**SUPPORTING INFORMATION**

# Discovery of 5-nitro-6-thiocyanatopyrimidines as inhibitors of *Cryptococcus neoformans* and *Cryptococcus gattii*

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**Short running title:** 5-Nitro-6-thiocyanatopyrimidine antifungals

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## Experimental Procedures

### Reagents and physicochemical analysis of the synthesized compounds

All reagents and solvents were purchased from commercial suppliers and used without further purification. $^{1}H$ spectra were measured on Bruker AC-300 (300 MHz). Chemical shifts were measured in DMSO-$d_6$, using tetramethylsilane as an internal standard, and reported as units (ppm) values. Mass spectra were recorded on Finnigan MAT INCO 50 mass spectrometer (EI, 70 eV) with direct injection. The purity of the final compounds were analyzed on an Agilent 1290 Infinity II HPLC system coupled to Agilent 6460 triple-quadrupole mass spectrometer equipped with an electrospray ionization source. All final compounds are > 95 % pure. Elemental analysis (% C, H, N) was carried out by an elemental analyzer EURO EA. Melting points were determined on Electrothermal 9001 (10 ºC per min) and are uncorrected. Merck KGaA silica gel 60 $F_{254}$ plates were used for analytical thin-layer chromatography. Yields refer to purified products and are not optimized.

2-Substituted 4,6-dihydroxy-5-nitropyrimidines **1-6** and 4,6-dichloro-5-nitropyrimidines **7-12** were synthesized with minimal changes according to the procedures described in Latli *et al.* [1]. Physicochemical data for intermediates **13-62** are reported in the literature [2-5] and are not shown in the present file. General synthetic procedures and physicochemical characteristics of the final 4-thiocyano-5-nitropyrimidines **63-112** are presented as follows.

### General procedure for the synthesis of 2-substituted 4-amino-6-chloro-5-nitropyrimidines

To a solution of 2-substituted 4,6-dichloro-5-nitropyrimidines (1 eqv.) in dioxane (40 mL), freshly prepared corresponding amine acetate (2.5 eqv.) was added at T < 15 ºC. The mixture was kept at room temperature for 3 h and then diluted with water (200 mL). The precipitate was collected, washed with cold water and used without purification.

**General procedure for the synthesis of 2-substituted 4-alkoxy-6-chloro-5-nitropyrimidines**

To a solution of 2-substituted 4,6-dichloro-5-nitropyrimidines (1 eqv.) in the corresponding anhydrous alcohol (40 mL), the corresponding freshly prepared sodium alkoxide (1 eqv., 20 mL) was added at T < 0 ºC, and the mixture was kept at room temperature from 2 h to 4 h. After completion of the reaction, the mixture was diluted with water (200 mL), and the precipitate of the corresponding products was collected, washed with cold water and recrystallized from benzene.

**General procedure for the synthesis of 2,4-disubstituted 5-nitro-6-thiocyanatopyrimidines**

A mixture of 2,4-substituted 6-chloro-5-nitropyrimidines (1 eqv.) in the corresponding alcohol (30 mL) and potassium thiocyanate (1.1 eqv.) was refluxed for 4 h. After completion of the reaction, activated carbon (0.1 g) was added, the hot mixture was stirred for 1 h and filtered off. The filtrate was cooled and then was diluted with water (100 mL). The precipitate of the final products was collected, washed with cold water and recrystallized from the corresponding solvent.

*4-Methoxy-5-nitro-6-thiocyanatopyrimidine 63*

Yield 75 %, 131-133 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 2.05 (3H, s, $CH_3$), 8.24 (1H, s, $CH_{ar}$). MS (EI): m/z 212 ($M^+$). Anal. calcd for $C_6H_4N_4O_3S$: C, 33.96; H, 1.90; N, 26.41. Found: C, 33.89; H, 1.96; N, 26.37.

*4-Propoxy-5-nitro-6-thiocyanatopyrimidine 64*

Yield 76 %, 107-109 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.21 (3H, t, $CH_3$), 1.86 (2H, m, $CH_2$), 4.46 (2H, t, $OCH_2$), 8.29 (1H, s, $CH_{ar}$). MS (EI): m/z 240 ($M^+$). Anal. calcd for $C_8H_8N_4O_3S$: C, 40.00; H, 3.36; N, 23.32. Found: C, 40.06; H, 3.30; N, 23.29.

*4-Isopropoxy-5-nitro-6-thiocyanatopyrimidine 65*

Yield 79 %, 96-99 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.24 (6H, s, $2CH_3$), 4.72 (1H, qui, CH), 8.27 (1H, s, $CH_{ar}$). MS (EI): m/z 240 ($M^+$). Anal. calcd for $C_8H_8N_4O_3S$: C, 40.00; H, 3.36; N, 23.32. Found: C, 40.08; H, 3.39; N, 23.38.

*5-Nitro-6-thiocyanatopyrimidin-4-amine 66*

Yield 84 %, 215 ºC (decomp.) (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 8.34 (1H, s, $CH_{ar}$), 8.53 (2H, brs, $NH_2$). MS (EI): m/z 197 ($M^+$). Anal. calcd for $C_5H_3N_5O_2S$: C, 30.46; H, 1.53; N, 35.52. Found: C, 30.42; H, 1.57; N, 35.47.

*N-Isopropyl-5-nitro-6-thiocyanatopyrimidin-4-amine 67*

Yield 81 %, 151-153 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.37 (6H, s, $2CH_3$), 4.86 (1H, m, CH), 8.16 (1H, s, $CH_{ar}$), 9.46 (1H, brs, NH). MS (EI): m/z 239 ($M^+$). Anal. calcd for $C_8H_9N_5O_2S$: C, 40.16; H, 3.79; N, 29.27. Found: C, 40.03; H, 3.88; N, 29.11.

*N-(3-Methylbutan-2-yl)-5-nitro-6-thiocyanatopyrimidin-4-amine 68*

Yield 78 %, 132-134 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.08 (6H, s, $2CH_3$), 1.17 (3H, s, $CH_3$), 1.55 (1H, m, CH), 2.53 (1H, qui, CH), 8.18 (1H, s, $CH_{ar}$), 9.39 (1H, brs, NH).

MS (EI): m/z 267 ($M^+$). Anal. calcd for $C_{10}H_{13}N_5O_2S$: C, 44.93; H, 4.90; N, 26.20. Found: C, 44.88; H, 4.97; N, 26.25.

