Supporting Information

3-SELENOCYANATE-INDOLES AS NEW AGENTS TO THE TREATMENT OF SUPERFICIAL AND MUCOCUTANEOUS INFECTIONS

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S1. Synthesis process

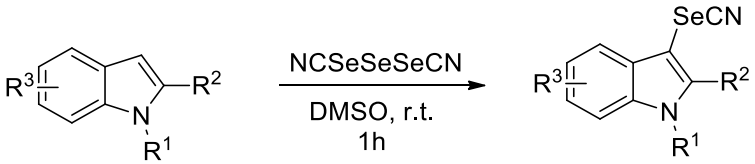
Materials and methods

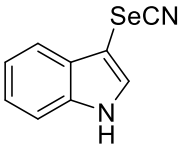
Chemicals

NMR spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Varian AS-400 or Bruker Avance 200 spectrometer. Chemical shifts (δ) are given in parts per million (ppm) downfield relative to TMS, and coupling constants (*J*) are in Hz. Residual solvent central signals were recorded as follows: $CDCl_3$, ^{1}H = 7.26, ^{13}C = 77.16; DMSO-d^6, ^{1}H = 2.50, ^{13}C = 39.52.[1] APPI-micrOTOF-Q II measurements were performed with a micrOTOF Q-II (Bruker Daltonics) mass spectrometer equipped with an automatic syringe pump (KD Scientific) for sample injection. The mass spectrometer was operated in the positive ion mode. The sample was injected using a constant flow (3 mL/min). The solvent was a chloroform/methanol mixture. The APPI-micrOTOF-Q II instrument was calibrated in the mass range of 50-3000 m/z using an internal calibration standard (low concentration tuning mix solution) supplied by Agilent Technologies. Data were processed employing Bruker Compass Data Analysis software (version 4.0). Column chromatography and thin layer chromatography (TLC) were conducted using silica gel 60 (230–400 mesh) and Merck Silica Gel GF254 (0.25mm thickness), respectively. For visualization, the TLC plates were either placed under ultraviolet light or stained with iodine vapor or sprayed with acidic vanillin. Melting points were determined using a microscope coverslip on a Micro Chemical MQA PF digital apparatus and are uncorrected.

General procedure

Malononitrile (3 mmol) and SeO_2 (6 mmol) were stirred in DMSO (5 mL) at room temperature for 15 minutes in a round bottomed flask.[2] Then, indole (3 mmol) was added in one portion. After 30 minutes, the mixture was diluted in AcOEt (20 mL) and washed with water (5x 20 mL) followed by brine (1x 20 mL). The organic phase was dried with $MgSO_4$, filtered, and the organic solvent was evaporated under reduced pressure. The crude obtained was purified by column chromatography using a mixture of AcOEt/Hexane (0 to 50% of AcOEt/Hexane) to give the 3-selenocyanate-indole **4a-g**.





3-selenocyanate-*1H*-indole (4a).[2] Brown solid, 91% yield. M.p.: 71-74°C (Lit. M.p. = 73-75°C);[2] **^{1}H NMR** ($CDCl_3$, 200 MHz): δ 8.82 (s, 1H), 7.67-7.72 (m, 1H), 7.22-7.37 (m, 4H). **^{13}C NMR** ($CDCl_3$, 50 MHz): δ 136.11, 132.08, 128.72, 123.70, 121.80, 119.45, 112.12, 102.50, 89.03.

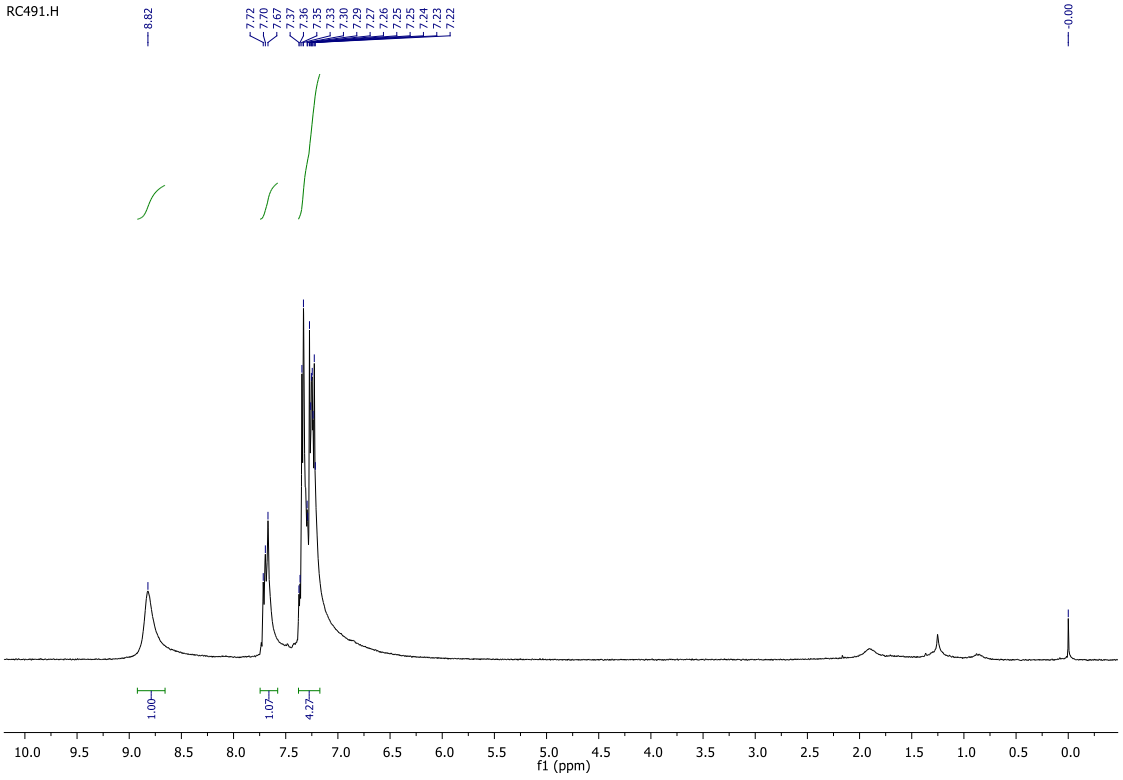


Figure S1. ^{1}H NMR spectrum of **4a** ($CDCl_3$, 200 MHz)

