used for CHN-analysis. The reported yield refers to the analytically pure substance and is not optimized.

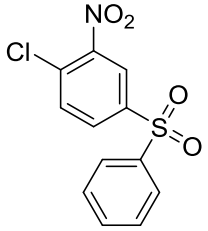
General methods for the preparation of sulfones and sulfoxides:

The sulfones and sulfoxides were synthesized according to known literature procedures.[1-3] To a solution of the corresponding phenol, thiophenol or thiophenolate and substituted nitrobenzene, an equimolar amount of base for the thiophenol or 0.3 equivalents for the thiophenolate was added and heated to reflux. EtOH and 0.1 M aqueous NaOH was used as the solvent and the base, respectively, if not otherwise mentioned. After complete consumption of the starting material the solvent was removed and a 1/1 mixture of water and EtOAc was added. The aqueous phase was extracted twice with EtOAc. The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuum to yield the crude sulfide.

Method A) The sulfide was dissolved in 5 ml acetic acid and an excess of 0.7 ml H_2O_2 was added drop wise if not otherwise mentioned. After complete addition the reaction mixture was heated to reflux. If necessary another equivalent of H_2O_2 was added. After complete reaction the solvent was removed in vacuum.

Method B) The sulfide was dissolved in CH_2Cl_2 cooled to 0°C and 1 equivalent of *m*-CPBA solution in CH_2Cl_2 was added drop wise. Saturated $NaHCO_3$ solution was added for neutralization and the organic phase was separated and the solvent removed to yield the crude sulfoxide.

1-Chloro-2-nitro-4-(phenylsulfonyl)benzene[4] (2):



$C_{12}H_8ClNO_4S$
M: 297.71 g mol^{-1}

$AlCl_3$ (1.56 g, 11.7 mmol) were suspended in benzene (5 ml) and 4-chloro-3-nitrobenzenesulfonyl chloride (2.5 g, 9.8 mmol) was added. The mixture was stirred for 24 h at ambient temperature and hydrolyzed with water (10 ml). The mixture was extracted three times with EtOAc (15 ml). The organic

layer was washed twice with 1 M NaOH (5 ml) and twice with brine (5 ml). After drying over $MgSO_4$ the solvent was removed in vacuum followed by column chromatographic purification (25 g SiO_2, PE/EE : 4/1) and recrystallization from EE/*n*-hexane to yield 1-chloro-2-nitro-4-(phenylsulfonyl)benzene as white crystals (1.36 g, 4.5 mmol, 46% yield).

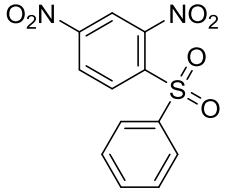
m.p: 124°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.41 (d, *J* = 2.1 Hz, 1H), 8.05 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.96 (m, 2H), 7.71 (d, *J* = 8.5 Hz, 1H), 7.68 – 7.61 (m, 1H), 7.61 – 7.53 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 148.14, 142.16, 139.98, 134.40, 133.37, 132.48, 131.67, 129.95, 128.11, 125.07.

CHN (calc/found %): C(48.41/48.42), H(2.71/2.73), N(4.70/4.62).

2,4-Dinitro-1-(phenylsulfonyl)benzene (3):



$C_{12}H_8N_2O_6S$

M: 308.26 g mol^{-1}

2,4-Dinitro-1-(phenylsulfonyl)benzene was synthesized according to Method A) using 2,4-dinitrochlorobenzene (288 mg, 1.4 mmol) and sodium thiophenolate (188 mg, 1.4 mmol). After column chromatographic pruficiation (25 g SiO_2, PE/EE : 3/1) and recrystallization from EE/hexane 2,4-dinitro-1-(phenylsulfonyl)benzene was received as off-white crystalls (110 mg, 0.3 mmol , 25% yield).

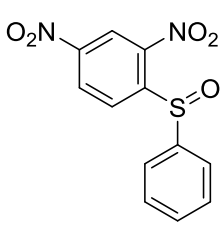
m.p.: 153°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.58 (d, *J* = 1.3 Hz, 2H), 8.53 (t, *J* = 1.3 Hz, 1H), 8.07 – 7.94 (m, 2H), 7.75 – 7.66 (m, 1H), 7.65 – 7.56 (m, 2H)

^{13}C NMR (101 MHz, Chloroform-*d*) δ 150.57, 148.88, 140.10, 139.15, 134.86, 133.47, 129.63, 128.84, 126.93, 120.31.

CHN (calc/found %): C(46.75/46.78), H(2.62/2.77), N(9.09/8.88).

2,4-Dinitro-1-(phenylsulfinyl)benzene (4):



$C_{12}H_8N_2O_5S$

M: 292.27 g mol^{-1}

2,4-Dinitro-1-(phenylsulfinyl)benzene was prepared according to Method B) using thiophenole (0.4 ml, 3.4 mmol) and 2,4-dinitrochlorobenzene (705 mg, 3.4 mmol). The crude sulfoxide was purified by column chromatography (90 g SiO_2, PE/EE : 9/1) followed by recrystallization from EtOH to yield 2,4-dinitro-1-(phenylsulfinyl)benzene as yellow crystalles (370 mg, 1.3 mmol, 38% yield).