*N-(Pentan-3-yl)-5-nitro-6-thiocyanatopyrimidin-4-amine 69*

Yield 72 %, 125-126 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.13 (6H, s, $2CH_3$), 1.51 (4H, brm, $2CH_2$), 2.43 (1H, qui, CH), 8.23 (1H, s, $CH_{ar}$), 9.41 (1H, brs, NH). MS (EI): m/z 267 ($M^+$). Anal. calcd for $C_{10}H_{13}N_5O_2S$: C, 44.93; H, 4.90; N, 26.20. Found: C, 44.97; H, 4.86; N, 26.25.

*N-Cyclopentyl-5-nitro-6-thiocyanatopyrimidin-4-amine 70*

Yield 77 %, 146-149 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.50-2.02 (8H, m, $4CH_2$), 2.64 (1H, t, CH), 8.21 (1H, s, $CH_{ar}$), 9.39 (1H, brs, NH). MS (EI): m/z 265 ($M^+$). Anal. calcd for $C_{10}H_{11}N_5O_2S$: C, 45.27; H, 4.18; N, 26.40. Found: C, 45.31; H, 4.13; N, 26.49.

*N-Cyclohexyl-5-nitro-6-thiocyanatopyrimidin-4-amine 71*

Yield 74 %, 141-142 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.11-1.21 (4H, m, $2CH_2$), 1.48-1.57 (6H, m, $3CH_2$), 2.59 (1H, qui, CH), 8.23 (1H, s, $CH_{ar}$), 9.38 (1H, brs, NH). MS (EI): m/z 279 ($M^+$). Anal. calcd for $C_{11}H_{13}N_5O_2S$: C, 47.30; H, 4.69; N, 25.07. Found: C, 47.37; H, 4.64; N, 25.01.

*4-(Piperidin-1-yl)-5-nitro-6-thiocyanatopyrimidine 72*

Yield 58 %, 152-154 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.51-1.60 (6H, m, $3CH_2$), 3.69-3.73 (4H, m, $2CH_2$), 8.25 (1H, s, $CH_{ar}$). MS (EI): m/z 265 ($M^+$). Anal. calcd for $C_{10}H_{11}N_5O_2S$: C, 45.27; H, 4.18; N, 26.40. Found: C, 45.35; H, 4.21; N, 26.36.

*4-(2-Methylpiperidin-1-yl)-5-nitro-6-thiocyanatopyrimidine 73*

Yield 54 %, 158-160 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.33 (3H, s, $CH_3$), 1.70 and 3.65 (9H, two brm, $CH(CH_2)_4$), 8.21 (1H, s, $CH_{ar}$). MS (EI): m/z 279 ($M^+$). Anal. calcd for $C_{11}H_{13}N_5O_2S$: C, 47.30; H, 4.69; N, 25.07. Found: C, 47.11; H, 4.54; N, 25.12.

*4-(2,6-Dimethylpiperidin-1-yl)-5-nitro-6-thiocyanatopyrimidine 74*

Yield 61 %, 115-116 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.18 (6H, s, $2CH_3$), 1.30-1.56 (6H, $3CH_2$), 3.19 (2H, m, 2CH), 8.28 (1H, s, $CH_{ar}$). MS (EI): m/z 293 ($M^+$). Anal. calcd for $C_{12}H_{15}N_5O_2S$: C, 49.13; H, 5.15; N, 23.87. Found: C, 49.17; H, 5.19; N, 23.80.

*4-(3,5-Dimethylpiperidin-1-yl)-5-nitro-6-thiocyanatopyrimidine 75*

Yield 67 %, 123-125 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.19 (6H, s, $2CH_3$), 1.21 (2H, m, $CH_2$), 1.97 (2H, m, 2CH), 3.02 and 3.97 (4H, 2 brm, $N(CH_2)_2$), 8.34 (1H, s, $CH_{ar}$). MS (EI): m/z 293 ($M^+$). Anal. calcd for $C_{12}H_{15}N_5O_2S$: C, 49.13; H, 5.15; N, 23.87. Found: C, 49.20; H, 5.12; N, 23.83.

*1-(5-Nitro-6-thiocyanatopyrimidin-4-yl)azepane 76*

Yield 71 %, 112 ºC (decomp.) (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.51-1.77 (8H, brm, $4CH_2$), 3.69 (4H, brm, $N(CH_2)_2$), 8.26 (1H, s, $CH_{ar}$). MS (EI): m/z 279 ($M^+$). Anal. calcd for $C_{11}H_{13}N_5O_2S$: C, 47.30; H, 4.69; N, 25.07. Found: C, 47.35; H, 4.64; N, 25.01.

*1-(5-Nitro-6-thiocyanatopyrimidin-4-yl)azocane 77*

Yield 74 %, 93-96 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.32-1.50 (10H, brm, $5CH_2$), 3.86 (4H, t, $N(CH_2)_2$), 8.30 (1H, s, $CH_{ar}$). MS (EI): m/z 293 ($M^+$). Anal. calcd for $C_{12}H_{15}N_5O_2S$: C, 49.13; H, 5.15; N, 23.87. Found: C, 49.08; H, 5.14; N, 23.80.

*N-Benzyl-5-nitro-6-thiocyanatopyrimidin-4-amine 78*

Yield 43 %, 144-146 ºC (iPrOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 4.41 (2H, s, $CH_2$), 7.38-7.25 (5H, m, Ph), 8.38 (1H, s, $CH_{ar}$), 9.40 (1H, brs, NH). MS (EI): m/z 287 ($M^+$). Anal. calcd for $C_{12}H_9N_5O_2S$: C, 50.17; H, 3.16; N, 24.38. Found: C, 50.29; H, 3.24; N, 24.29.

*5-Nitro-6-thiocyanato-N-(3-(trifluoromethyl)benzyl)pyrimidin-4-amine 79*

Yield 51 %, 114-116 ºC (iPrOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 4.99 (2H, d, $CH_2$), 7.48-7.70 (4H, m, Ph), 8.59 (1H, s, $CH_{ar}$), 9.20 (1H, brs, NH). MS (EI): m/z 355 ($M^+$). Anal. calcd for $C_{13}H_8F_3N_5O_2S$: C, 43.95; H, 2.27; N, 19.71. Found: C, 43.99; H, 2.21; N, 19.78.