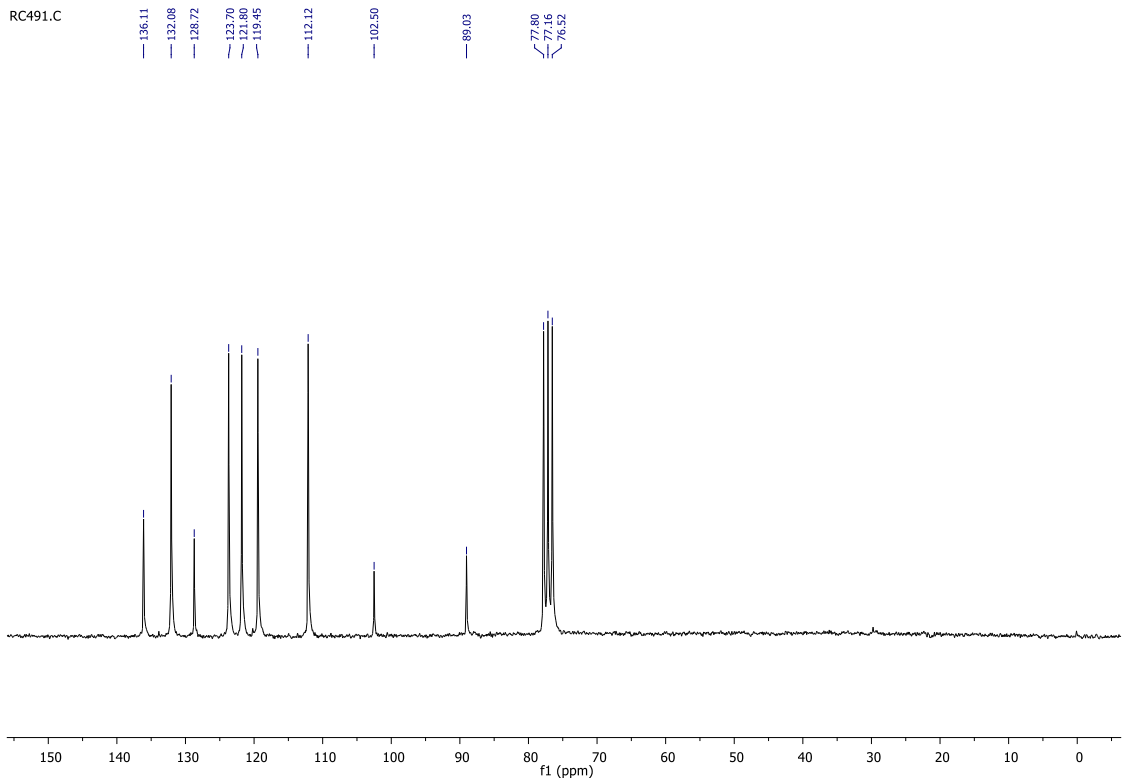
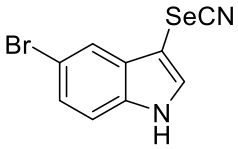


Figure S2. ^{13}C NMR spectrum of **4a** ($CDCl_3$, 50 MHz)



5-Bromo-3-selenocyanate-*1H*-indole (4b).[3] Brown solid, 90% yield. M.p.: 138-141°C (Lit. M.p. = 141-142°C);[3] **^{1}H NMR** (DMSO-d6, 200 MHz): δ 12.06 (s, 1H), 7.93 (d, *J* = 2.8 Hz, 1H), 7.71 (d, *J* = 1.5 Hz, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.37 (dd, *J* = 8.6, 1.8 Hz, 1H); **^{13}C NMR** (DMSO-d6, 100 MHz): δ 134.98, 134.62, 130.51, 125.12, 120.89, 114.52, 113.30, 104.20, 88.90.

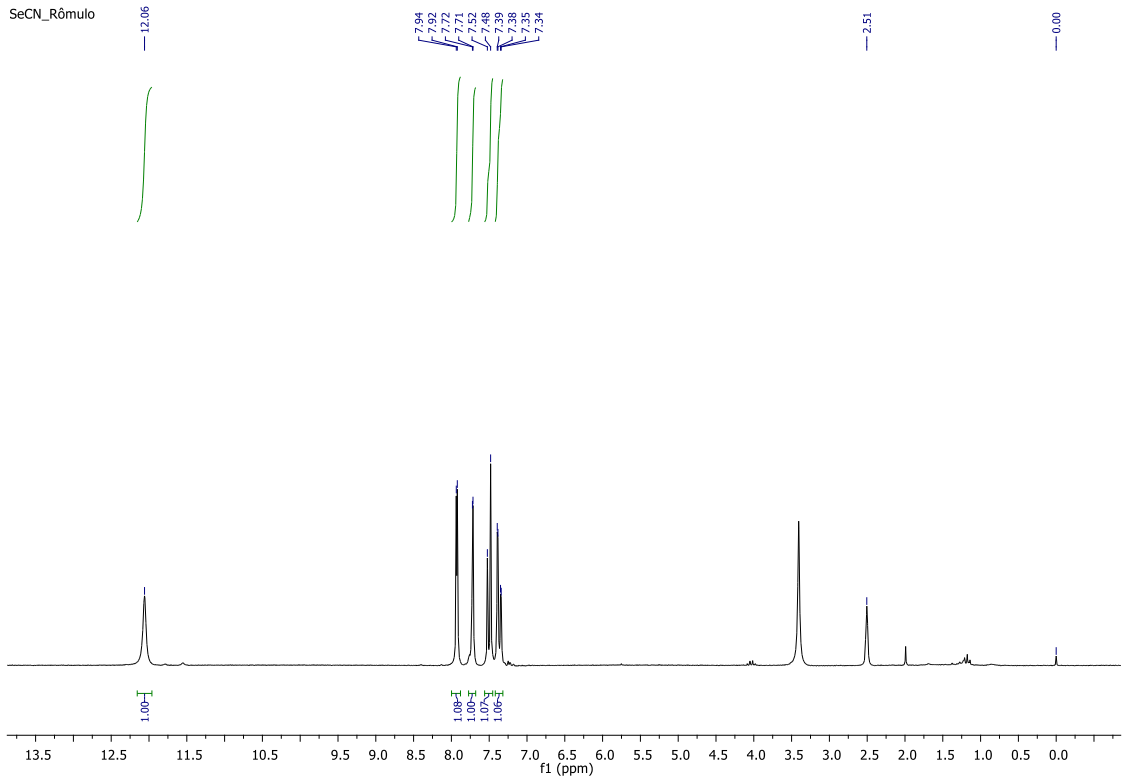


Figure S3. ^{1}H NMR spectrum of **4b** (DMSO-d6, 200 MHz)

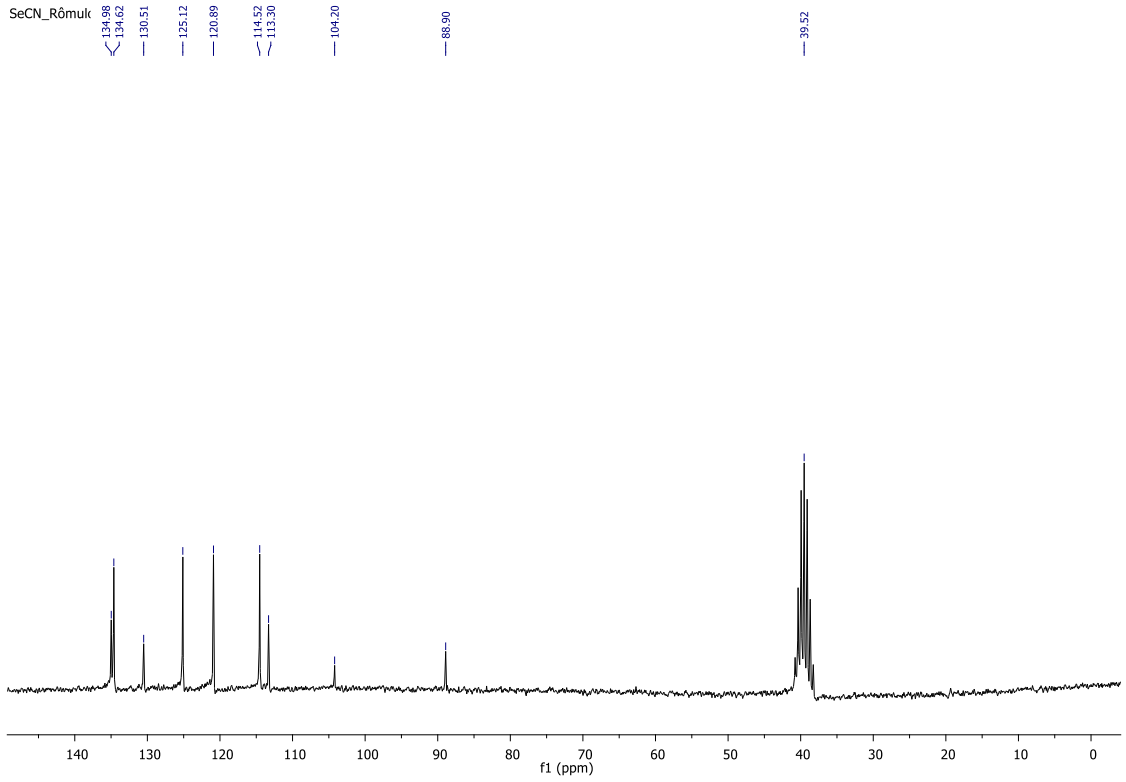
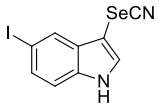


