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Supplementary data

Bis-guanyldrazones as efficient anti-*Candida* compounds through DNA interaction

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Experimental section

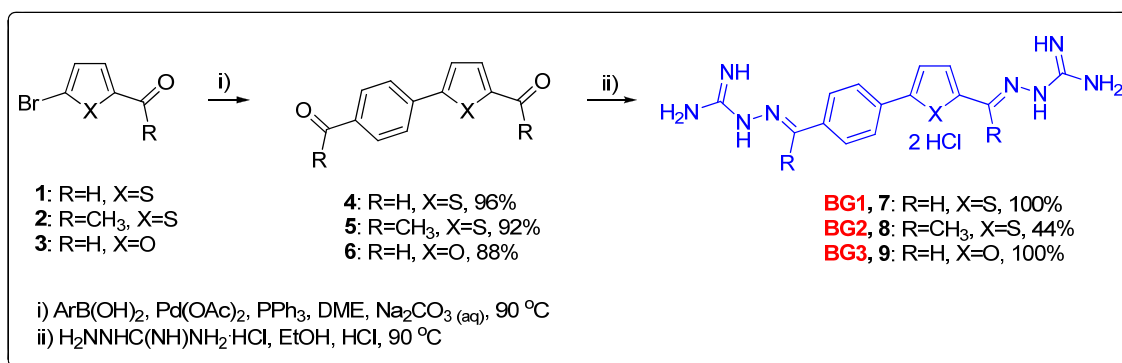


Fig. S1 Synthesis of compounds **BG1-BG3** (7-9)

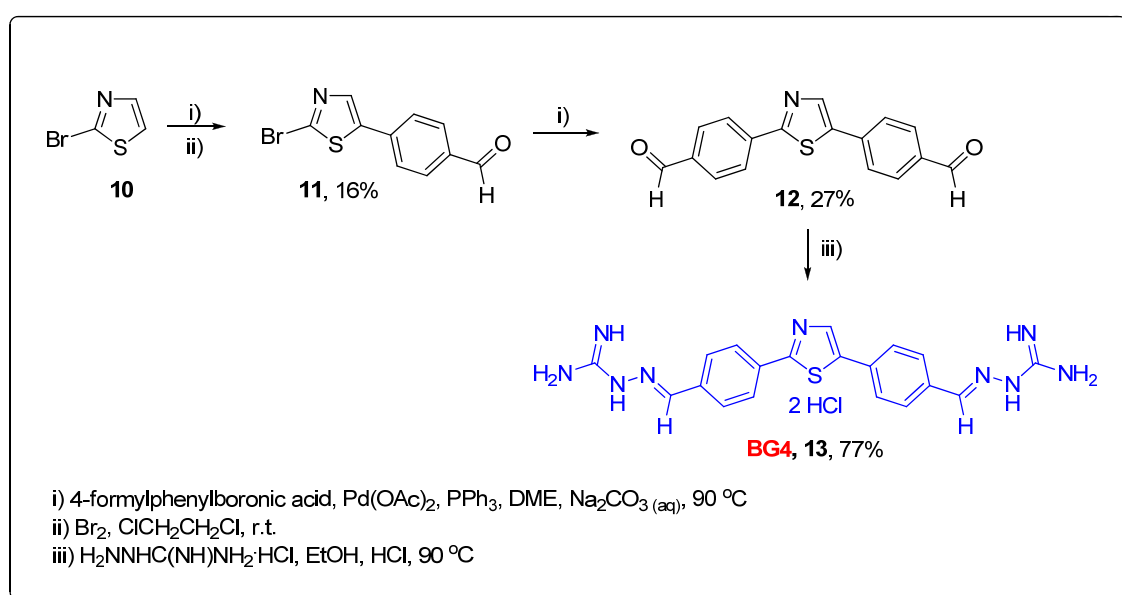


Fig. S2 Synthesis of the compound **BG4** (13)

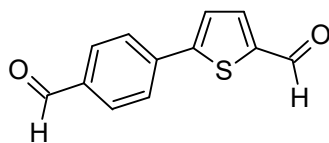
Instrumentation

Dry-flash chromatography was performed on SiO₂ (0.018–0.032 mm). Melting points were determined on a Boetius PMHK apparatus and were not corrected. IR spectra were recorded on a Thermo-Scientific Nicolet 6700 FT-IR Diamond Crystal. NMR: ¹H and ¹³C NMR spectra were recorded on a Bruker Ultrashield Advance III spectrometer (at 500 and 125 MHz, respectively) in the indicated solvent using TMS as the internal standard. Chemical shifts are expressed in parts per million (ppm) on the (δ) scale, and coupling constants (*J*) in Hz. ESI MS spectra of the synthesized compounds were recorded on an Agilent Technologies 6210 Time-of-Flight LC/MS instrument in positive ion mode using