*N-(4-Chlorobenzyl)-5-nitro-6-thiocyanatopyrimidin-4-amine 80*

Yield 45 %, 126-128 ºC (iPrOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 4.81 (2H, d, $CH_2$), 7.25-7.45 (4H, m, Ph), 8.59 (1H, s, $CH_{ar}$), 9.18 (1H, brs, NH). MS (EI): m/z 321 ($M^+$). Anal. calcd for $C_{12}H_8ClN_5O_2S$: C, 44.80; H, 2.51; N, 21.77. Found: C, 44.85; H, 2.47; N, 21.72.

*5-Nitro-N-phenethyl-6-thiocyanatopyrimidin-4-amine 81*

Yield 44 %, 126-128 ºC (iPrOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 2.97 (2H, t, $CH_2$), 3.81 (2H, m, $CH_2$), 7.25 (5H, m, Ph), 8.60 (1H, s, $CH_{ar}$), 9.42 (1H, brs, NH). MS (EI): m/z 301 ($M^+$). Anal. calcd for $C_{13}H_{11}N_5O_2S$: C, 51.82; H, 3.68; N, 23.24. Found: C, 51.76; H, 3.73; N, 23.29.

*N,N-Dimethyl-5-nitro-6-thiocyanatopyrimidin-4-amine 82*

Yield 78 %, 142-144 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 3.48 (6H, s, $N(CH_3)_2$), 8.37 (1H, s, $CH_{ar}$). MS (EI): m/z 225 ($M^+$). Anal. calcd for $C_7H_7N_5O_2S$: C, 37.33; H, 3.13; N, 31.10. Found: C, 37.23; H, 3.27; N, 29.97.

*N,N-Diethyl-5-nitro-6-thiocyanatopyrimidin-4-amine 83*

Yield 74 %, 114-116 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.01 (6H, t, $2CH_3$), 3.46 (4H, m, $2CH_2$), 8.31 (1H, s, $CH_{ar}$). MS (EI): m/z 253 ($M^+$). Anal. calcd for $C_9H_{11}N_5O_2S$: C, 42.68; H, 4.38; N, 27.65. Found: C, 42.60; H, 4.33; N, 27.74.

*N-Ethyl-N-isopropyl-5-nitro-6-thiocyanatopyrimidin-4-amine 84*

Yield 79 %, 103-105 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.17 (9H, m, $3CH_3$), 2.83 (1H, m, NCH), 3.12 (2H, q, $NCH_2$), 8.23 (1H, s, $CH_{ar}$). MS (EI): m/z 267 ($M^+$). Anal. calcd for $C_{10}H_{13}N_5O_2S$: C, 44.93; H, 4.90; N, 26.20. Found: C, 44.87; H, 4.95; N, 26.13.

*N-Cyclohexyl-N-methyl-5-nitro-6-thiocyanatopyrimidin-4-amine 85*

Yield 34 %, 112-114 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.0-2.0 (10H, brm, $5CH_2$), 3.12 (3H, s, $CH_3$), 3.64 (1H, m, NCH), 8.32 (1H, s, $CH_{ar}$). MS (EI): m/z 293 ($M^+$). Anal. calcd for $C_{12}H_{15}N_5O_2S$: C, 49.13; H, 5.15; N, 23.87. Found: C, 49.17; H, 5.08; N, 23.84.

*N-Benzyl-N-methyl-5-nitro-6-thiocyanatopyrimidin-4-amine 86*

Yield 46 %, 127-129 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 3.15 (3H, s, $CH_3$), 4.34 (2H, s, $CH_2$), 7.20-7.40 (5H, m, Ph), 8.31 (1H, s, $CH_{ar}$). MS (EI): m/z 301 ($M^+$). Anal. calcd for $C_{13}H_{11}N_5O_2S$: C, 51.82; H, 3.68; N, 23.24. Found: C, 51.76; H, 3.57; N, 23.19.

*N-Benzyl-N-isopropyl-5-nitro-6-thiocyanatopyrimidin-4-amine 87*

Yield 51 %, 110-112 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.18 (6H, s, $2CH_3$), 2.85 (1H, m, NCH), 4.71 (2H, m, $NCH_2$), 7.25-7.35 (5H, m, Ph), 8.29 (1H, s, $CH_{ar}$). MS (EI): m/z 329 ($M^+$). Anal. calcd for $C_{15}H_{15}N_5O_2S$: C, 54.70; H, 4.59; N, 21.26. Found: C, 54.71; H, 4.52; N, 21.29.

*4-Ethoxy-2-methyl-5-nitro-6-thiocyanatopyrimidine 88*

Yield 40 %, 60-62 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.43 (3H, t, $CH_3$), 2.66 (3H, s, $CH_3$), 4.64 (2H, q, $OCH_2$). MS (EI): m/z 240 ($M^+$). Anal. calcd for $C_8H_8N_4O_3S$: C, 40.00; H, 3.36; N, 23.32. Found: C, 39.93; H, 3.46; N, 23.31.

*2-Methyl-5-nitro-4-propoxy-6-thiocyanatopyrimidine 89*

Yield 26 %, 50-52 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.17 (3H, t, $CH_3$), 1.77 (2H, m, $CH_2$), 2.58 (3H, s, $CH_3$), 4.32 (2H, t, $OCH_2$). MS (EI): m/z 254 ($M^+$). Anal. calcd for $C_9H_{10}N_4O_3S$: C, 42.51; H, 3.96; N, 22.04. Found: C, 42.45; H, 3.89; N, 22.09.

*4-Isopropoxy-2-methyl-5-nitro-6-thiocyanatopyrimidine 90*

Yield 33 %, 54-56 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.18 (6H, s, $2CH_3$), 2.39 (3H, s, $CH_3$), 4.65 (1H, m, OCH). MS (EI): m/z 254 ($M^+$). Anal. calcd for $C_9H_{10}N_4O_3S$: C, 42.51; H, 3.96; N, 22.04. Found: C, 42.45; H, 3.92; N, 21.97.

*2-Methyl-5-nitro-6-thiocyanatopyrimidin-4-amine 91*

Yield 39 %, 209-212 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 2.43 (3H, s, $CH_3$), 8.19 (2H, brs, $NH_2$). MS (EI): m/z 211 ($M^+$). Anal. calcd for $C_6H_5N_5O_2S$: C, 34.12; H, 2.39; N 33.16. Found: C, 34.25; H, 2.47; N, 33.04.

*N,2-Dimethyl-5-nitro-6-thiocyanatopyrimidin-4-amine 92*

Yield 76 %, 200-202 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 2.35 (3H, s, $CH_3$), 3.12 (3H, s, $CH_3$), 9.29 (1H, brs, NH). MS (EI): m/z 225 ($M^+$). Anal. calcd for $C_7H_7N_5O_2S$: C, 37.33; H, 3.13; N, 31.10. Found: C, 37.21; H, 3.27; N, 31.22.