Figure S4. ^{13}C NMR spectrum of **4b** (DMSO-d6, 100 MHz)



5-Iodo-3-selenocyanate-*1H*-indole (4c). Brown solid, 78% yield. M.p.: 125-129°C; **^{1}H NMR** (DMSO-d6, 200 MHz): δ 12.03 (s, 1H), 7.78-7.96 (m, 2H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.38 (d, *J* = 8.5 Hz, 1H). **^{13}C NMR** (DMSO-d6, 50 MHz): δ 135.36, 134.14, 131.23, 130.58, 127.09, 114.87, 104.28, 88.51, 84.69. **HRMS** (APPI-TOF, M^{+}) calcd. 347.8663, found 347.8661.

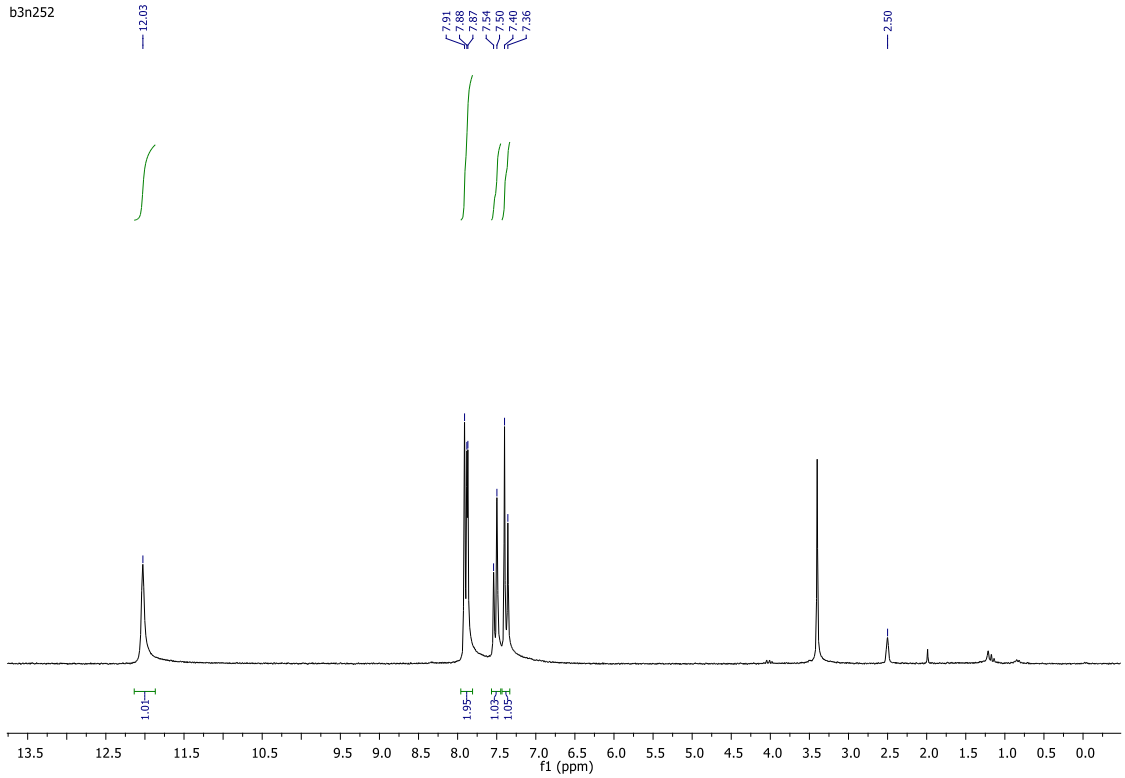


Figure S5. ^{1}H NMR spectrum of **4c** (DMSO-d6, 200 MHz)

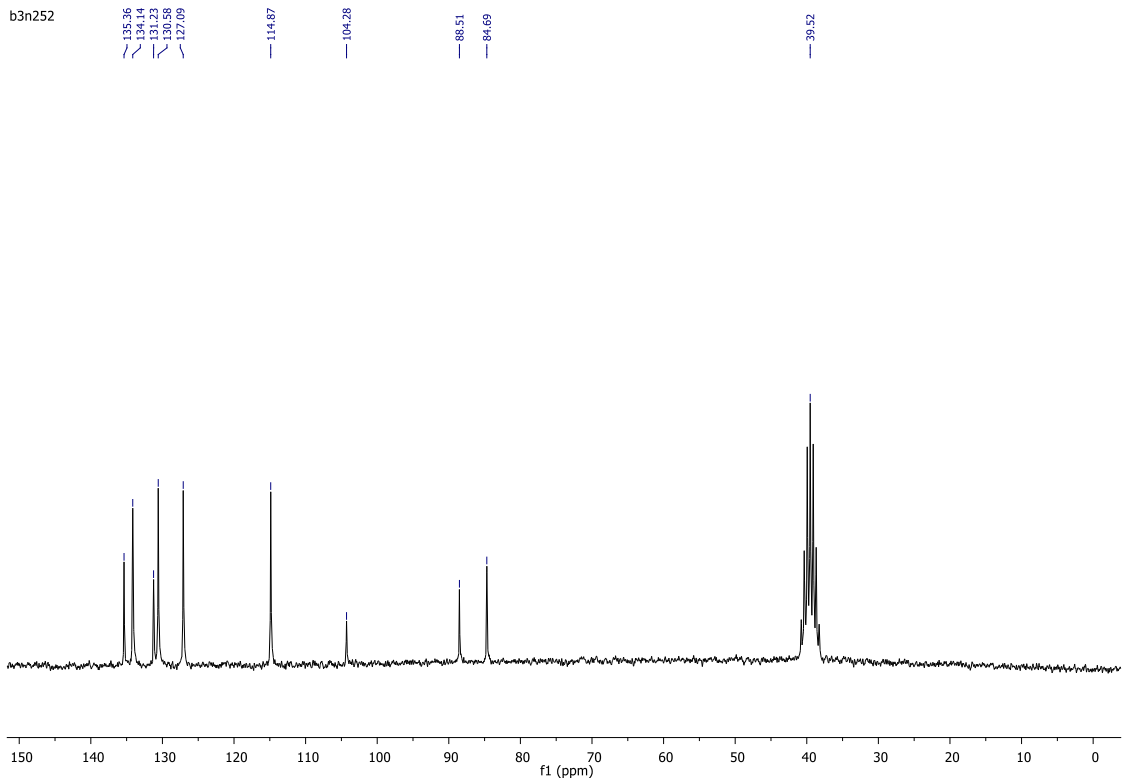
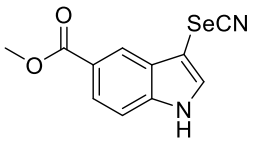