MeOH/H₂O = 1:1 with 0.2% HCOOH as the carrying solvent solution. The samples were dissolved in pure MeOH (HPLC grade). The selected values were as follows: capillary voltage = 4 kV; gas temperature = 350 °C; drying gas N₂ = 12 L/min; nebulizer pressure = 45 psig; fragmentator voltage = 70 V. All the yields reported refer to isolated yields. GC–MS spectra of the synthesized compounds were acquired on an Agilent Technologies 7890A apparatus equipped with a DB-5 MS column (30 m × 0.25 mm × 0.25 μm), a 5975C MSD and FID detector. The selected values are as follows: carrier gas was He (1.0 mL/min), temperature linearly increased from 40–315 °C (10 °C/min), injection volume = 1 μL, temperature = 250 °C, temperature (FID detector) = 300 °C, and EI mass spectra range: m/z 40–550. Compounds were analyzed for purity using: Agilent 1200 HPLC system equipped with Quat Pump (G1311B), Injector (G1329B) 1260 ALS, TCC 1260 (G1316A) and Detector 1260 DAD VL+(G1315C), and Waters 1525 HPLC dual pump system equipped with an Alltech Select degasser system, and a dual λ 2487 UV–VIS detector. All compounds were >95% pure. *Method A*: Zorbax Eclipse Plus C18 4.6 × 150 mm, 1.8μ, S.N. USWKY01594 was used as the stationary phase. Eluent was made from the following solvents: 0.2% formic acid in water (A) and acetonitrile (B). The analysis was performed at the UV max of the compounds to maximize selectivity. Compounds were dissolved in methanol, final concentrations were 1 mg/mL. Flow rate was 0.5 mL/min. Compounds **7-9** and **13** were eluted using gradient protocol: 0 – 0.5 min 95%A, 0.5 - 3 min 95%A→ 5%A, 3 - 13 min 5%A, 13 – 14 min 5%A→ 95%A, 14 – 16 min 95%A. *Method B*: Poroshell 120 EC-C18 4.6 × 50mm, 2.7μ, S.N. USWKY01594 was used as the stationary phase. Eluent was made from the following solvents: 0.2% formic acid in water (A) and acetonitrile (B). The analysis were performed at the UV max of the compounds to maximize selectivity. Compounds were dissolved in methanol, final concentrations were 1 mg/mL. Flow rate was 0.5 mL/min. Compounds **7-9** and **13** were eluted using gradient protocol: 0 – 0.5 min 95%A, 0.5 - 3 min 95%A→ 5%A, 3 - 13 min 5%A, 13 – 14 min 5%A→ 95%A, 14 – 16 min 95%A.

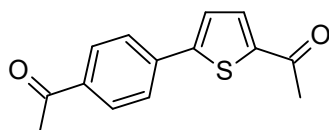
General procedure for Suzuki coupling reactions

5-(4-Formylphenyl)thiophene-2-carbaldehyde (**4**)¹



To a dry glass flask purged with argon were added Pd(OAc)₂ (2.9 mg, 0.013 mmol), PPh₃ (14 mg, 0.050 mmol) and dry DME (2 mL). The resultant solution was stirred at room temperature for 10 min, and 5-bromothiophene-2-carbaldehyde **1** (30 μL, 0.26 mmol) and Na₂CO₃ (aq) (2M, 0.30 mL, 0.6 mmol) were added. After 5 min, 4-formylphenylboronic acid (49 mg, 0.33 mmol) was added and the reaction mixture was purged with argon and refluxed at 90 °C for 3 h under argon. The solution was cooled to room temperature and filtered through a pad of Celite, washed with CH₂Cl₂ and dried with anhydrous Na₂SO₄. The organic solvent was removed under reduced pressure and the crude product was purified by dry-flash chromatography (SiO₂: hexane/EtOAc = 9:1 to 6:4) to afford the title compound **4** (54 mg, 96%) as a yellow amorphous powder, mp = 130–132 °C. IR (ATR): 3078, 3047, 2844, 2757, 1693, 1658, 1601, 1566, 1506, 1447, 1396, 1318, 1291, 1224, 1183, 1059, 961, 840m, 806, 761, 696, 675 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): δ 10.06 (s, 1H), 9.95 (s, 1H), 7.99-7.94 (m, 2H), 7.88-7.83 (m, 2H), 7.80 (d, *J* = 4.0 Hz, 1H), 7.55 (d, *J* = 4.0 Hz, 1H) ppm. ¹³C NMR (125 MHz, CDCl₃): δ 191.2, 182.8, 151.8, 144.0, 138.5, 137.1, 136.5, 130.5, 126.8, 125.7 ppm. GC/MS (*m/z*(%)): 214.9 ([M]⁺, 100).