*2-Methyl-5-nitro-N-(pentan-3-yl)-6-thiocyanatopyrimidin-4-amine 93*

Yield 79 %, 95-97 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.07 (6H, t, $2CH_3$), 1.46 (4H, brm, $2CH_2$), 2.40 (4H, m, CH, $CH_3$), 9.32 (1H, brs, NH). MS (EI): m/z 281 ($M^+$). Anal. calcd for $C_{11}H_{15}N_5O_2S$: C, 46.96; H, 5.37; N, 24.89. Found: C, 47.02; H, 5.32; N, 24.81.

*N-Cyclopropyl-2-methyl-5-nitro-6-thiocyanatopyrimidin-4-amine 94*

Yield 67 %, 104-107 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 0.83-1.12 (4H, brm, $2CH_2$), 2.25 (1H, brm, CH), 2.39 (3H, s, $CH_3$), 9.41 (1H, brs, NH). MS (EI): m/z 251 ($M^+$). Anal. calcd for $C_9H_9N_5O_2S$: C, 43.02; H, 3.61; N, 27.87. Found: C, 43.06; H, 3.68; N, 27.80.

*N-Cyclopentyl-2-methyl-5-nitro-6-thiocyanatopyrimidin-4-amine 95*

Yield 70 %, 124-125 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.57-1.82 (8H, m, $4CH_2$), 2.39 (3H, s, $CH_3$), 2.64 (1H, t, CH), 9.35 (1H, brs, NH). MS (EI): m/z 279 ($M^+$). Anal. calcd for $C_{11}H_{13}N_5O_2S$: C, 47.30; H, 4.69; N, 25.07. Found: C, 47.37; H, 4.75; N, 25.10.

*N-Cyclohexyl-2-methyl-5-nitro-6-thiocyanatopyrimidin-4-amine 96*

Yield 69 %, 95-96 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.01-1.13 (4H, m, $2CH_2$), 1.45-1.63 (6H, m, $3CH_2$), 2.39 (3H, s, $CH_3$), 2.57 (1H, qui, CH), 9.38 (1H, brs, NH). MS (EI): m/z 293 ($M^+$). Anal. calcd for $C_{12}H_{15}N_5O_2S$: C, 49.13; H, 5.15; N, 23.87. Found: C, 49.18; H, 5.21; N, 23.81.

*2-Methyl-5-nitro-4-(pyrrolidin-1-yl)-6-thiocyanatopyrimidine 97*

Yield 68 %, 142-144 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.91 (4H, m, $CH_2CH_2$), 2.41 (3H, s, $CH_3$), 3.50 (4H, m, $N(CH_2)_2$). MS (EI): m/z 265 ($M^+$). Anal. calcd for $C_{10}H_{11}N_5O_2S$: C, 45.27; H, 4.18; N, 26.40. Found: C, 45.29; H, 4.34; N, 26.43.

*2-Methyl-4-(2-methylpiperidin-1-yl)-5-nitro-6-thiocyanatopyrimidine 98*

Yield 76 %, 75-78 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.17 (3H, s, $CH_3$), 1.45-1.55 (6H, m, $2CH_2$), 2.37 (3H, s, $CH_3$), 3.04-3.19 (3H, m, $CH_2$, NCH). MS (EI): m/z 293 ($M^+$). Anal. calcd for $C_{12}H_{15}N_5O_2S$: C, 49.13; H, 5.15; N, 23.87. Found: C, 49.17; H, 5.19; N, 23.81.

*4-(3,5-Dimethylpiperidin-1-yl)-2-methyl-5-nitro-6-thiocyanatopyrimidine 99*

Yield 74 %, 122-125 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.15 (6H, s, $2CH_3$), 1.25-1.60 (4H, m, $HCCH_2CH$), 2.39 (3H, s, $CH_3$), 2.90 and 3.15 (4H, 2 brm, $N(CH_2)_2$). MS (EI): m/z 307 ($M^+$). Anal. calcd for $C_{13}H_{17}N_5O_2S$: C, 50.80; H, 5.58; N, 22.79. Found: C, 50.89; H, 5.63; N, 22.73.

*1-(2-Methyl-5-nitro-6-thiocyanatopyrimidin-4-yl)azepane 100*

Yield 68 %, 71-73 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.32-1.48 (8H, brm, $4CH_2$), 2.39 (3H, s, $CH_3$), 3.64 (4H, brm, $N(CH_2)_2$). MS (EI): m/z 293 ($M^+$). Anal. calcd for $C_{12}H_{15}N_5O_2S$: C, 49.13; H, 5.15; N, 23.87. Found: C, 49.21; H, 5.18; N, 23.92.

*1-(2-Methyl-5-nitro-6-thiocyanatopyrimidin-4-yl)azocane 101*

Yield 76 %, 95-98 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.18-1.82 (10H, brm, $5CH_2$), 2.56 (3H, s, $CH_3$), 3.91 (4H, brs, $N(CH_2)_2$). MS (EI): m/z 307 ($M^+$). Anal. calcd for $C_{13}H_{17}N_5O_2S$: C, 50.80; H, 5.58; N, 22.79. Found: C, 50.89; H, 5.63; N, 22.72.

*N-Benzyl-2-methyl-5-nitro-6-thiocyanatopyrimidin-4-amine 102*

Yield 58 %, 175-177 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 2.69 (3H, s, $CH_3$), 4.82 (2H, d, $CH_2$), 7.45 (5H, m, Ph), 9.04 (1H, brs, NH). MS (EI): m/z 301 ($M^+$). Anal. calcd for $C_{13}H_{11}N_5O_2S$: C, 51.82; H, 3.68; N, 23.24. Found: C, 51.90; H, 3.63; N, 23.26.

*2-Methyl-N-(4-methylbenzyl)-5-nitro-6-thiocyanatopyrimidin-4-amine 103*

Yield 67 %, 160-162 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 2.27 (3H, s, $CH_3$), 2.71 (3H, s, $CH_3$), 4.79 (2H, d, $CH_2$), 7.18 and 7.26 (2H, m, Ph), 9.70 (1H, brs, NH). MS (EI): m/z 315 ($M^+$). Anal. calcd for $C_{14}H_{13}N_5O_2S$: C, 53.32; H, 4.16; N, 22.21. Found: C, 53.39; H, 4.18; N, 22.27.