Figure S6. ^{13}C NMR spectrum of **4c** (DMSO-d6, 50 MHz)



Methyl 3-selenocyanate-*1H*-indole-5-carboxylate (4d). Brown solid, 87% yield. M.p.: 159-162°C; **^{1}H NMR** (DMSO-d6, 200 MHz): δ 12.22 (s, 1H), 8.27 (s, 1H), 8.01 (d, *J* = 2.0 Hz, 1H), 7.86 (d, *J* = 8.6 Hz, 1H), 7.60 (d, *J* = 8.6 Hz, 1H), 3.89 (s, 3H). **^{13}C NMR** (DMSO-d6, 50 MHz): δ 166.84, 138.92, 135.10, 128.38, 123.38, 122.21, 121.05, 112.62, 104.42, 91.18, 51.97. **HRMS** (APPI-TOF, M^{+}) calcd 279.9751, found 279.9755.

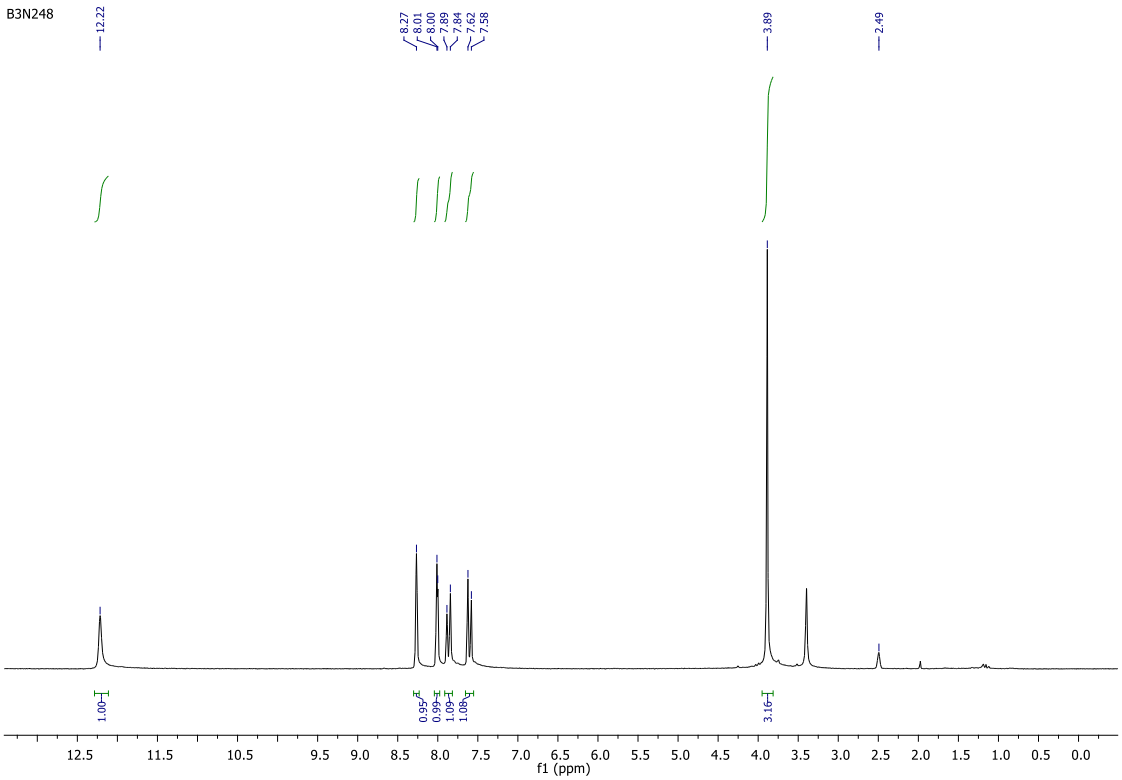


Figure S7. ^{1}H NMR spectrum of **4d** (DMSO-d6, 200 MHz)

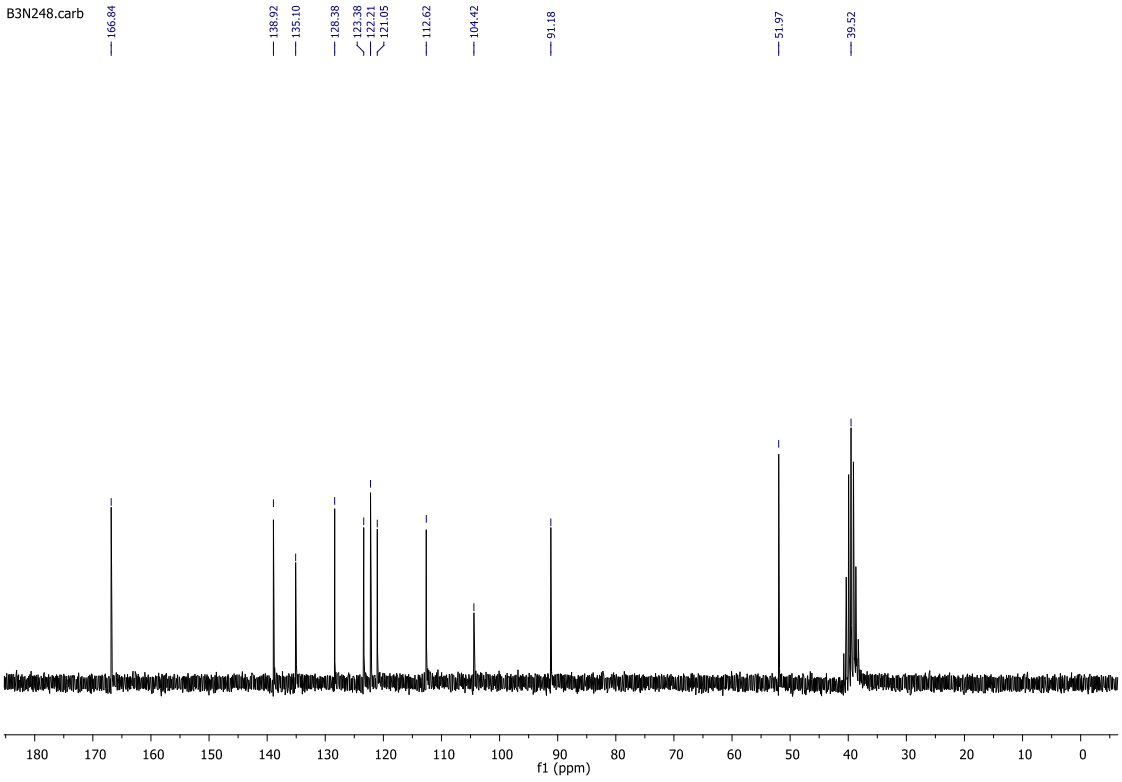
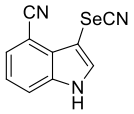


Figure S8. ^{13}C NMR spectrum of **4d** (DMSO-d6, 50 MHz)



3-selenocyanate-*1H*-indole-4-carbonitrile (4e). Brown solid, 84% yield. M.p.: 91-93°C; **^{1}H NMR** (DMSO-d6, 200 MHz): δ 12.44 (s, 1H), 8.12 (d, *J* = 2.1 Hz, 1H), 7.85 (d, *J* = 8.2 Hz, 1H), 7.64 (d, *J* = 7.4 Hz, 1H), 7.35 (t, *J*= 7.8 Hz, 1H). **^{13}C NMR** (DMSO-d6, 50 MHz): δ 137.29, 136.63, 127.90, 126.92, 122.46, 117.98, 117.67, 105.07, 101.47, 88.91. **HRMS** (APPI-TOF M^{+}) calcd. 246.9649, found 246.9654.