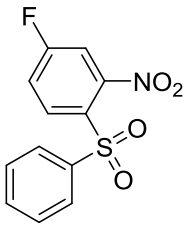
m.p.: 108°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 9.04 (d, *J* = 1.9 Hz, 1H), 8.86 – 8.76 (m, 2H), 7.77 – 7.68 (m, 2H), 7.51 – 7.40 (m, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 151.24, 149.58, 144.96, 144.06, 132.41, 129.78, 129.31, 128.24, 126.94, 120.81.

CHN (calc/found %): C(49.31/49.23), H(2.76/2.85), N(9.58/9.44).

4-Fluoro-2-nitro-1-(phenylsulfonyl)benzene(5):



$C_{12}H_8FNO_4S$

M: 281.26 g mol^{-1}

4-Fluoro-2-nitro-1-(phenylsulfonyl)benzene was synthesized according to Method A) using 1-chloro-4-fluoro-2-nitrobenzene (500 mg, 2.8 mmol) and sodium thiophenolate (380 mg, 2.8 mmol). After column chromatography (20 g SiO_2, *n*-Hexane/EE : 3/1) and 4-fluoro-2-nitro-1-(phenylsulfonyl)benzene was received as a white solid (50 mg, 0.2 mmol, 12% yield).

m.p.: 126°C

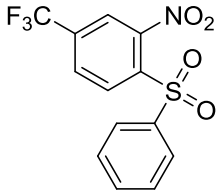
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.44 – 8.37 (m, 1H), 7.99 – 7.92 (m, 2H), 7.69 – 7.62 (m, 1H), 7.61 – 7.54 (m, 2H), 7.51 – 7.43 (m, 2H).

^{19}F NMR (376 MHz, Chloroform-*d*) δ: -99.24.

^{13}C NMR (151 MHz, Chloroform-*d*) δ: 164.92 (d, *J* = 261.8 Hz), 149.77 (d, *J* = 8.8 Hz), 140.26 , 134.21 (d, *J* = 9.4 Hz), 133.96 , 130.94 (d, *J* = 4.1 Hz), 129.20 , 128.24 , 119.55 (d, *J* = 21.4 Hz), 113.08 (d, *J* = 27.2 Hz).

CHN (calc/found %): C(51.24/51.05), H(2.87/2.99), N(4.98/5.16).

2-Nitro-1-(phenylsulfonyl)-4-(trifluoromethyl)benzene (6):



$C_{13}H_8F_3NO_4S$
M: 331.27 g mol^{-1}

2-Nitro-1-(phenylsulfonyl)-4-(trifluoromethyl)benzene was prepared following Methode A) using 1-chloro-2-nitro-4-(trifluoromethyl)benzene (321 mg, 1.4 mmol) and sodium thiophenolate (188 mg, 1.4 mmol). After column chromatography (25 g SiO_2, PE/EE : 3/1) 2-nitro-1-(phenylsulfonyl)-4-(trifluoromethyl)benzene was recrystallized from EE/hexane and recieved as white crystalls (120 mg, 0.3 mmol, 26% yield).

m.p.: 142°C

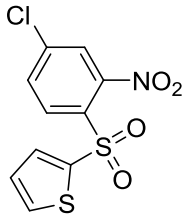
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.52 (d, *J* = 8.2 Hz, 1H), 8.08 – 8.01 (m, 1H), 8.01 – 7.95 (m, 3H), 7.73 – 7.65 (m, 1H), 7.64 – 7.55 (m, 2H).

^{19}F NMR (376 MHz, Chloroform-*d*) δ: -63.33.

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 148.73 , 139.61 , 138.23 , 136.63 (q, *J* = 34.9 Hz), 134.54 , 132.77 , 129.50 , 129.70 – 129.26 (m), 128.69 , 122.26 (q, *J* = 3.7 Hz), 122.03 (q, *J* = 273.8 Hz).

CHN (calc/found %): C(47.13/46.89), H(2.43/2.63), N(4.23/4.46).

2-((4-Chloro-2-nitrophenyl)sulfonyl)thiophene (7):



$C_{10}H_6ClNO_4S_2$
M: 303.73 g mol^{-1}

2-((4-Chloro-2-nitrophenyl)sulfonyl)thiophene was synthesized according to Method A) using thiophene-2-thiol 163 mg, 1.4 mmol) and 1,4-dichloro-2-nitrobenzene (269 mg, 1.4 mmol) and 1 M aq. NaOH as

base. After column chromatography (30 g SiO_2, PE/EE : 3/1) 2-((4-Chloro-2-nitrophenyl)sulfonyl)thiophene was received as off-white crystalls (290 mg, 0.95 mmol, 68% yield).

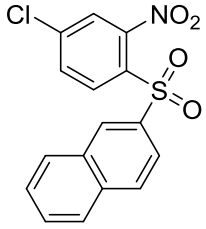
m.p.: 134°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.21 (d, *J* = 8.4 Hz, 1H), 7.92 (dd, *J* = 3.9, 1.3 Hz, 1H), 7.78 (dd, *J* = 5.0, 1.3 Hz, 1H), 7.71 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.69 (d, *J* = 1.9 Hz, 1H), 7.17 (dd, *J* = 4.9, 3.9 Hz, 1H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 148.77, 141.12, 140.66, 136.27, 135.76, 133.58, 132.70, 132.46, 128.15, 124.95.

CHN (calc/found %): C(39.54/39.44), H(1.99/2.14), N(4.61/4.85).