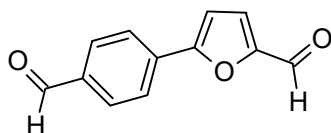
1-[5-(4-acetylphenyl)thiophen-2-yl]ethanone (**5**)



Following the general procedure for Suzuki coupling, compound **2** (100 mg, 0.450 mmol) was transformed to the title compound **5** (109 mg, 92%) using 4-acetylphenylboronic acid (100 mg, 0.610 mmol), Pd(OAc)₂ (5.5 mg, 0.024 mmol) and PPh₃ (26 mg, 0.097 mmol). The crude product was purified by dry-flash chromatography (SiO₂: hexane/EtOAc = 8:2 to 6:4) to afford the title compound **5** as a yellow amorphous powder, mp = 140–144 °C. IR (ATR): 3332, 3291, 3076, 3051, 3028, 3003, 2962, 2919, 2857, 2708, 1676, 1655, 1600, 1561, 1533, 1503, 1446, 1406, 1357, 1314, 1270, 1188, 1120, 1076, 1040, 959, 930, 832, 754, 590 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): δ 8.03-7.98 (m, 2H), 7.78-7.72 (m, 2H), 7.69

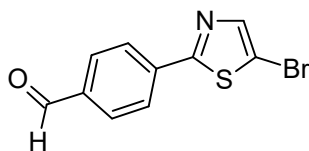
(d, $J = 4.0$ Hz, 1H), 7.43 (d, $J = 4.0$ Hz, 1H), 2.63 (s, 3H), 2.59 (s, 3H) ppm. ^{13}C NMR (125 MHz, CDCl_3): δ 197.1, 190.5, 150.7, 144.4, 137.5, 136.9, 133.3, 129.1, 126.2, 125.2, 26.6 ppm. GC/MS ($m/z(\%)$): 244.0 ($[\text{M}]^+$, 100).

5-(4-formylphenyl)furan-2-carbaldehyde (**6**)



Following the general procedure for Suzuki coupling, compound **3** (100 mg, 0.570 mmol) was transformed to the title compound **6** (101.3 mg, 88%) using 4-formylphenylboronic acid (107 mg, 0.710 mmol), $\text{Pd}(\text{OAc})_2$ (6.4 mg, 0.028 mmol) and PPh_3 (30 mg, 0.11 mmol). The crude product was purified by dry-flash chromatography (SiO_2 : hexane/EtOAc = 8:2 to 3:7) to afford the title compound **6** as an orange amorphous powder, mp = 138–140 °C. IR (ATR): 3113, 3060, 2862, 2827, 2738, 1675, 1603, 1527, 1487, 1425, 1391, 1362, 1315, 1294, 1258, 1215, 1172, 1047, 967, 922, 834, 808, 779, 687 cm^{-1} . ^1H NMR (500 MHz, CDCl_3): δ 10.04 (s, 1H), 9.71 (s, 1H), 8.00–7.95 (m, 4H), 7.35 (d, $J = 3.5$ Hz, 1H), 7.01 (d, $J = 4.0$ Hz, 1H) ppm. ^{13}C NMR (125 MHz, CDCl_3): δ 191.3, 177.5, 157.5, 152.7, 136.5, 134.1, 130.3, 125.6, 110.0 ppm. GC/MS ($m/z(\%)$): 200.0 ($[\text{M}]^+$, 100).