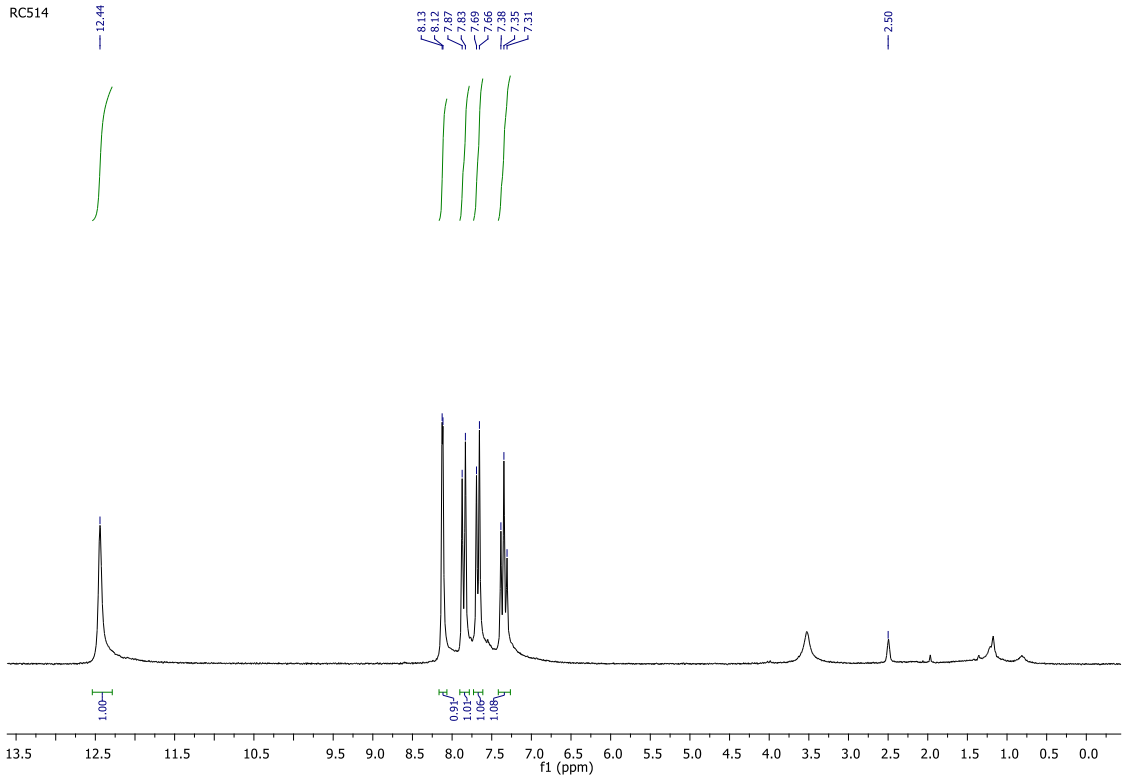


Figure S9. ^{1}H NMR spectrum of **4e** (DMSO-d6, 200 MHz)

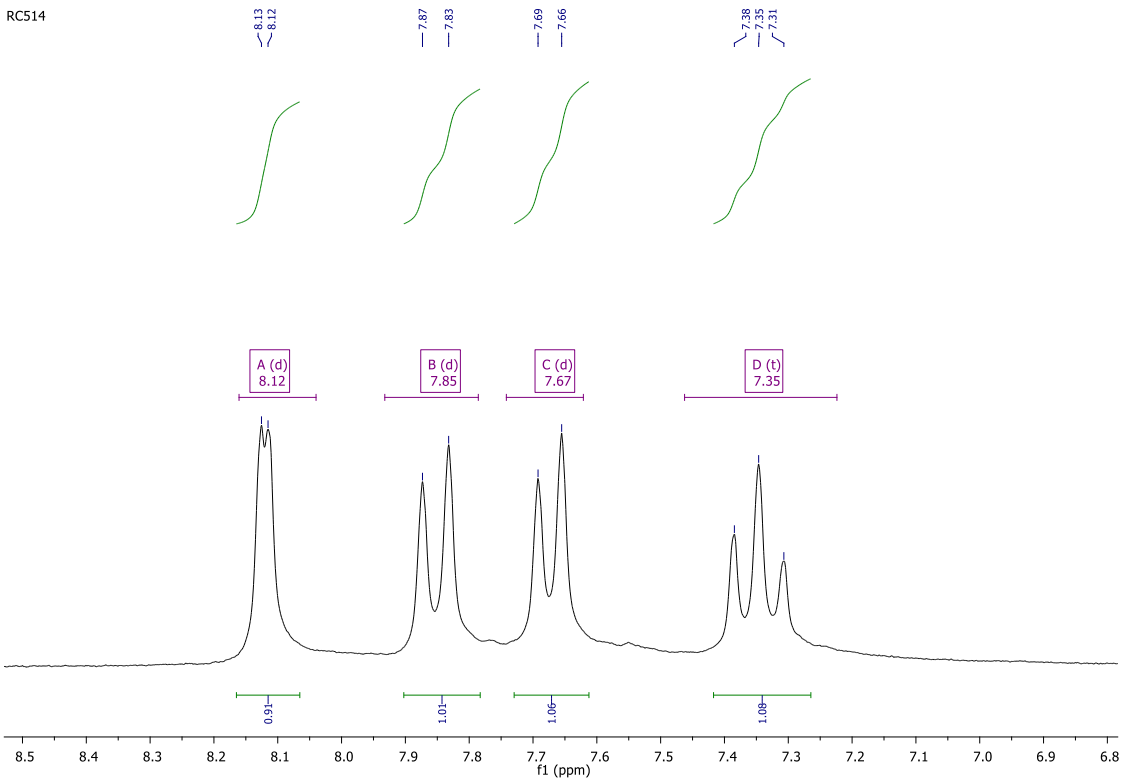


Figure S10. Expansion from δ 6.8 to 8.5 ppm of ^{1}H NMR spectrum of **4e** (DMSO-d6, 200 MHz)