2-((4-Chloro-2-nitrophenyl)sulfonyl)naphthalene (8):



$C_{16}H_{10}ClNO_4S$
M: 347.77 g mol^{-1}

2-((4-Chloro-2-nitrophenyl)sulfonyl)naphthalene was prepared according to Method A) using 2-thionaphthalene (224 mg, 1.4 mmol) and 1,4-dichloro-2-nitrobenzene (269 mg, 1.4 mmol). The crude sulfone was purified by column chromatography (35 mg, SiO_2, *n*-hexane/EE : 3/1) followed by recrystallization from EE/ *n*-hexane to yield 2-((4-chloro-2-nitrophenyl)sulfonyl)naphthalene as off-white solid (30 mg, 0.08 mmol, 6% yield).

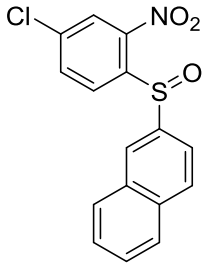
m.p.: 155°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.60 (d, *J* = 8.5 Hz, 1H), 8.35 (s, 1H), 8.23 (d, *J* = 2.0 Hz, 1H), 7.99 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.95 – 7.89 (m, 1H), 7.88 – 7.78 (m, 2H), 7.65 – 7.52 (m, 3H), 1.55 (s, 2H, H_2O).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 142.70, 141.54, 138.07, 135.54, 134.68, 132.71, 129.97, 129.08, 128.55, 128.34, 128.06, 127.91, 127.55, 125.59, 121.66.

CHN (calc/found %): C(55.26/54.76), H(2.90/3.07), N(4.03/4.10).

2-((4-Chloro-2-nitrophenyl)sulfinyl)naphthalene (9):



$C_{16}H_{10}ClNO_3S$
M: 331.77 g mol^{-1}

2-((4-Chloro-2-nitrophenyl)sulfinyl)naphthalene was synthesized following Methode B) using 2-thionaphthalene (224 mg, 1.4 mmol) and 1,4-dichloro-2-nitrobenzene (269 mg, 1.4 mmol). The crude sulfoxide was received as yellow oil which crystallizes upon cooling. The yellow crystalls were washed with *n*-hexane to yield the pure 2-((4-chloro-2-nitrophenyl)sulfinyl)naphthalene (306 mg, 0.9 mmol, 66% yield).

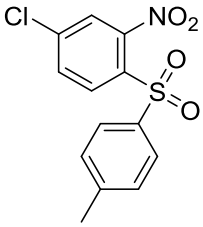
m.p.: 126°C

^{1}H NMR (600 MHz, Chloroform-*d*) δ: 8.59 (d, *J* = 8.5 Hz, 1H), 8.35 (d, *J* = 1.8 Hz, 1H), 8.21 (d, *J* = 2.1 Hz, 1H), 7.98 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.93 – 7.88 (m, 1H), 7.85 (d, *J* = 8.7 Hz, 1H), 7.83 – 7.79 (m, 1H), 7.59 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.58 – 7.54 (m, 2H).

^{13}C NMR (151 MHz, Chloroform-*d*) δ: 145.11, 142.62, 141.47, 138.00, 135.50, 134.60, 132.64, 129.92, 129.03, 128.50, 128.30, 128.01, 127.85, 127.50, 125.53, 121.63.

CHN (calc/found %): C(57.92/57.97), H(3.04/3.11), N(4.22/4.43).

4-Chloro-2-nitro-1-tosylbenzene (10):



$C_{13}H_{10}ClNO_4S$
M: 311.74 g mol^{-1}

4-Chloro-2-nitro-1-tosylbenzene was synthesized according to Method A) using 4-methylbenzenethiol (174 mg, 1.4 mmol) and 1,4-dichloro-2-nitrobenzene (269 mg, 1.4 mmol). After column chromatography (35 mg, SiO_2, *n*-hexane/EE : 3/1) the product was received as light brown solid (30 mg, 0.09 mmol, 7%).

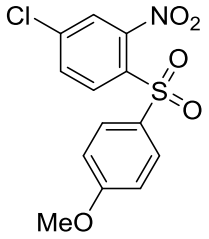
m.p.: 117°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.26 (d, *J* = 8.5 Hz, 1H), 7.88 – 7.81 (m, 2H), 7.71 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.68 (d, *J* = 2.1 Hz, 1H), 7.40 – 7.32 (m, 2H), 2.44 (s, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 148.92, 145.47, 140.89, 137.20, 133.58, 132.78, 132.58, 130.00, 128.58, 125.01, 21.85.

CHN (calc/found %): C(50.09/50.05), H(3.23/3.53), N(4.49/4.53).

4-Chloro-1-((4-methoxyphenyl)sulfonyl)-2-nitrobenzene (11):



$C_{13}H_{10}ClNO_5S$
M: 327.74 g mol^{-1}

4-Chloro-1-((4-methoxyphenyl)sulfonyl)-2-nitrobenzene was synthesized according to Methode A) using 4-methoxybenzenethiol (196 mg, 1.4 mmol) and 1,4-dichloro-2-nitrobenzene (269 mg, 1.4 mmol). After column chromatography (30 mg, SiO_2, PE/EE : 3/1) the product was received as off-white solid (54 mg, 0.16 mmol, 12%).