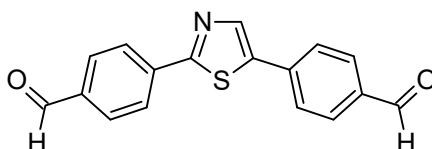
4-(5-bromo-1,3-thiazol-2-yl)benzaldehyde (**11**)



To a dry glass flask purged with argon were added $\text{Pd}(\text{OAc})_2$ (12 mg, 0.050 mmol), PPh_3 (55 mg, 0.21 mmol) and dry DME (7 mL). The resultant solution was stirred at room temperature for 10 min and 2-bromo-1,3-thiazole (**10**) (172 mg, 1.05 mmol) and Na_2CO_3 (aq) (2M, 1 mL, 2 mmol) were added. After 5 min of stirring at room temperature, 4-formylphenylboronic acid (196 mg, 1.31 mmol) was added and the reaction mixture was purged with argon and refluxed at 90 °C for 3 h under argon. The solution was cooled to room temperature and filtered through a pad of Celite, washed with CH_2Cl_2 and dried with anhydrous Na_2SO_4 . The organic solvent was removed under reduced pressure and the crude product was purified by dry-flash chromatography (SiO_2 : hexane/EtOAc = 9:1 to 7:3). The solvent was removed under reduced pressure, the crude product was dissolved in 1,2-dichloroethane (1.2 mL) and a solution of bromine (30 μL , 0.52 mmol) in 1,2-dichloroethane

(1.2 mL) was added. The reaction mixture was stirred at room temperature for 18 h. The reaction was stopped with the addition of saturated $\text{Na}_2\text{S}_2\text{O}_3$ (aq) (5 mL), washed with CH_2Cl_2 and dried with anhydrous Na_2SO_4 . The organic solvent was removed under reduced pressure and the crude product was purified by dry-flash chromatography (SiO_2 : hexane/EtOAc = 8:2 to 3:7) to afford the title compound **11** (44 mg, 16%) as a white amorphous powder, mp = 129 °C. IR (ATR): 3987, 3920, 2848, 2744, 1699, 1605, 1570, 1477, 1422, 1266, 1211, 1173, 1106, 972, 827 cm^{-1} . ^1H NMR (500 MHz, CDCl_3): δ 10.06 (s, 1H), 8.09-8.01 (m, 2H), 7.99-7.93 (m, 2H), 7.82 (s, 1H) ppm. ^{13}C NMR (125 MHz, CDCl_3): δ 191.3, 167.6, 145.6, 138.0, 137.3, 130.4, 126.7, 110.5 ppm. GC/MS (m/z (%)): 268.9 ($[\text{M}]^+$, 100).

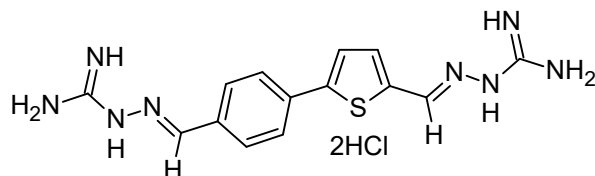
4,4'-(1,3-thiazole-2,5-diyl)dibenzaldehyde (**12**)



Following the general procedure for Suzuki coupling, compound **11** (118 mg, 0.440 mmol) was transformed to the title compound **12** (35 mg, 27%) using 4-formylphenylboronic acid (82 mg, 0.55 mmol), $\text{Pd}(\text{OAc})_2$ (5 mg, 0.02 mmol) and PPh_3 (23 mg, 0.09 mmol). The crude product was purified by dry-flash chromatography (SiO_2 : hexane/EtOAc = 7:3 to 1:9) to afford the title compound **12** as a yellow amorphous powder, mp = 178–180 °C. IR (ATR): 3370, 2955, 2920, 2851, 1738, 1701, 1602, 1462, 1428, 1389, 1287, 1212, 1168, 1107, 823 cm^{-1} . ^1H NMR (500 MHz, CDCl_3): δ 10.08 (s, 1H), 10.05 (s, 1H), 8.23 (s, 1H), 8.20-8.12 (m, 2H), 8.15-7.90 (m, 4H), 7.85-7.75 (m, 2H) ppm. ^{13}C NMR (125 MHz, CDCl_3): δ 191.4, 191.2, 166.8, 141.4, 139.4, 138.3, 137.3, 136.7, 136.0, 130.6, 130.4, 127.1, 127.0 ppm. GC/MS (m/z (%)): 292.9 ($[\text{M}]^+$, 100).

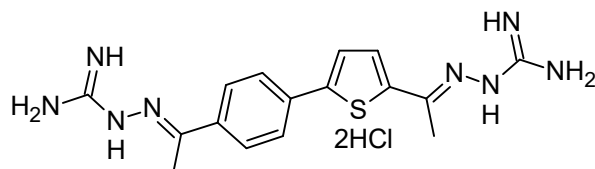
General procedure for preparation of guanylhydrazones 7-9 and 13

(2E)-2-[(5-{4-[(E)-2-Carbamimidoylhydrazinylidene)methyl]phenyl}thiophen-2-yl)methylidene]hydrazinecarboximidamide dihydrochloride (7, BG1)¹