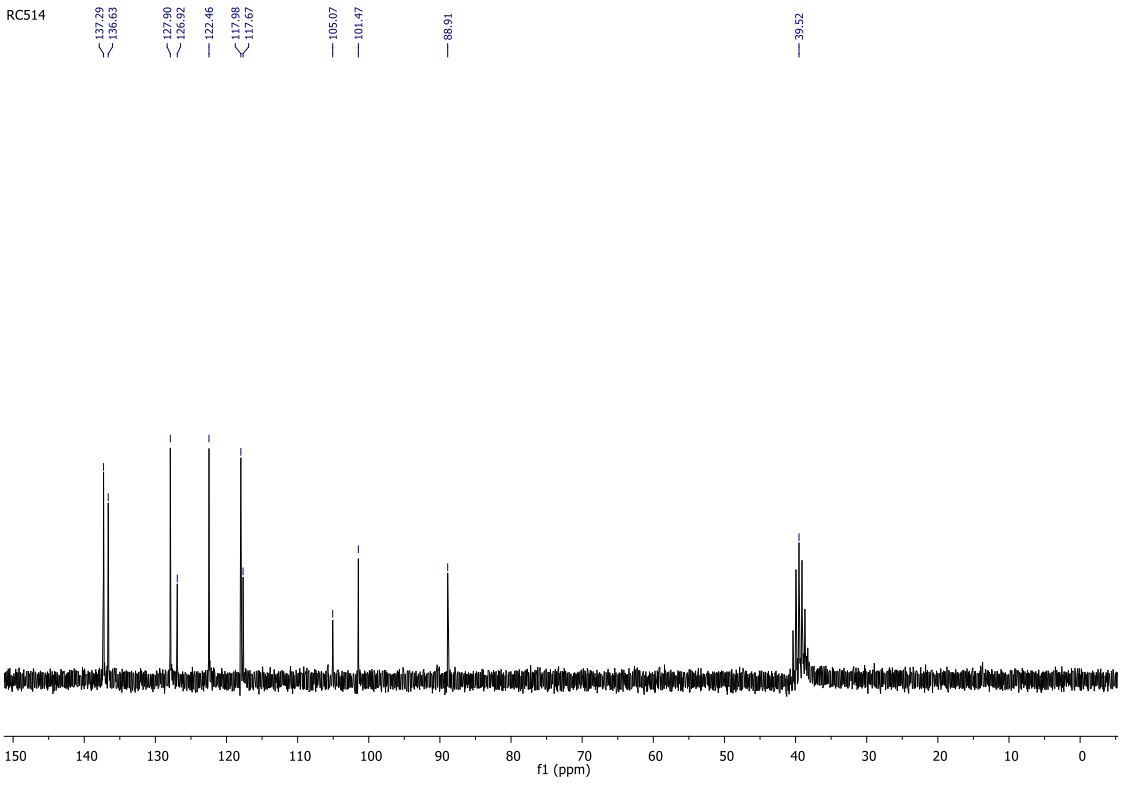
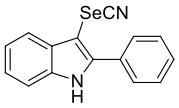


Figure S11. ^{13}C NMR spectrum of **4e** (DMSO-d6, 50 MHz)



2-Phenyl-3-selenocyanate-*1H*-indole (4f).[4] Brown solid, 88% yield m.p.: 81-85°C (Lit. M.p. = 170-172°C);[4] **^{1}H NMR** (DMSO-d6, 200 MHz): δ 12.25 (s, 1H), 7.81-7.95 (m, 2H), 7.44-7.71 (m, 5H), 7.20-7.36 (m, 2H). **^{13}C NMR** (DMSO-d6, 50 MHz): δ 142.77, 136.12, 130.96, 130.45, 129.13, 128.94, 128.64, 123.03, 121.02, 119.21, 112.12, 104.60, 88.11. **HRMS** (APPI-TOF, M^{+}) calc. 298.0009, found 298.0002.

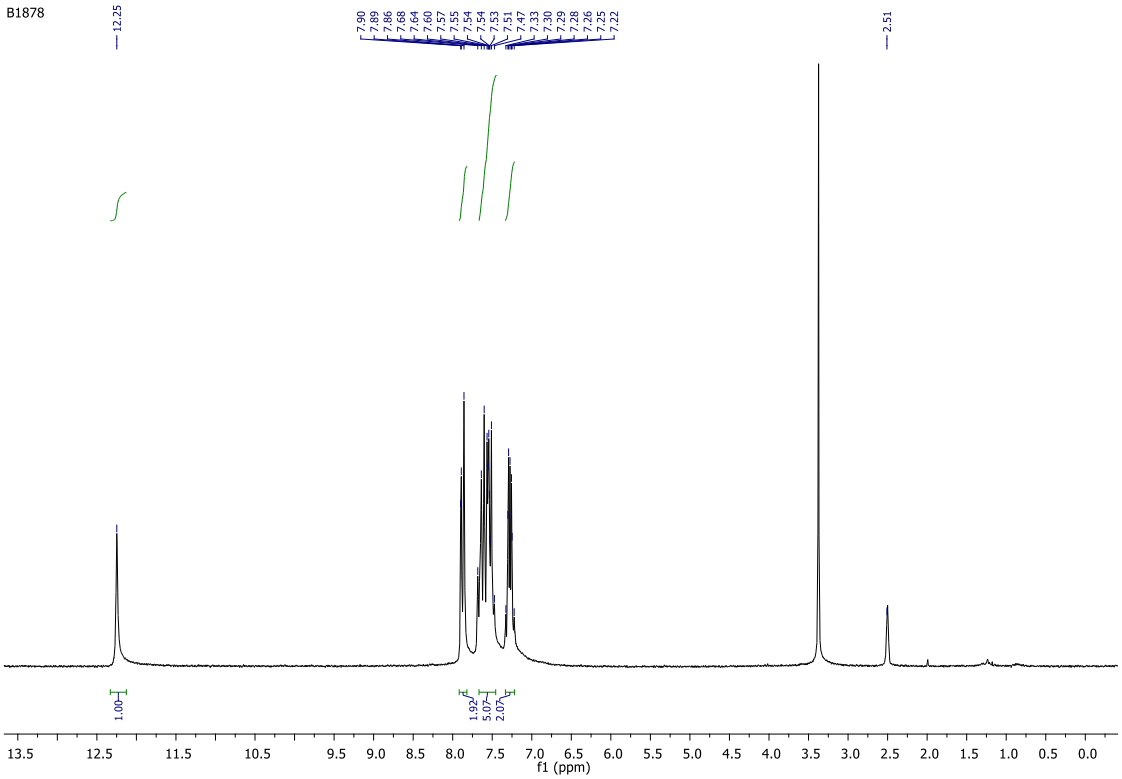


Figure S12. ^{1}H NMR spectrum of **4f** (DMSO-d6, 200 MHz)

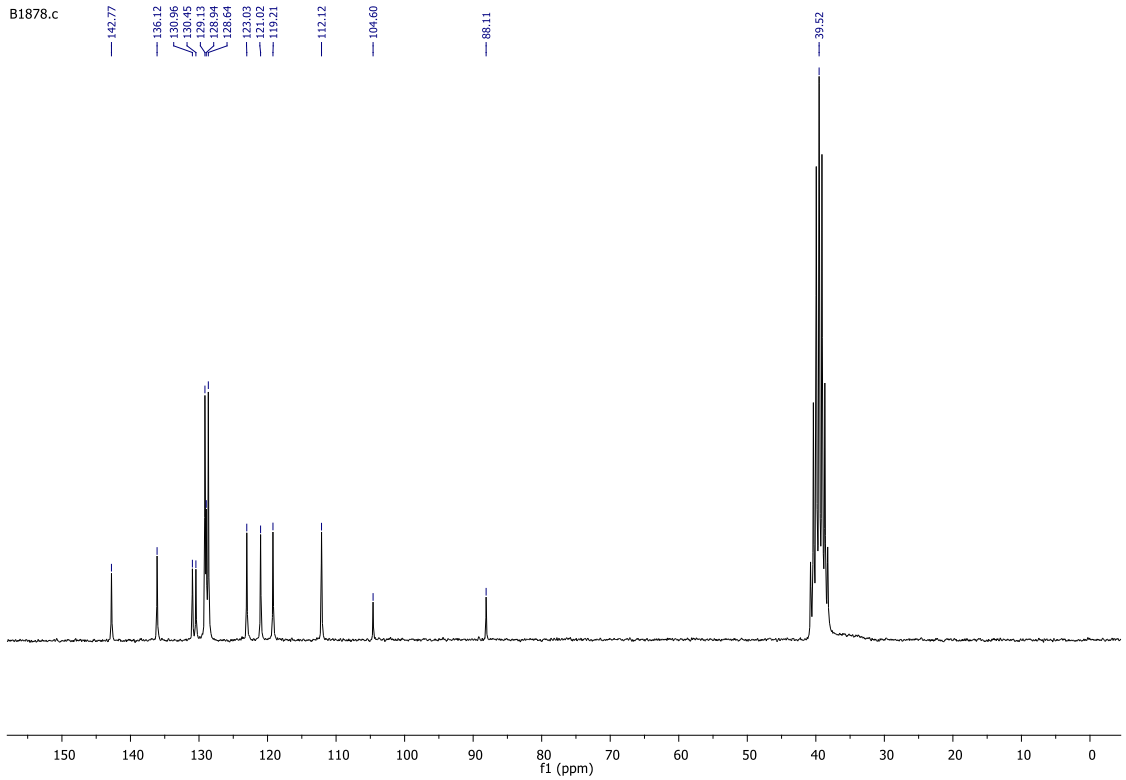
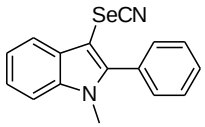