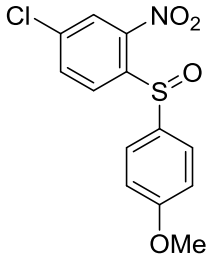
m.p.: 104°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.23 (d, *J* = 8.5 Hz, 1H), 7.95 – 7.86 (m, 2H), 7.70 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.66 (d, *J* = 2.0 Hz, 1H), 7.08 – 6.95 (m, 2H), 3.88 (s, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 164.27, 148.84, 140.68, 133.95, 132.59, 132.52, 131.40, 131.06, 124.93, 114.62, 55.91.

CHN (calc/found %): C(47.64/47.65), H(3.08/3.16), N(4.27/4.38).

4-Chloro-1-((4-methoxyphenyl)sulfinyl)-2-nitrobenzene (12):



$C_{13}H_{10}ClNO_4S$
M: 311.74 g mol^{-1}

4-Chloro-1-((4-methoxyphenyl)sulfinyl)-2-nitrobenzene was synthesized according to Method B) using 4-methoxybenzenethiol (350 mg, 2.5 mmol) and 1,4-dichloro-2-nitrobenzene (480 mg, 2.5 mmol). The crude sulfoxide was crystallized from EE/PE. Due to traces of *m*-chlorbenzoic acid the crude product was dissolved in CH_2Cl_2, washed with sat. K_2CO_3 solution and dried over $MgSO_4$. After removal of the

solvent 4-chloro-1-((4-methoxyphenyl)sulfinyl)-2-nitrobenzene was received as yellow solid (560 mg, 1.8 mmol, 72% yield).

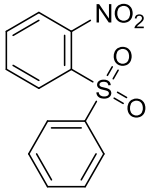
m.p.: 118°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.53 (d, *J* = 8.5 Hz, 1H), 8.22 (d, *J* = 2.1 Hz, 1H), 7.95 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.69 – 7.52 (m, 2H), 6.94 – 6.85 (m, 2H), 3.80 (s, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 162.39, 145.04, 143.01, 137.78, 135.97, 135.35, 128.90, 127.71, 125.54, 114.86, 55.66.

CHN (calc/found %): C(50.09/49.94), H(3.23/3.32), N(4.49/4.63).

1-Nitro-2-(phenylsulfonyl)benzene (13):



$C_{12}H_9NO_4S$
M: 263.27 g mol^{-1}

According to Methode A) thiophenol (1.47 g, 13.3 mmol) and 1-chloro-2-nitrobenzene (2.1 g 13.3 mmol) was converted to 1-nitro-2-(phenylsulfonyl)benzene. The crude sulfide was recrystallized from water. After oxidation the crude sulfon was purified with column chromatography (PE/EE : 1/1) and recrystalized from EtOH to yield 1-nitro-2-(phenylsulfonyl)benzene as white solid (120 mg, 0.4 mmol, 3% yield).

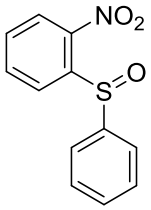
m.p.: 145°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.41 – 8.33 (m, 1H), 8.03 – 7.94 (m, 2H), 7.84 – 7.70 (m, 3H), 7.68 – 7.60 (m, 1H), 7.60 – 7.52 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 148.68, 140.61, 134.78, 134.70, 133.94, 132.63, 131.75, 129.25, 128.39, 124.91.

CHN (calc/found %): C(54.75/54.64), H(3.45/3.52), N(5.32/5.45).

1-Nitro-2-(phenylsulfinyl)benzene (14):



$C_{12}H_9NO_3S$
M: 247.27 g mol^{-1}

According to Method B According to Methode A) thiophenol (1.47 g, 13.3 mmol) and 1-chloro-2-nitrobenzene (2.1 g 13.3 mmol) was converted to 1-nitro-2-(phenylsulfinyl)benzene. The crude sulfide was recrystallized from water. After oxidation the crude sulfoxide was purified with column chromatography (EE/PE : 1/1) and recrystallized from EtOH to yield 1-nitro-2-(phenylsulfinyl)benzene as yellow crystalls(1.7 mmol, 427 mg, 13% yield).

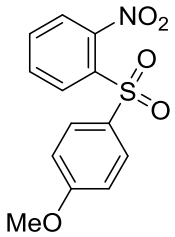
m.p.: 88°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.59 (dd, *J* = 7.9, 1.4 Hz, 1H), 8.26 (dd, *J* = 8.1, 1.2 Hz, 1H), 8.01 (m), 7.78 – 7.65 (m, 3H), 7.49 – 7.36 (m, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 145.29, 144.76, 144.05, 135.52, 131.69, 131.57, 129.41, 126.92, 126.45, 125.46.

CHN (calc/found %): C(58.29/58.20), H(3.67/3.65), N(5.66/5.86).

1-((4-Methoxyphenyl)sulfonyl)-2-nitrobenzene (15):



$C_{13}H_{11}NO_5S$
M: 293.29 g mol^{-1}

4-Methoxybenzenethiol (0.89 g, 6.3 mmol) and 1-chloro-2-nitrobenzene (1.00 g, 6.3 mmol) were dissolved in EtOH. 0.8 g KOH was dissolved in EtOH and was added to the reaction mixture followed by heating to 70°C. After filtration water was added to the filtrate and extracted twice with 30 ml CH_2Cl_2. The organic layer was washed with 30 ml of 2M aq. KOH and dried over Na_2SO_4. After removal of the solvent the crude sulfide was oxidized to the corresponding sulfone following Method A). The crude product was washed with EE yielding 1-((4-methoxyphenyl)sulfonyl)-2-nitrobenzene as a white solid (100 mg, 0.34 mmol, 5% yield).