To a solution of aldehyde **4** (20 mg, 0.092 mmol) in absolute ethanol (6 mL) aminoguanidine hydrochloride (26 mg, 0.23 mmol) was added. The resultant solution was stirred at room temperature for 5 min, and solution of concentrated HCl in absolute EtOH (40 μ L, 1:25 v/v) was added. The reaction mixture was heated to reflux for 18 h and allowed to cool to room temperature. The solvent was removed under reduced pressure, and the crude product was washed with CH₂Cl₂ (1 mL) and then crystallized from EtOH/hexane (9:1) to provide the title compound **7** (37 mg, 100%) as a pale-yellow solid, mp = 248–250 °C. IR (ATR): 3352, 3275, 3153, 2872, 1668, 1612, 1536, 1437, 1350, 1237, 1181, 1141, 1011, 829 cm⁻¹. ¹H NMR (500 MHz, CD₃OD): δ 8.30 (s, 1H), 8.15 (s, 1H), 7.86 (d, 2H, J = 8.0 Hz), 7.78 (d, 2H, J = 8.0 Hz), 7.52 (d, 1H, J = 4.0 Hz), 7.45 (d, 1H, J = 4.0 Hz) ppm. ¹³C NMR (125 MHz, CD₃OD): δ 157.3, 157.0, 148.8, 148.3, 144.1, 139.1, 137.2, 134.8, 134.3, 129.7, 127.3, 126.0 ppm. (+)ESI-HRMS (*m/z*): [M+H]⁺ 329.12823 (error -2.77 ppm). HPLC purity: method A: RT 6.322, area 99.08%; method B: RT 2.544, area 95.62%.

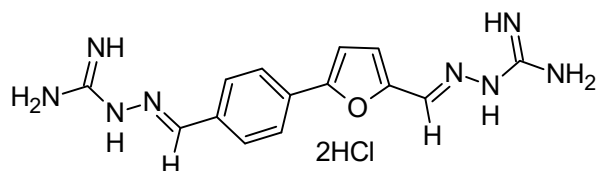
(2E)-2-[1-(5-{4-[(1E)-1-(2-carbamimidoylhydrazinylidene)ethyl]phenyl}thiophen-2-yl)ethylidene]hydrazinecarboximidamide dihydrochloride (8, BG2)



Following the general procedure for guanylhydrazone formation, compound **5** (100 mg, 0.410 mmol) was transformed to the title compound **8** (76 mg, 44%) using aminoguanidine hydrochloride (113 mg, 1.02 mmol). The product was obtained as a pale-yellow solid, mp = 226–228 °C. IR (ATR): 3137, 1674, 1619, 1463, 1409, 1372, 1311, 1133, 835, 585 cm⁻¹. ¹H NMR (500 MHz, CD₃OD): δ 8.00-7.95 (m, 2H), 7.78-7.74 (m, 2H), 7.55 (d, J = 4.0 Hz, 1H), 7.48 (d, J = 4.0 Hz, 1H), 2.40 (s, 3H), 2.39 (s, 3H) ppm. ¹³C NMR (125 MHz, *d*-

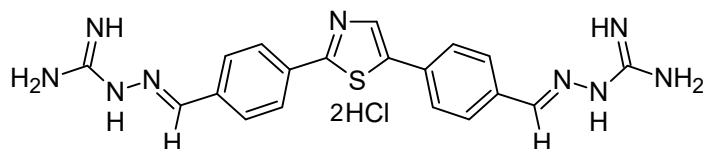
DMSO): δ 158.1, 157.7, 153.0, 150.1, 147.0, 143.4, 138.4, 136.3, 132.0, 129.6, 127.2, 126.9, 17.0, 16.5 ppm. (+)ESI-HRMS m/z : $[M + H]^+$ 357.16018 (error -0.72 ppm). HPLC purity: method A: RT 6.324, area 99.18%; method B: RT 2.640, area 95.32%.

(2Z)-2-(4-{5-[(E)-(2-carbamimidoylhydrazinylidene)methyl]furan-2-yl}benzylidene)hydrazinecarboximidamidedihydrochloride (9, BG3)



Following the general procedure for guanylylhydrazone formation, compound **6** (70 mg, 0.35mmol) was transformed to the title compound **9** (135 mg, 100%) using aminoguanidine hydrochloride (97 mg, 0.87 mmol). The product was obtained as an orange solid, mp = 186 °C. IR (ATR): 3326, 1673, 1617, 1277, 1226, 1141, 1025, 789 cm^{-1} . ^1H NMR (500 MHz, CD_3OD): δ 8.14 (s, 1H), 8.06 (s, 1H), 7.93-7.82 (m, 4H), 7.10-7.05 (m, 2H) ppm. ^{13}C NMR (125 MHz, D_2O): δ 154.5, 153.8, 153.6, 146.6, 136.2, 131.2, 129.6, 126.9, 123.3, 118.5, 108.6 ppm. (+)ESI-HRMS m/z : $[M + H]^+$ 313.15171 (error -0.86 ppm). HPLC purity: method A: RT 6.328, area 97.71%; method B: RT 2.518, area 96.31%.