Figure S13. ^{13}C NMR spectrum of **4f** (DMSO-d6, 50 MHz)



1-Methyl-2-phenyl-3-selenocyanate-*1H*-indole (4g). Brown solid, 97% yield. M.p.: 69-72°C; **^{1}H NMR** (DMSO-d6, 200 MHz): δ 7.52-7.75 (m, 7H), 7.26-7.43 (m, 2H), 3.71 (s, 3H). **^{13}C NMR** (DMSO-d6, 50 MHz): δ 145.73, 137.05, 130.82, 129.75, 129.24, 128.99, 128.46, 123.03, 121.37, 119.18, 111.04, 104.36, 89.45, 31.78. **HRMS** (APPI-TOF $M+H^+$) calc. 313.0244, found 313.0243.

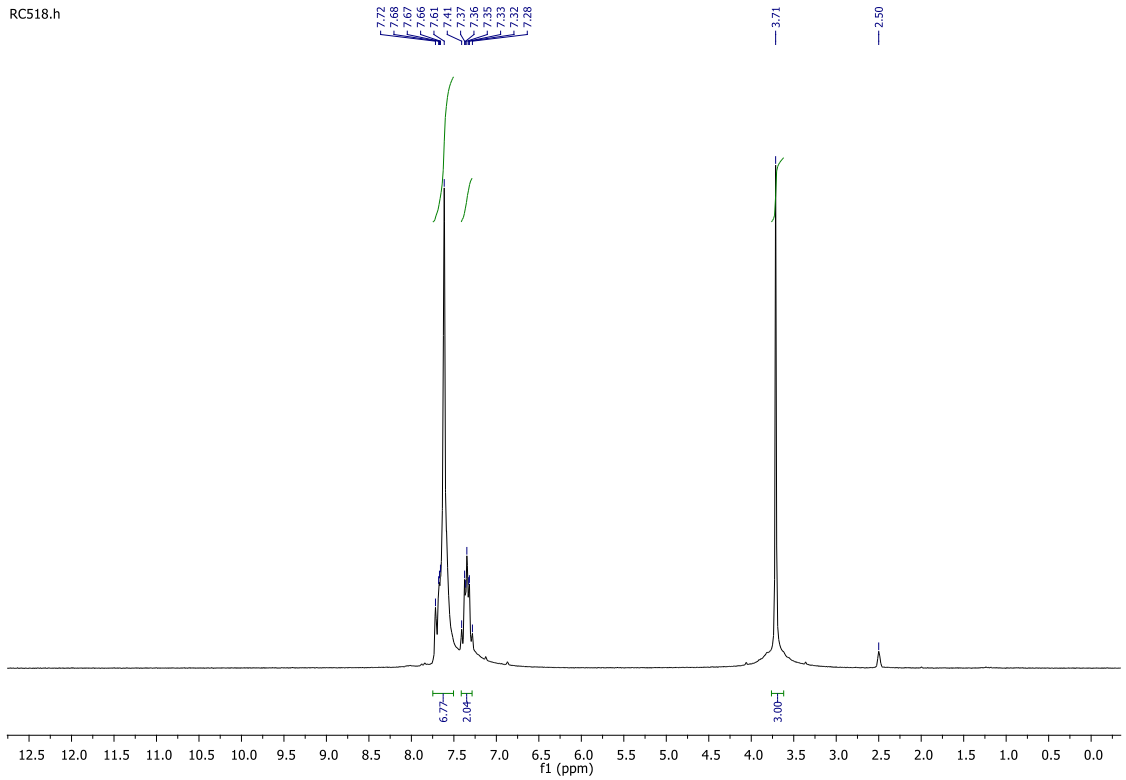


Figure S14. ^{1}H NMR spectrum of **4g** (DMSO-d6, 200 MHz)

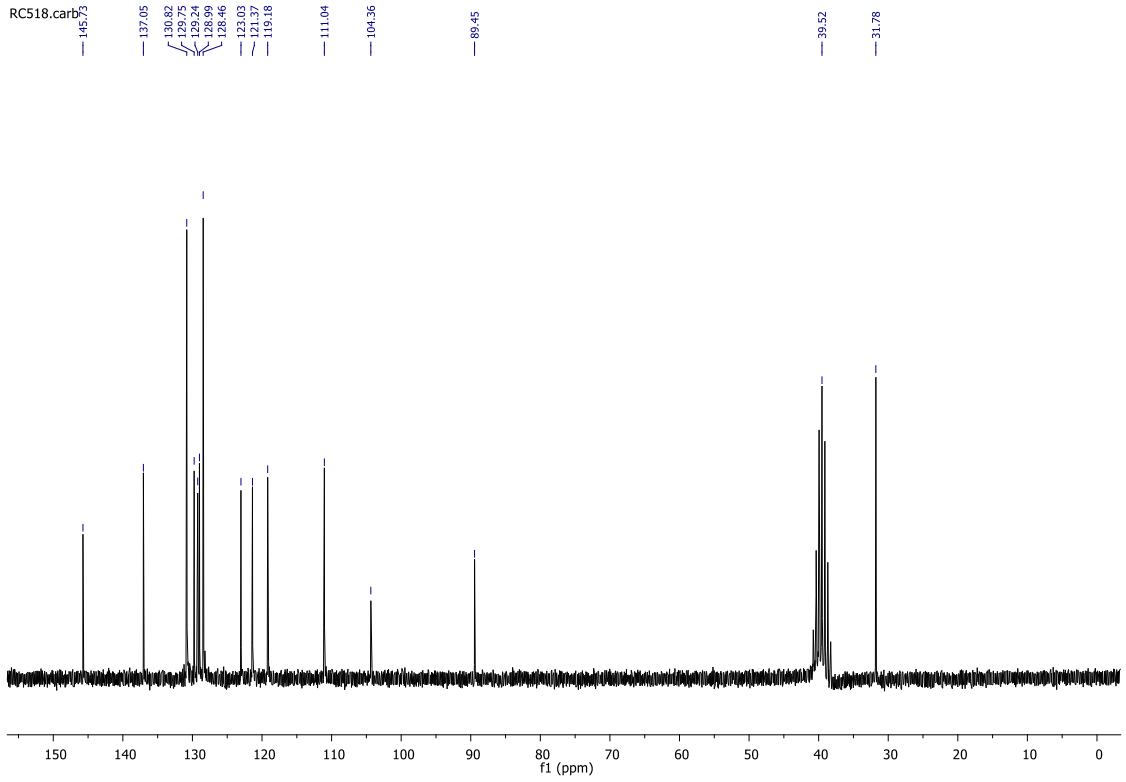


Figure S15. ^{13}C NMR spectrum of **4g** (DMSO-d6, 50 MHz)

S2. Determination of fugal mechanism of action

Table S1. Sorbitol Protection Assay: MICs (µg.ml^{-1}) against eight fungi isolates for the **4a**, **4b,** and MEC (µg.ml^{-1}) for AFG before and after adding sorbitol.