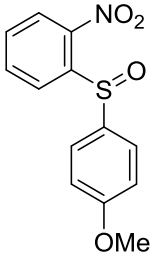
m.p: 148°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.35 – 8.25 (m, 1H), 7.99 – 7.87 (m, 2H), 7.78 – 7.64 (m, 3H), 7.09 – 6.94 (m, 2H), 3.87 (s, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 164.08, 148.54, 135.46, 134.32, 132.47, 131.81, 131.40, 131.01, 124.69, 114.51, 55.87.

CHN (calc/found %): C(53.24/53.26), H(3.78/3.72), N(4.78/4.94).

1-((4-Methoxyphenyl)sulfinyl)-2-nitrobenzene (16):



$C_{13}H_{11}NO_4S$
M: 277.29 g mol^{-1}

4-Methoxybenzenethiol (1.78 g, 12.7 mmol) and 1-chloro-2-nitrobenzene (2.00 g, 12.7 mmol) were dissolved in EtOH. 0.8 g KOH was dissolved in EtOH and was added to the reaction mixture followed by heating to 70°C. After filtration water was added to the filtrate and extracted twice with 30 ml CH_2Cl_2. The organic layer was washed with 30 ml of 2 M aq. KOH and dried over Na_2SO_4. After removal of the solvent the crude sulfide was oxidized to the corresponding sulfoxide following Method B). The sulfoxide was purified via cholumn chromatography (EE/PE : 2/1) yielding 1-((4-methoxyphenyl)sulfinyl)-2-nitrobenzene as yellow crystals (70 mg, 0.25 mmol, 2%).

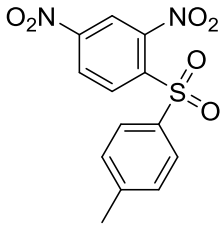
m.p: 94°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.57 (dd, *J* = 7.9, 1.2 Hz, 1H), 8.24 (dd, *J* = 8.1, 1.0 Hz, 1H), 8.05 – 7.95 (m, 1H), 7.70 – 7.64 (m, 1H), 7.63 – 7.57 (m, 2H), 6.87 (dd, *J* = 9.4, 2.5 Hz, 2H), 3.77 (s, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 162.29, 144.80, 144.49, 136.49, 135.49, 131.47, 129.04, 126.44, 125.58, 114.83, 55.70.

CHN (calc/found %): C(56.31/56.32), H(4.00/4.00), N(5.05/5.16).

2,4-Dinitro-1-tosylbenzene (17):



$C_{13}H_{10}N_2O_6S$
M: 322.29 g mol^{-1}

2,4-Dinitro-1-tosylbenzene was synthesized according to Method A) using 1-chloro-2,4-dinitrobenzene (910 mg, 4.5 mmol) and 4-methylbenzenethiol (558 mg, 4.5 mmol). After column chromatographic purification (EE/PE : 1/1) 2,4-dinitro-1-tosylbenzene was received as an off-white solid (241 mg, 0.8 mmol, 18%).

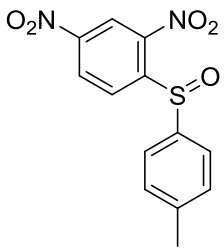
m.p: 181°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.59 – 8.47 (m, 3H), 7.93 – 7.77 (m, 2H), 7.39 (d, *J* = 8.1 Hz, 2H), 2.46 (s, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 150.42, 148.79, 146.36, 140.44, 136.07, 133.31, 130.28, 128.99, 126.84, 120.19, 21.92.

CHN (calc/found %): C(48.45/48.31), H(3.13/3.25), N(8.69/8.81).

2,4-Dinitro-1-(*p*-tolylsulfinyl)benzene (18):



$C_{13}H_{10}N_2O_5S$
M: 306.29 g mol^{-1}

2,4-Dinitro-1-(*p*-tolylsulfinyl)benzene was synthesized according to Method B) using 1-chloro-2,4-dinitrobenzene (910 mg, 4.5 mmol) and 4-methylbenzenethiol (558 mg, 4.5 mmol). After column chromatographic purification (EE/PE : 1/2) and recrystallization from EE/*n*-hexane 4-dinitro-1-(*p*-tolylsulfinyl)benzene was received as yellow crystalls (260 mg, 0.9 mmol, 20%).

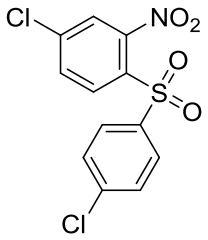
m.p: 134°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 9.03 (d, *J* = 1.9 Hz, 1H), 8.81 (d, *J* = 8.6 Hz, 1H), 8.78 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.61 – 7.55 (m, 2H), 7.25 – 7.20 (m, 2H), 2.35 (s, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 151.49, 149.50, 144.93, 143.27, 140.87, 130.41, 129.23, 128.22, 127.00, 120.79, 21.58.

CHN (calc/found %): C(50.98/50.90), H(3.29/3.36), N(9.15/9.22).