(2E)-2-[4-(2-{4-[(Z)-(2-carbamimidoylhydrazinylidene)methyl]phenyl}-1,3-thiazol-5-yl)benzylidene]hydrazinecarboximidamide (13, BG4)



Following the general procedure for guanylylhydrazone formation, compound **12** (30 mg, 0.10 mmol) was transformed to the title compound **13** (38 mg, 77%) using aminoguanidine hydrochloride (28 mg, 0.26 mmol). The product was obtained as a bright-yellow solid, mp = 230–231 °C. IR (ATR): 3363, 3265, 3131, 2952, 2919, 2877, 2803, 1672, 1622, 1540, 1486, 1428, 1352, 1235, 1183, 1150, 867, 715 cm^{-1} . ^1H NMR (500 MHz, d -DMSO): δ 12.20-12.00 (m, 2H), 8.49 (s, 1H), 8.24 (s, 1H), 8.22 (s, 1H), 8.10-7.90 (m, 4H), 7.99-7.94 (m, 2H), 7.85-7.80 (m, 2H) ppm. ^{13}C NMR (125 MHz, d -DMSO): δ 165.6, 155.4, 146.0, 145.9, 141.2, 138.7, 135.2, 134.2, 133.5, 132.4, 128.5, 128.4, 126.6, 126.3 ppm. (+)ESI-HRMS m/z : $[M + H]^+$ 406.15468 (error -2.48 ppm). HPLC purity: method A: RT 6.312, area 99.05%; method B: RT 2.667, area 96.62%.

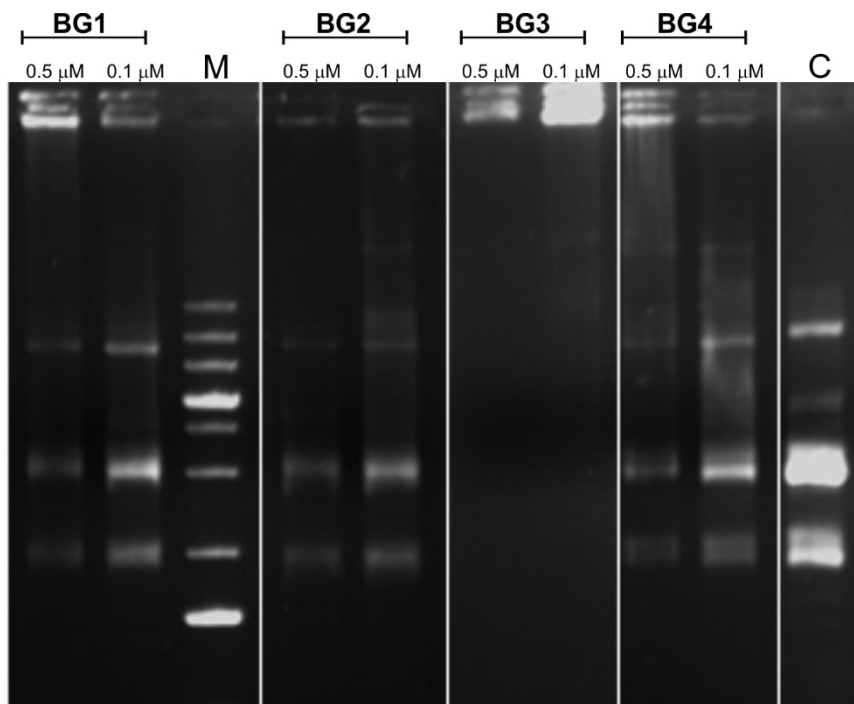


Fig. S3 *In-vitro* interaction of BG1-4 with pUC19 plasmid DNA. (M = molecular marker peqGOLD 1 kb DNA-Leiter Plus; C = DMSO treatment)