*2-Methyl-5-nitro-N-phenethyl-6-thiocyanatopyrimidin-4-amine 104*

Yield 52 %, 151-153 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 2.37 (3H, s, $CH_3$), 2.90 (2H, t, $CH_2$), 3.81 (2H, m, $CH_2$), 7.25 (5H, brm, Ph), 9.29 (1H, brs, NH). MS (EI): m/z 315 ($M^+$). Anal. calcd for $C_{14}H_{13}N_5O_2S$: C, 53.32; H, 4.16; N, 22.21. Found: C, 53.41; H, 4.11; N, 22.16.

*N,N,2-Trimethyl-5-nitro-6-thiocyanatopyrimidin-4-amine 105*

Yield 86 %, 159-161 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 2.36 (3H, s, $CH_3$), 3.48 (6H, s, $2CH_3$). MS (EI): m/z 239 ($M^+$). Anal. calcd for $C_8H_9N_5O_2S$: C, 40.16; H, 3.79; N, 29.27. Found: C, 40.26; H, 3.88; N, 29.34.

*N,N-Diethyl-2-methyl-5-nitro-6-thiocyanatopyrimidin-4-amine 106*

Yield 53 %, 69-70 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.10 (6H, m, $2CH_3$), 2.56 (3H, s, $CH_3$), 3.44 (4H, m, $N(CH_2)_2$). MS (EI): m/z 267 ($M^+$). Anal. calcd for $C_{10}H_{13}N_5O_2S$: C, 44.93; H, 4.90; N, 26.20. Found: C, 45.08; H, 4.93; N, 26.32.

*N-Benzyl-2-(methylthio)-5-nitro-6-thiocyanatopyrimidin-4-amine 107*

Yield 69 %, 157-159 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 2.15 (3H, s, $CH_3S$), 4.77 (2H, d, $CH_2$), 7.27 (5H, brm, Ph), 9.81 (1H, brs, NH). MS (EI): m/z 333 ($M^+$). Anal. calcd for $C_{13}H_{11}N_5O_2S_2$: C, 46.84; H, 3.33; N, 21.01. Found: C, 46.89; H, 3.40; N, 20.97.

*N-(4-Methylbenzyl)-2-(methylthio)-5-nitro-6-thiocyanatopyrimidin-4-amine 108*

Yield 80 %, 115-117 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 2.25 (3H, s, $CH_3$), 2.61 (3H, s, $CH_3S$), 4.75 (2H, d, $CH_2$), 7.07-7.27 (4H, m, Ph), 9.79 (1H, brs, NH). MS (EI): m/z 347 ($M^+$). Anal. calcd for $C_{14}H_{13}N_5O_2S_2$: C, 48.40; H, 3.77; N, 20.16. Found: C, 48.49; H, 3.71; N, 20.21.

*2-(Methylthio)-5-nitro-N-phenethyl-6-thiocyanatopyrimidin-4-amine 109*

Yield 71 %, 93-95 ºC (MeOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 2.59 (3H, s, $CH_3S$), 2.87 (2H, t, $CH_2$), 3.78 (2H, t, $CH_2$), 7.25 (5H, brm, Ph), 9.46 (1H, brs, NH). MS (EI): m/z 347 ($M^+$). Anal. calcd for $C_{14}H_{13}N_5O_2S_2$: C, 48.40; H, 3.77; N, 20.16. Found: C, 48.51; H, 3.86; N, 20.13.

*N-Isopropyl-5-nitro-6-thiocyanato-2-(trifluoromethyl)pyrimidin-4-amine 110*

Yield 75 %, 74-76 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.24 (6H, s, $2CH_3$), 3.97 (1H, m, CH), 9.59 (1H, brs, NH). MS (EI): m/z 307 ($M^+$). Anal. calcd for $C_9H_8F_3N_5O_2S$: C, 35.18; H, 2.62; N, 22.79. Found: C, 35.25; H, 2.67; N, 22.72.

*N-Isopropyl-5-nitro-2-phenyl-6-thiocyanatopyrimidin-4-amine 111*

Yield 76 %, 156-158 ºC (EtOH). $^1$H NMR (DMSO-$d_6$; δ, ppm): 1.18 (6H, s, $2CH_3$), 3.85 (1H, m, CH), 7.50 (3H, m, $CH_{ar}$), 8.36 (2H, m, $CH_{ar}$). 9.47 (1H, brs, NH). MS (EI): m/z 315 ($M^+$). Anal. calcd for $C_{14}H_{13}N_5O_2S$: C, 53.32; H, 4.16; N, 22.21. Found: C, 53.39; H, 4.23; N, 22.16.

*N-Methyl-5-nitro-2-styryl-6-thiocyanatopyrimidin-4-amine 112*

Yield 79 %, 215-217 ºC (EtOH/DMF). $^1$H NMR (DMSO-$d_6$; δ, ppm): 2.73 (3H, s, $CH_3$), 6.95-6.99 (2H, m, HC=CH), 7.36-7.49 (3H, brm, $CH_{ar}$), 7.60 (2H, m, $CH_{ar}$), 9.57 (1H, brs, NH). MS (EI): m/z 313 ($M^+$). Anal. calcd for $C_{14}H_{11}N_5O_2S$: C, 53.67; H, 3.54; N, 22.35. Found: C, 53.74; H, 3.49; N, 22.30.

**Strains and media**

KN99α, a strain of *C. neoformans* serotype A [6], was used as the wild-type strain and was obtained from Dr. Jennifer Lodge, Washington University. The *C. neoformans*

fluconazole-resistant DUMC-158.03, and *C. gattii,* RSA-3615, clinical strains were obtained from Dr. John Perfect, Duke University [7]. *Candida albicans* (Robin) Berkhout strain was purchased from the ATCC (ATCC #90028).

Strains were grown on YPD (1% yeast extract, 2% bacto-peptone, and 2% dextrose). Solid media contained 2% bacto-agar. YNB-02 (0.67% yeast nitrogen base, 0.2% dextrose, pH 7.0 with 50 mM MOPs) was used for all limiting dilution inhibition assays unless otherwise noted. The media is prepared in deionized water passed through ion exchange Milli Q filter system (Sigma-Millipore, USA).