4-Chloro-1-((4-chlorophenyl)sulfonyl)-2-nitrobenzene (19):



$C_{12}H_7Cl_2NO_4S$
MW: 332.15 g mol^{-1}

4-Chloro-1-((4-chlorophenyl)sulfonyl)-2-nitrobenzene was synthesized according to Method A) using 4-chlorobenzenethiol (750 mg, 5.18 mmol), 1,4-dichloro-2-nitrobenzene (1.00 g, 5.18 mmol), MeOH as the

solvent and 1 M aqueous NaOH as the base. The crude reaction mixture was evaporated to dryness and the residue dissolved in CH_2Cl_2 and washed with H_2O. The aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and the solvent was removed in order to recieve the crude sulfide as a yellow solid. After oxidation the crude product was purified by column chromatography (*n*-hexane/EE : 3/1) yielding 4-chloro-1-((4-chlorophenyl)sulfonyl)-2-nitrobenzene as a white powder (850 mg, 2.83 mmol, 55% yield).

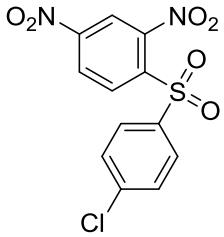
m.p.: 134°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.29 (d, *J* = 8.3 Hz, 1H), 7.94 – 7.85 (m, 2H), 7.79 – 7.71 (m, 2H), 7.59 – 7.49 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 148.97, 141.48, 141.05, 138.70, 132.92, 132.82, 129.96, 129.79, 129.70, 125.30.

CHN (calc/found %): C(43.39/43.34), H(2.12/2.28), N(4.22/4.29).

1-((4-Chlorophenyl)sulfonyl)-2,4-dinitrobenzene (20):



$C_{12}H_7ClN_2O_6S$
M: 342.71 g mol^{-1}

1-((4-Chlorophenyl)sulfonyl)-2,4-dinitrobenzene was synthesized according to Method A) using 1-chloro-2,4-dinitrobenzene (284 mg, 1.4 mmol) and 4-chlorobenzenethiol (202 mg, 4.5 mmol). Upon cooling after the oxidation with H_2O_2 1-((4-chlorophenyl)sulfonyl)-2,4-dinitrobenzene crystallizes from glacial acetic acid (368 mg, 1.1 mmol, 77% yield).

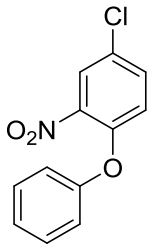
m.p.: 166°C

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.55 – 8.49 (m, 2H), 8.48 (dd, J = 2.0, 0.8 Hz, 1H), 7.92 – 7.78 (m, 2H), 7.53 – 7.47 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 150.72, 148.92, 141.91, 139.79, 137.58, 133.49, 130.36, 130.00, 127.07, 120.44.

CHN (calc/found %): C(42.06/41.90), H(2.06/2.34), N(8.17/7.99).

4-Chloro-2-nitro-1-phenoxybenzene (21)[5]:



$C_{12}H_8ClNO_3$
M: 249.65 g mol^{-1}

To a solution of phenol (508 mg, 5.4 mmol) in DMSO (30 ml) was added KOH (303 mg, 5.4 mmol) and the reaction mixture was heated to 50°C for 30 min. After cooling to ambient temperature a solution of 1,4-dichloro-2-nitrobenzene (1.037 g, 5.4 mmol) in DMSO (7 ml) was added and heated to 90°C for 16 h. After addition of H_2O (15 ml) the aqueous phase was extracted three times with EtO_2 (50 ml). The organic layer was washed with H_2O and dried over $MgSO_4$. After removal of the solvent 4-chloro-2-nitro-1-phenoxybenzene was received as brown oil (1.300 g, 5.2 mmol, 96% yield).

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.95 (d, *J* = 2.6 Hz, 1H), 7.45 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.43 – 7.36 (m, 2H), 7.24 – 7.18 (m, 1H), 7.07 – 7.02 (m, 2H), 6.96 (d, *J* = 8.9 Hz, 1H).

^{13}C NMR (151 MHz, Chloroform-*d*) δ: 155.53, 149.68, 141.42, 134.22, 130.35, 128.23, 125.74, 125.12, 121.62, 119.43.

CHN (calc/found %): C(57.37/57.48), H(3.23/3.35), N(5.61/5.72).

S2 Protein expression and purification from bacteria via poly-histidine (His) tag:

All kinesin motor domains were expressed and purified as described previously.[1] Bacteria of the *E.coli* strain BL21RIL were transformed with the plasmids coding for poly-histidine-tagged motors in the presence of selective antibiotics (ampicillin 100 μg ml^{-1} and chloramphenicol 34 μg ml^{-1}) and incubated over night at 37°C. Multiple colonies were used to inoculate 25 to 100 ml culture of LB medium, grown over night at 37°C including antibiotics as above. 10 ml per liter of pre-inoculum were used to inoculate a new liquid culture (2 to 4 liters) plus antibiotics as above, which was grown at 37°C until OD600nm 0.6 units was reached. Afterwards the expression of the recombinant protein was induced with 0.5 mM IPTG and allowed for 18 h at 18°C and the bacteria were harvested by centrifugation (4500 x g, 15 min, 4°C). The pellet was first frozen (-80°C, 20 min) then thawed out and resuspended with 10 ml lysis buffer (20 mM Tris pH 8.0, 300 mM NaCl, 5 mM imidazole, 0.1% Triton X 100, Complete protease inhibitor Roche) per liter of original culture. The cells were lysed by high-pressure treatment using the EmulsiFlex-C5 or C3 homogenizer (Avestin) and then cleared by centrifugation (40000 x g, 30 min, 4°C). The lysate was incubated with Ni-NTA beads (Qiagen) for 2 h at 4°C on a rotating wheel (0.25 to 0.5 ml resin per liter of original culture), washed 3 times in batch with 20 ml washing buffer (20 mM Tris pH 8.0, 300 mM NaCl, 20 mM imidazole, +/- 1 mM NaATP and 1 mM $MgCl_2$) and finally eluted over a Poly- Prep Chromatography Column (Bio-Rad) in 0.5 to 1 ml fractions (elution buffer: 20 mM Tris pH 8.0, 300 mM NaCl, 200 mM imidazole). The fractions containing the recombinant protein were pooled together and

dialyzed o/n at 4°C (dialysis buffer: 10 mM β-mercaptoethanol, 10% glycerol, 25 mM Tris pH 7.4, 300 mM NaCl) then aliquoted, snap-frozen and stored at -80°C.

S3.1 BTB-1 Analogs Screening:

Experimental procedure

A mix of 650 µM MgATP, 3 µM taxol stabilized microtubules, 200 µM NADH, 5 mM PEP (pH 7), 4 U ml^{-1} Pyruvate kinase, 8 U ml^{-1} Lactate dehydrogenase and assay buffer (10 mM Imidazole/acetate pH 7.2, 5 mM Mg-acetate, 2 mM EGTA, 20 µM taxol) was supplemented with DMSO (0.5%) or BTB-1 and analogs (5µM final concentration). The reaction was started by addition of $Kif18A^{MD}$ (1-467aa, 20 nM final concentration) and absorbance readings were taken every 30 seconds at 340 nm for 10 minutes using the Tecan 500 plate reader. DMSO was used as solvent control and the activity of the $Kif18A^{MD}$ was set to 100%.

S3.2 IC50 (Kif18A):

Experimental procedure

Increasing concentrations of BTB-1 analogs were used to determine the residual activity of 20 nM His-$Kif18A^{MD}$ in the presence of 650 µM ATP and 3 µM taxol stabilized microtubules by enzyme-coupled assay (see above). DMSO was used as solvent control and the activity of the protein in its presence was set to 100%. The assays were performed in 384 well plates and the oxidation of NADH over time was monitored using a plate reader as reported above.

S3.3 Basal Kif18A ATPase activity:

Experimental procedure

The ATPase activity of 300 nM $Kif18A^{MD}$ without microtubules was determined using the enzyme coupled assay (as described above) in the presence of 50 µM BTB-1 (**1**), **5** and **13** or 0.5% DMSO as solvent control (Figure S1 and S2).

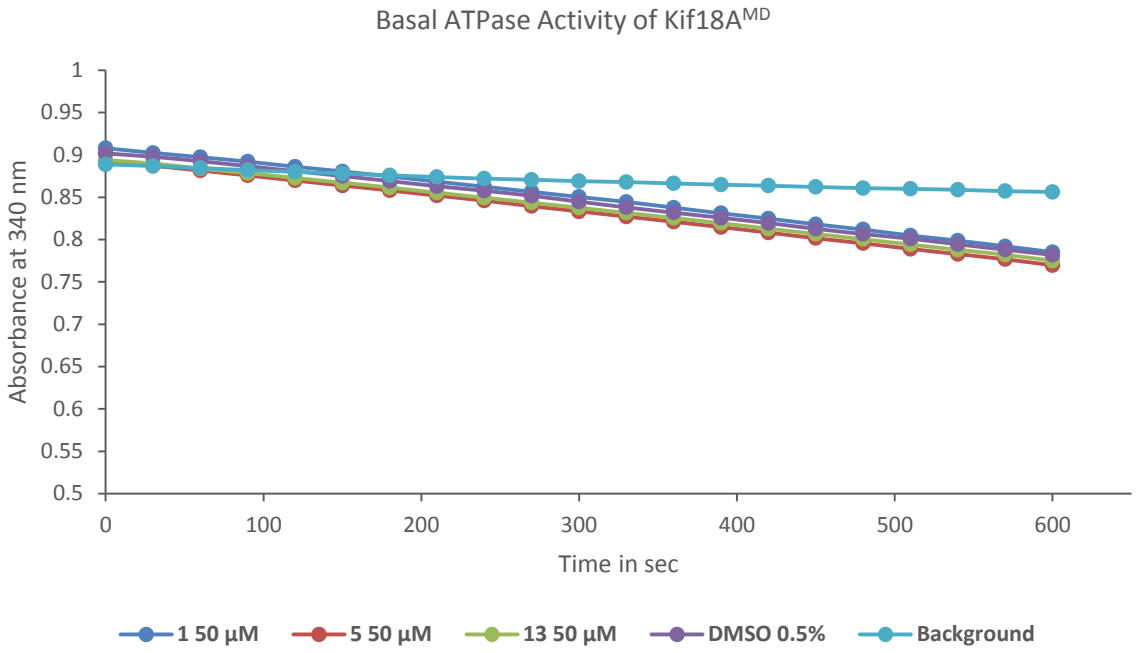


Figure S1: Experimental data received for basal ATPase activity of Kif18A^{MD} in the presence of 50 µM BTB-1 (**1**) (blue line), **5** (red line) and **13** (green line) or 0.5% DMSO (purple line).