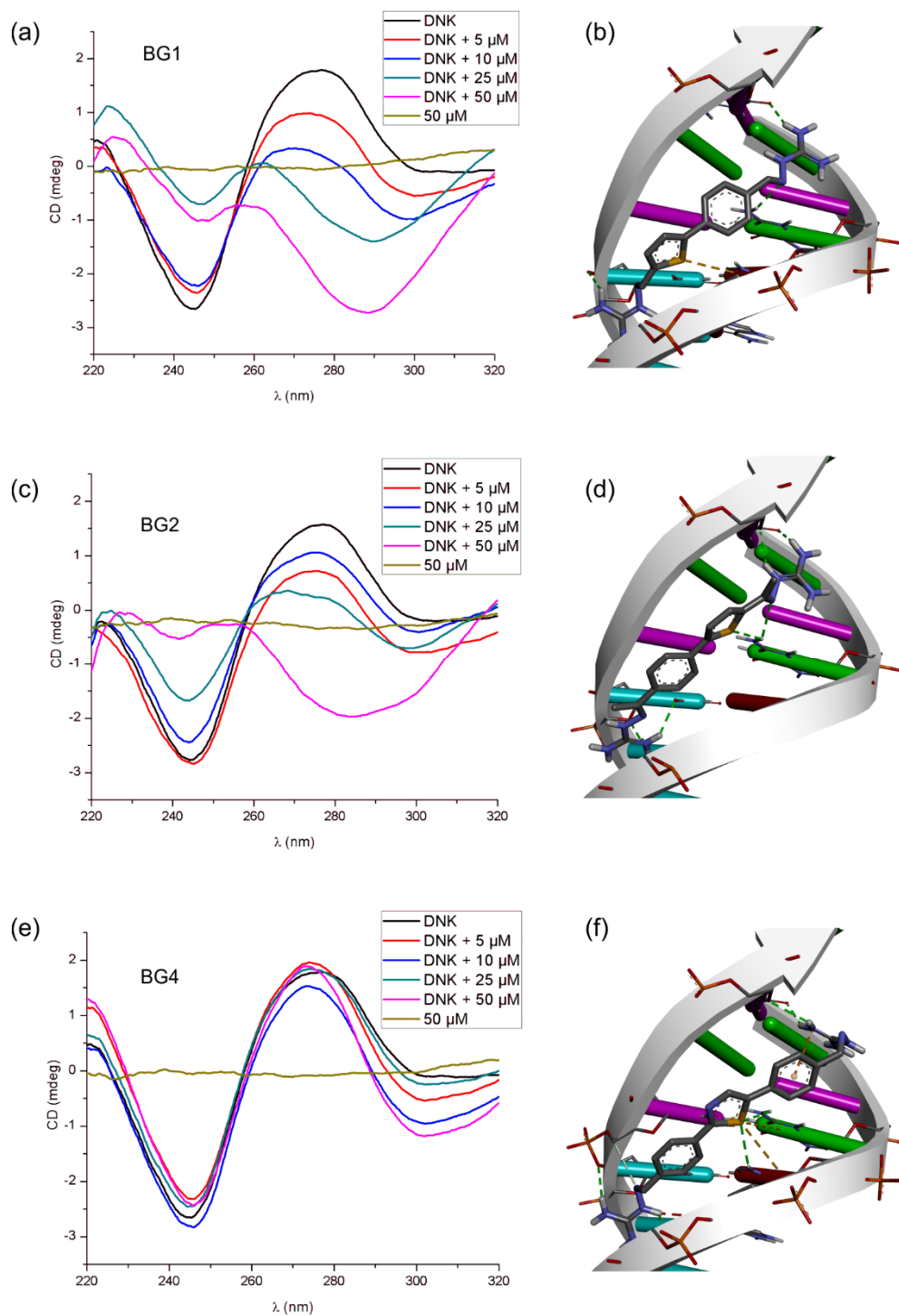


Fig. S4 BG1, BG2 and BG4 interaction with DNA. CD spectra (a, c, e) and molecular modeling (b, d, f).