**Determination of minimal inhibitory concentration**

Cells were grown overnight in 4 mL cultures of YPD at 30°C with shaking and then diluted to with an optical density (650 nM) of 0.001 (~5 x $10^5$ cells/mL) in YNB-02 + 1% DMSO for the limiting dilution assay. The compound dilution series and cells were prepared in round-bottom clear 96-well plates (Costar #3488), incubated without shaking for 48 hours at 35°C and the optical density measured. The minimal inhibitory concentration (MIC) was determined using compound concentrations from 0.19 to 50 mM, unless otherwise noted, of the compound in YNB-02 + 1% DMSO. Each assay was done in triplicate and all values are the average of two or more independent assays. The data are presented as the average cell density as a percent of DMSO-only treated cells. MICs are reported as the minimal concentration needed to inhibit 80% of *C. neoformans* or *C. gatti* growth relative to vehicle-treated controls.

**Drug combinations**

Drug combinations were assessed using the checkerboard assay [8, 9]. The MIC of the selected compounds were measured using compound concentrations from 0.19 to 12.5 μM and MICs for fluconazole (FLC) and amphotericin B (AMB) were measured using concentrations from 0.08 to 12.5 μM. Each assay was performed in triplicate and all values are the average of two or more independent assays. The fractional inhibitory concentration index (FICI) model is expressed as $\Sigma FIC = FIC_A + FIC_B = MIC_{A'}/MIC_A + MIC_{B'}/MIC_B$, where $MIC_A$, and $MIC_B$ are the MIC values of agents A and B used alone and $MIC_{A'}$ and $MIC_{B'}$ are the MICs of agents A and B used in combination. The interaction between FLC or AMB and the test compounds was interpreted as synergistic when FICI was ≤ 0.5, as indifferent when FICI was between > 0.5 and 4 and as antagonistic when FICI was > 4 [10].

**Cytotoxicity in hepatoma cells**

HepDES19 cells ($1.0 \times 10^4$ cells per well) were seeded in 96-well plates and incubated in DMEM with 10% fetal bovine serum (FBS) plus 1% penicillin and streptomycin, 1% nonessential amino acids, and 1% glutamine. The compounds were diluted in the medium at concentrations ranging from 0.78 to 100 μM plus 1% DMSO and added to the cells 48 hours after plating, with each concentration tested in triplicate. Cells were incubated with the compound for 72 hours and cytotoxicity was measured using a mitochondrial metabolic assay with MTS (Promega). The data were transformed to log[inhibitor] and fit to a 4-variable slope curve using GraphPad Prism (v8, www.graphpad.com). The concentration at which 50% of cells were inhibited relative to vehicle-treated control is reported as the $CC_{50}$ value.

**Assay Central**

Assay Central is a proprietary software developed from open-source descriptors and algorithms that have been previously described [11-17] combined with additional proprietary scripts. Structure-activity datasets for the molecules previously described were collated in Molecular Notebook (Molecular Materials Informatics, Inc. in Montreal, Canada) and are thoroughly curated to generate a Bayesian machine learning model with multiple scripts. We employed a series of rules to detect any problematic data, corrections were implemented by a combination of automated and human re-curation for structure standardization. This approach produces a high-quality dataset and a Bayesian model to predict activities for proposed compounds. These Bayesian models utilize extended-connectivity fingerprints of maximum diameter 6 (ECFP6) descriptors generated from the Chemistry Development Kit library [18]. These descriptors have widely been previously noted for their ability to map structure-activity relationships [19]. From all the training set molecules, the Assay Central software enumerates all possible fingerprints from the training set and determines a given fingerprint's contribution to a binary activity classification from the ratio of its presence in active and inactive molecules. Assay Central also uses the summation of these contributions for a given molecule to produce a probability-like score. Metrics such as Receiver Operator Characteristic (ROC), Recall, Precision, F1 Score, Cohen's Kappa and Matthew's Correlation Coefficient are generated from internal five-fold cross-validation of the model. To maximize these internal performance statistics, the software can select a reasonable activity threshold, and generate predictions as well as applicability scores for any desired compound. Higher

prediction scores are desirable as scores higher than 0.5 are assigned to active compounds (inhibitors). Higher applicability scores are also desirable as it ensures the representation of the drug in the training set [19]. Assay Central has been used in various drug discovery projects and the applicability of the model statistics have also been previously described [11-13, 16, 20-25].

**NIAID ChemDB Curation of Training Data**

ChemDB was initially received as a transfer from NIAID as a series of comma-separate value files (.csv) [26]. Each measurement was assigned to a molecule identifier (AIDSNo) and depicted in a structure file (.sdf) with additional molecular information. We utilized workflows to combine the structures with measurements into new datasheet structures files (.ds) with the AIDSNo, restricted to *Cryptoccocus neoformans* data.

**Supplemental References**


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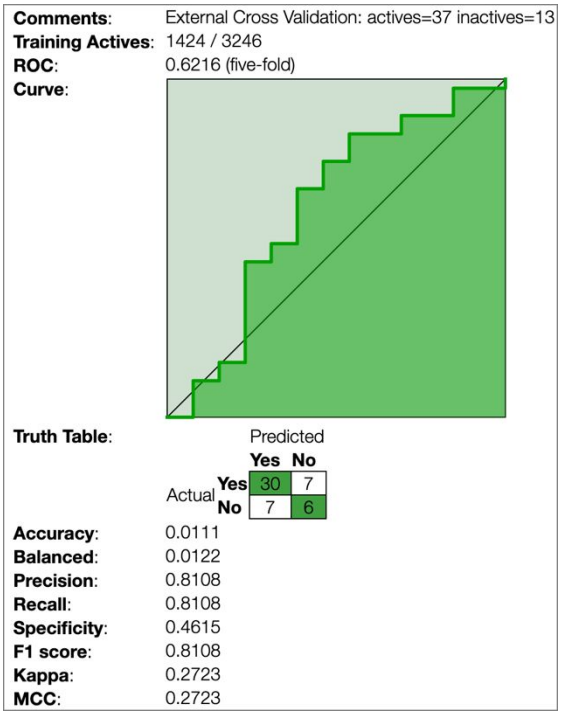
**Figure S1**. “Subvalidations” of the compounds from Table 1 using the literature cryptococcus model derived from the NIAID ChemDB database at thresholds of 10 μM (A) and 3.2μM (B).