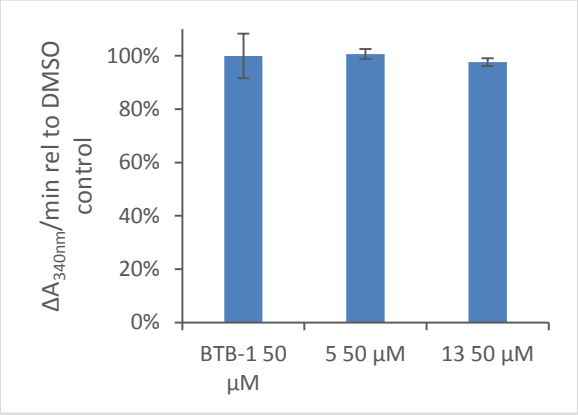


Figure S2: Comparison of basal ATPase activity of Kif18A^{MD} relative to DMSO control in the presence of 50 µM BTB-1 (**1**), **5** and **13**.

S3.4 Inhibition mode of 13:

Increasing concentrations of **13** and ATP were used in an enzym coupled assay (as described above) to elucidate the inhibition mode. According to the Michaelis-Menten kinetics each data set was fit to competitive inhibitory model using the software Prism 5 (GraphPad).

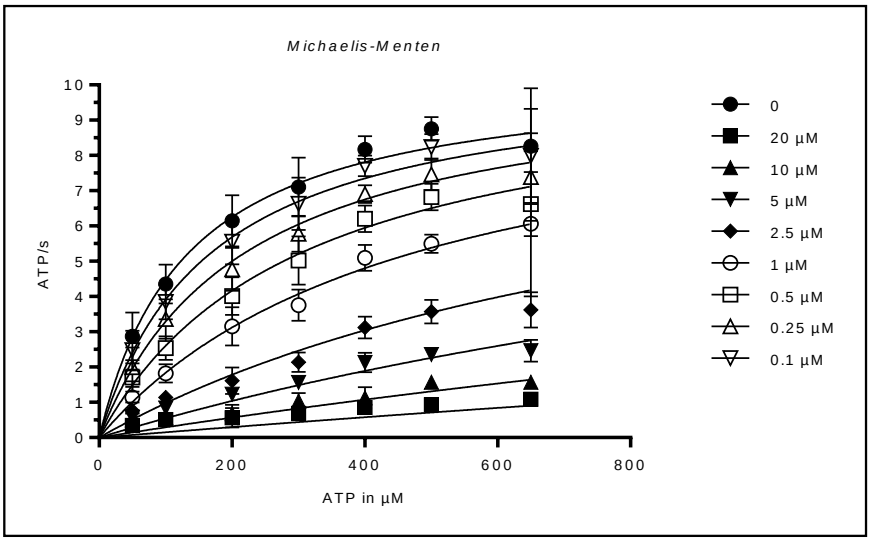


Figure S3: Different concentrations of **13** were used and the ATPase activity of Kif18A^{MD} was determined by increasing concentrations of ATP.

S4 Cell culture and immunofluorescence:

Experimental procedure

HeLa cells stably expressing GFP labeled Kif18A, as previously described in Häfner et al.[6] were synchronized using 2 mM thymidine (18 hours) and plated on coverslips in 6 well plates (200,000 cells/well). The coverslips were treated with different concentrations of BTB-1 or its analogs or DMSO as solvent control for 30 min. The cells were then treated and immunostained as described in previously to visualize microtubules and DNA.[7]

S5.1 Tubulin polymerization assay:

Experimental procedure

A mix of 0.8 µM glutamate (pH 6.6), 100 µM MgCl2 and 10 µM tubulin was supplemented with DMSO (0.5%), compound (50µM) or Nocodazole, which was used as a control tubulin polymerization inhibitor, and incubated for 15 min at ambient temperature. Afterwards the mixtures were cooled on an ice bath for 5 min and the polymerization was started by addition of GTP (final conc. 0.4 mM) and incubated for 30 sec at 30°C. The assays were performed in quartz cuvettes with the final assay volume of 150 µL and the polymerization was measured with VarianInc Cary100bio spectrophotometer for 20 min at 30°C and the depolymerization for 15 min at 15°C. The tubulin polymerization velocity was determined by linear regression and compared to the DMSO control which was set to 100%.

S5.2 IC50 (Tub.Polym.):

The experiments were performed using the conditions described above with increasing concentrations of BTB-1 and its analogs.

S6 Alamar Blue Assay and EC_{50} values

Experimental procedure[8]

Cells were seeded in 96-well plates (4,000 cells/well) and allowed to attach for 24 h. Compounds to be tested were dissolved in a suitable amount of DMSO and different concentrations were prepared to give final concentrations with a maximum DMSO content of 1%. The cells were incubated for 48 h with different concentrations of the compound. AlamarBlue (10 µL) was added and the cells were incubated for another hour. After excitation at 530 nm, fluorescence at 590 nm was measured using a FL600 Fluorescence Microplate Reader (Bio-TEK). Cell viability is expressed in percent with respect to a control containing 1% DMSO.

S7 References:

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