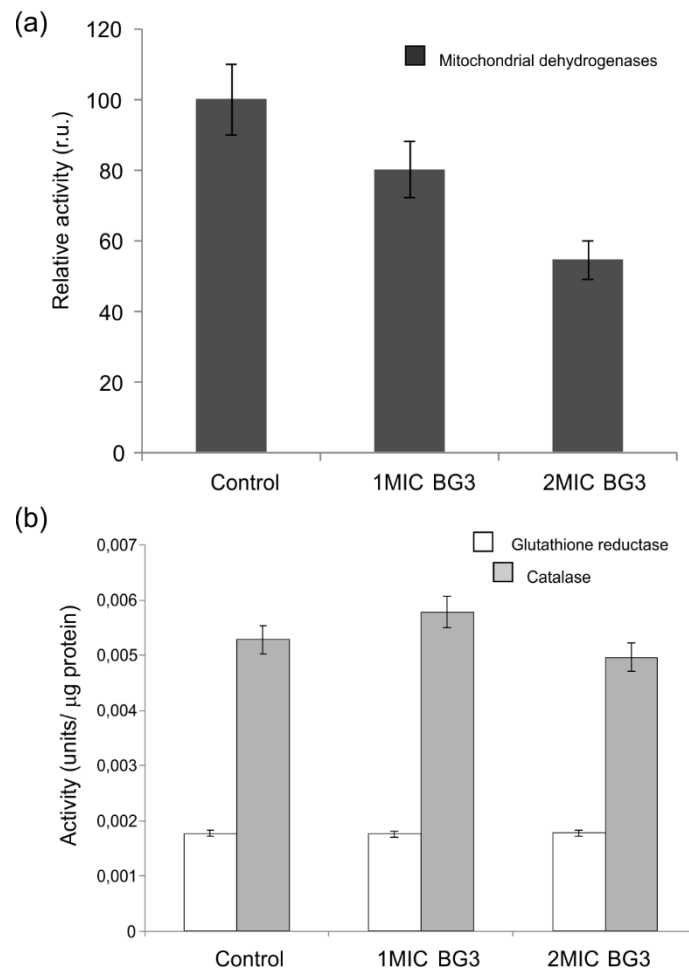


Fig. S5 BG3 effect on the activity of *C. albicans* (a) mitochondrial dehydrogenases and (b) glutathione reductase and catalase. DMSO treated cells were used as control.

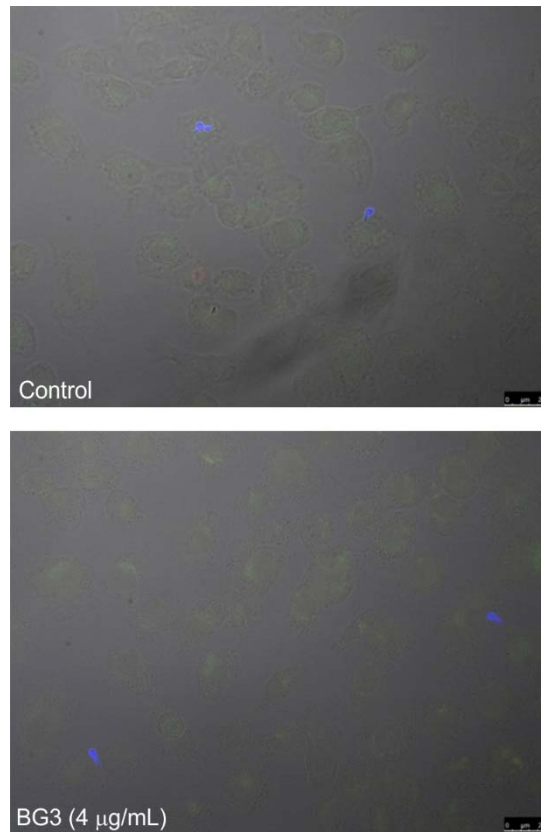


Fig. S6 BG3 effect on the *C. albicans* adhesion in the epithelial infection model.

Table S1. FACS Analysis of apoptotic markers in *C. albicans* treated with BG3 and AmB

	Annexin V⁻/PI⁻ (%)	Annexin V⁺/PI⁺ (%)	Annexin V⁻/PI⁺ (%)
Control	1.8	3.5	1.8
BG3	2.8	6.6	3.8
AmB	2.9	5.5	1.6

Table S2. FACS Analysis of oxidative stress markers in *C. albicans* treated with BG3 and AmB

	ROS (%)	JC-1 (%)
Control	0.1	1.2
BG3	0.4	4.3
AmB	9.2	44.1
2MIC BG3	6.1	17.1
2MIC AmB	27.8	/

REFERENCES

1. Ajdačić, V.; Senerovic, L.; Vranić, M.; Pekmezovic, M.; Arsic-Arsenijevic, V.; Veselinovic, A.; Veselinovic, J.; Šolaja, B. A.; Nikodinovic-Runic, J.; Opsenica, I. M. Synthesis and evaluation of thiophene-based guanylhydrazones (iminoguanidines) efficient against panel of voriconazole-resistant fungal isolates, *Bioorg. Med. Chem.*, 2016, *24*, 1277–1291.