A




B

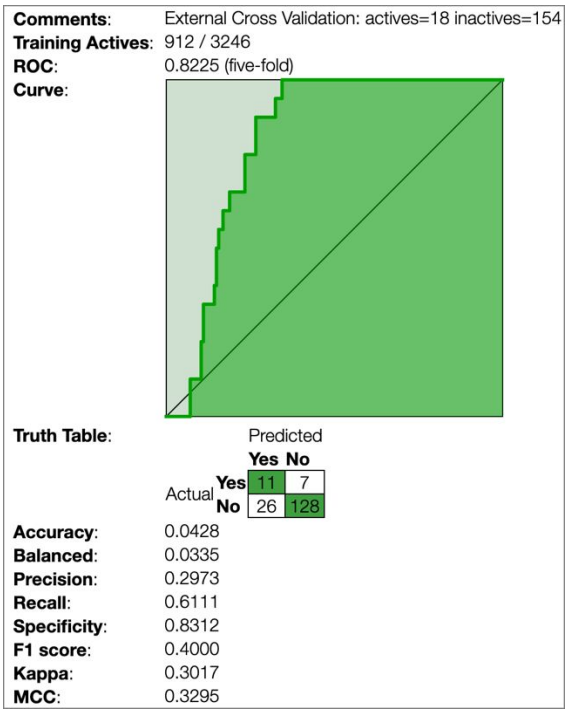
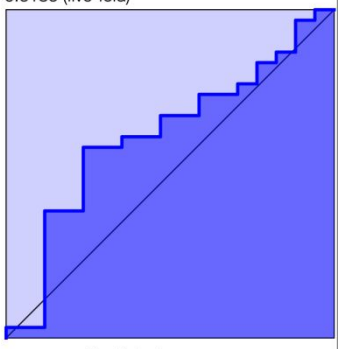


**Figure S2.** Bayesian Model 5-fold cross validation statistics for *C. gatti* model with an $MIC_{80}$ threshold for activity threshold of MIC 1.52-3.12 μM.

| | |
|---|---|
| **Origin**: | Assay Central |
| **Field**: | donlin/Gatti_120120 |
| **Comments**: | Threshold < 3.1200 μM |
| **Training Actives**: | 31 / 48 |
| **ROC**: | 0.6186 (five-fold) |
| **Curve**: |  |

**Truth Table**:

| | | Predicted | |
|---|---|---|---|
| | | **Yes** | **No** |
| Actual | **Yes** | 18 | 13 |
| | **No** | 4 | 13 |

| | |
|---|---|
| **Accuracy**: | 0.6458 |
| **Balanced**: | 0.6727 |
| **Precision**: | 0.8182 |
| **Recall**: | 0.5806 |
| **Specificity**: | 0.7647 |
| **F1 score**: | 0.6792 |
| **Kappa**: | 0.3085 |
| **MCC**: | 0.3315 |

**Table S1**. Model 5-fold cross validation or subvalidation statistics for models described in each figure.

| Model | ROC | truePos | trueNeg | falsePos | falseNeg | accuracy | balanced | precision | recall | specificity | F1 | kappa | MCC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fig 3A | 0.70 | 8 | 103 | 4 | 6 | 0.92 | 0.77 | 0.67 | 0.57 | 0.96 | 0.62 | 0.57 | 0.57 |
| Fig 3B | 0.51 | 13 | 32 | 4 | 2 | 0.33 | 0.49 | 0.29 | 0.87 | 0.11 | 0.43 | -0.01 | -0.03 |
| Fig 3C | 0.90 | 18 | 120 | 34 | 0 | 0.80 | 0.89 | 0.35 | 1.00 | 0.78 | 0.51 | 0.42 | 0.52 |
| Fig 3D | 0.87 | 1202 | 1347 | 475 | 222 | 0.79 | 0.79 | 0.72 | 0.84 | 0.74 | 0.78 | 0.57 | 0.58 |
| Fig 4 | 0.82 | 36 | 31 | 96 | 9 | 0.77 | 0.78 | 0.54 | 0.80 | 0.76 | 0.64 | 0.48 | 0.50 |
| Fig S1A | 0.62 | 30 | 7 | 6 | 7 | 0.72 | 0.64 | 0.81 | 0.81 | 0.46 | 0.81 | 0.27 | 0.27 |
| Fig S1B | 0.64 | 14 | 21 | 13 | 2 | 0.54 | 0.63 | 0.40 | 0.88 | 0.38 | 0.55 | 0.20 | 0.26 |
| Fig S2 | 0.62 | 18 | 13 | 4 | 13 | 0.65 | 0.67 | 0.82 | 0.58 | 0.76 | 0.68 | 0.31 | 0.33 |

**Table S2.** Prediction validation for the 50 highlighted compounds using the NIAID *C. neoformans* training set.

| Molecule Number | Prediction Score | Domain | Predicted Active | Active (10 μM Threshold) | *C. neoformans* $MIC_{80}$ μM (μg/ml) | MIC80 (μM) | Predicted Correctly | Category |
|---|---|---|---|---|---|---|---|---|
| 63 | 0.508 | 0.585 | yes | yes | 9.37 ± 3.12 (1.98 ± 0.66) | 9.3 ± 3.1 | yes | TP |
| 64 | 0.507 | 0.625 | yes | yes | 3.9 ± 0 (0.93 ± 0) | 3.8 ± 0 | yes | TP |
| 65 | 0.513 | 0.556 | yes | yes | 1.9 ± 0 (0.45 ± 0) | 1.9 ± 0 | yes | TP |
| 66 | 0.516 | 0.553 | yes | yes | 4.68 ± 1.56 (0.92 ± 0.3) | 4.6 ± 1.5 | yes | TP |
| 67 | 0.517 | 0.578 | yes | yes | 2.34 ± 0.78 (0.55 ± 0.18) | 2.3 ± 0.7 | yes | TP |
| 68 | 0.524 | 0.521 | yes | yes | 3 ± 0 (0.8 ± 0) | 3 ± 0 | yes | TP |

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|---|---|---|---|---|---|---|---|---|
| 69 | 0.520 | 0.571 | yes | no | 18.5 ± 0<br>(4.94 ± 0) | 18.5 ± 6.5 | no | FP |
| 70 | 0.526 | 0.571 | yes | yes | 4.9 ± 0<br>(1.29 ± 0) | 2.2 ± 0.7 | yes | TP |
| 71 | 0.531 | 0.585 | yes | yes | 6 ± 0<br>(1.67 ± 0) | 4.5 ± 1.5 | yes | TP |
| 72 | 0.512 | 0.571 | yes | yes | 2.34 ± 0.78<br>(0.61 ± 0.2) | 2.3 ± 0.7 | yes | TP |
| 73 | 0.505 | 0.614 | yes | yes | 9.37 ± 3.12<br>(2.61 ± 0.86) | 9.3 ± 3.1 | yes | TP |
| 74 | 0.494 | 0.566 | no | yes | 9.37 ± 3.12<br>(2.74 ± 0.9) | 9.3 ± 3.1 | no | FN |
| 75 | 0.509 | 0.528 | yes | yes | 2.34 ± 0.78<br>(0.68 ± 0.22) | 2.3 ± 0.7 | yes | TP |
| 76 | 0.517 | 0.571 | yes | yes | 2.34 ± 0.78<br>(0.65 ± 0.22) | 2.3 ± 0.7 | yes | TP |

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|---|---|---|---|---|---|---|---|---|
| 77 | 0.519 | 0.580 | yes | yes | 4.68 ± 1.56 (1.37 ± 0.46) | 4.6 ± 1.5 | yes | TP |
| 78 | 0.510 | 0.636 | yes | yes | 2.34 ± 0.78 (0.66 ± 0.22) | 2.3 ± 1.5 | yes | TP |
| 79 | 0.552 | 0.667 | yes | no | 9.37 ± 12.5 (3.32 ± 4.44) | 18.5 ± 0 | no | FP |
| 80 | 0.505 | 0.632 | yes | yes | 5.85 ± 7.8 (1.88 ± 2.5) | 9 ± 0 | yes | TP |
| 81 | 0.492 | 0.603 | no | yes | 3.51 ± 4.68 (1.05 ± 1.4) | 4.5 ± 0 | no | FN |
| 82 | 0.517 | 0.512 | yes | yes | 4.68 ± 1.56 (1.05 ± 0.34) | 4.6 ± 1.5 | yes | TP |
| 83 | 0.517 | 0.511 | yes | yes | 9.37 ± 3.12 (2.37 ± 0.78) | 9.3 ± 3.1 | yes | TP |
| 84 | 0.529 | 0.490 | yes | yes | 4.55 ± 4.24 (1.4 ± 1.12) | 0.9 ± 0.3 | yes | TP |

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|---|---|---|---|---|---|---|---|---|
| 85 | 0.538 | 0.509 | yes | yes | 6 ± 0<br>(1.76 ± 0) | 9 ± 3 | yes | TP |
| 86 | 0.527 | 0.596 | yes | yes | 2.34 ± 0.78<br>(0.7 ± 0.22) | 2.3 ± 1.5 | yes | TP |
| 87 | 0.526 | 0.550 | yes | no | >50<br>(>16.46) | 50 ± 0 | no | FP |
| 88 | 0.499 | 0.533 | no | yes | 1.17 ± 0.38<br>(0.27 ± 0.08) | 1.1 ± 0.3 | no | FN |
| 89 | 0.494 | 0.563 | no | yes | 0.6 ± 0<br>(0.15 ± 0) | 0.6 ± 0 | no | FN |
| 90 | 0.500 | 0.489 | yes | yes | 1 ± 0<br>(0.25 ± 0) | 1.2 ± 0 | yes | TP |
| 91 | 0.505 | 0.487 | yes | yes | 2.34 ± 0.78<br>(0.48 ± 0.16) | 2.3 ± 0.7 | yes | TP |
| 92 | 0.513 | 0.463 | yes | yes | 2.34 ± 0.78<br>(0.52 ± 0.16) | 2.3 ± 0.7 | yes | TP |

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|---|---|---|---|---|---|---|---|---|
| 93 | 0.507 | 0.510 | yes | yes | 9 ± 0<br>(2.53 ± 0) | 9 ± 3 | yes | TP |
| 94 | 0.507 | 0.500 | yes | yes | 0.6 ± 0<br>(0.15 ± 0) | 0.6 ± 0 | yes | TP |
| 95 | 0.513 | 0.520 | yes | yes | 9 ± 0<br>(2.51 ± 0) | 9 ± 3 | yes | TP |
| 96 | 0.472 | 0.583 | no | no | >50<br>(>24.62) | 50 ± 0 | yes | TN |
| 97 | 0.501 | 0.522 | yes | yes | 4.68 ± 1.56<br>(1.23 ± 0.4) | 4.6 ± 1.5 | yes | TP |
| 98 | 0.493 | 0.561 | no | no | 18.75 ± 6.24<br>(5.49 ± 1.82) | 18.7 ± 6.2 | yes | TN |
| 99 | 0.498 | 0.472 | no | no | 18.75 ± 6.24<br>(5.76 ± 1.92) | 18.7 ± 6.2 | yes | TN |
| 100 | 0.506 | 0.520 | yes | no | 18.75 ± 6.24<br>(5.49 ± 1.82) | 18.7 ± 6.2 | no | FP |

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|---|---|---|---|---|---|---|---|---|
| 101 | 0.508 | 0.529 | yes | no | 18.75 ± 6.24 (5.76 ± 1.92) | 18.7 ± 6.2 | no | FP |
| 102 | 0.497 | 0.596 | no | no | 50 ± 0 (15.06 ± 0) | 50 ± 0 | yes | TN |
| 103 | 0.504 | 0.561 | yes | no | 50 ± 0 (15.76 ± 0) | 50 ± 0 | no | FP |
| 104 | 0.479 | 0.567 | no | no | 50 ± 0 (15.76 ± 0) | 50 ± 0 | yes | TN |
| 105 | 0.508 | 0.463 | yes | yes | 4.68 ± 1.56 (1.11 ± 0.36) | 4.6 ± 1.5 | yes | TP |
| 106 | 0.508 | 0.467 | yes | yes | 9.37 ± 3.12 (2.5 ± 0.82) | 9.3 ± 3.1 | yes | TP |
| 107 | 0.482 | 0.661 | no | yes | 4.68 ± 4.68 (1.56 ± 1.56) | 4.5 ± 0 | no | FN |
| 108 | 0.489 | 0.633 | no | yes | 9.37 ± 3.12 (3.25 ± 1.08) | 9 ± 0 | no | FN |

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|---|---|---|---|---|---|---|---|---|
| 109 | 0.465 | 0.629 | no | yes | 9.37 ± 9.36<br>(3.25 ± 3.24) | 9 ± 0 | no | FP |
| 110 | 0.518 | 0.531 | yes | yes | 12.25 ± 6.5<br>(4.4 ± 2) | 4.5 ± 1.5 | yes | TP |
| 111 | 0.493 | 0.571 | no | no | 50 ± 0<br>(15.76 ± 0) | 50 ± 0 | yes | TN |
| 112 | 0.511 | 0.552 | yes | no | >50<br>(>15.66) | 50 ± 0 | no | FP |