	4a		**4b**		**AFG (MEC)**	
	-/Sorbitol	**+/Sorbitol**	**-/Sorbitol**	**+/Sorbitol**	**-/Sorbitol**	**+/Sorbitol**
TRU 45						
Day 4	1.5	1.5	3.1	3.1	0.03	>1
Day 8	1.5	1.5	3.1	3.1	>1	>1
TME 46						
Day 4	1.5	1.5	6.2	6.2	0.03	>1
Day 8	1.5	3.1	6.2	6.2	>1	>1
MGY 50						
Day 4	0.8	0.8	1.5	1.5	0.007	0.01
Day 8	1.5	1.5	3.1	3.1	>1	>1
CA 18804						
Day 2	3.1	1.5	6.2	6.2	0.03	0.007
Day 7	6.2	6.2	25	12.5	>1	0.007
CG RL37						
Day 2	1.5	0.8	6.2	6.2	0.003	0.31
Day 7	12.5	12.5	25	50	0.015	0.015
CK 01						
Day 2	3.1	0.8	12.5	12.5	0.015	0.015
Day 7	12.5	6.2	25	6.2	0.015	0.03
CT 750						
Day 2	0.8	1.5	12.5	12.5	0.003	0.007
Day 7	6.2	3.1	25	12.5	0.003	>1
CP RL13						
Day 2	0.4	0.8	3.1	12.5	0.06	0.1
Day 7	3.1	1.5	6.2	3.1	0.1	0.5

AFG: Anidulafungin; MEC: Minimum effective concentration; -/Sorbitol: without addition of commercial sorbitol; +/Sorbitol: with addition of sorbitol.

Table S2. Ergosterol Binding Assay: MICs (µg.ml^{-1}) against eight fungi isolates for the **4a**, **4b,** and AFB before and after adding ergosterol.

Fungi strains	**Reading**				
4a	**MIC^1**	**MIC^2**	**MIC^3**	**MIC^4**	**MIC^5**
TRU 45	0.8	0.8	0.8	0.8	0.8
TME 46	0.8	1.5	1.5	1.5	1.5
MGY 50	0.8	0.8	0.8	0.8	0.8
CA ATCC 18804	3.1	3.1	3.1	3.1	3.1
CG Rl37	3.1	6.2	0.4	0.4	6.2
CK 01	12.5	12.5	12.5	12.5	12.5
CT ATCC 750	12.5	12.5	12.5	12.5	12.5
CG RL13	3.1	0.8	1.5	1.5	1.5
4b	**MIC^1**	**MIC^2**	**MIC^3**	**MIC^4**	**MIC^5**
TRU 45	3.1	1.5	1.5	1.5	1.5
TME 46	3.1	1.5	1.5	1.5	1.5
MGY 50	1.5	1.5	1.5	1.5	1.5
CA ATCC 18804	6.2	3.1	6.2	6.2	6.2
CG Rl37	6.2	3.1	3.1	3.1	3.1
CK 01	6.2	6.2	6.2	6.2	6.2
CT ATCC 750	6.2	6.2	6.2	6.2	6.2
CG RL13	6.2	3.1	3.1	3.1	3.1
AFB	**MIC^1**	**MIC^2**	**MIC^3**	**MIC^4**	**MIC^5**
TRU 45	0.2	2	2	2	2
TME 46	0.2	2	2	2	2
MGY 50	0.1	2	2	2	2
CA ATCC 18804	0.1	2	4	4	4
CG RL37	0.06	2	2	2	2
CK 01	0.5	0.5	2	0.5	0.5
CT ATCC 750	0.03	0.03	1	0.06	0.06
CG RL13	<0.015	0.8	1.5	1.5	1.5

AFB = Amphotericin B; MIC^1 corresponds to MIC without addition of ergosterol; MIC^2, MIC^3, MIC^4, and MIC^5, correspond to MIC with addition of ergosterol at the concentration of 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL, respectively.

S3. Antifungal susceptibility testing

The minimum inhibitory concentrations (MICs) was defined by the broth microdilution method, according to the M38-A2 protocol established for filamentous fungi.[5] The conidial inocula (1x 10^3 to 3x 10^3 CFU/mL) were prepared from cultures grown on potato dextrose agar (PDA) at 32°C for seven days. The final suspension was made 2x more concentrated than density needed for testing. The assays were conducted with RPMI medium, containing L-glutamine (without sodium bicarbonate), buffered to pH 7.0 with 0.165 mol l^{-1} MOPS. The experiments were carried out in duplicate, incubating the microplates at 32°C for 4 days (96 h) and reading the MIC visually. The M27-A3 protocol was established for the *Candida* species.[6] The final yeast suspension was obtained after dilutions 1:50 and 1:20 that were performed with RPMI medium to obtain the two times test inoculum (1x 10^3 to 5x 10^3 CFU/mL), and were prepared from cultures grown on sabouraud dextrose agar at 35°C. This concentration will be diluted 1:1 when inoculated into the wells, resulting in a final inoculum (0.5x 10^3 to 2.5x 10^3). The microplates were incubated at 35°C for 2 days (48 h) and reading the MIC visually. This assay was performed in duplicate. Controls were used in parallel in the tests: sterility control (negative control as a drug-free medium) and positive control for fungal cells viability.

S4. Antibacterial susceptibility testing

Staphylococus Aureus Methicillin-susceptible (ATCC 25923) was obtained by donation from Instituto Oswaldo Cruz, RJ, Brazil. Evaluation of MICs followed the CLSI microdilution method using BBL™ Mueller Hinton II broth (Interlab, Brazil) as described previously.[7] Briefly, two-fold serial dilutions of each compound were prepared in triplicate in 96-well plates and inoculated with 5x 10^5 CFU/ml of the bacterial suspension. Plates were incubated at 37 °C for 16-20 h. Oxaxicilin (OXA) and Ampicilin (AMP) purity ≥ 97% were purchased from Sigma-Aldrich (Sao Paulo, Brazil) and used as controls.

S5. HET-CAM assay

The irritation score, when based on this formula, presents a maximum value of 21. The following classification criterion is used: 0 to 4.9 non-irritant (or practically no irritation); 5.0 to 21 irritant (moderate to severe or extreme irritation).[8]

Equation 1. Determination of irritation score.

$$IS = \left(\left(\frac{(301 - \text{Hemorrhage Time})}{300}\right)x5\right) + \left(\left(\frac{(301 - \text{Lysis Time})}{300}\right)x7\right) + \left(\left(\frac{(301 - \text{Coagulation Time})}{300}\right)x9\right)$$

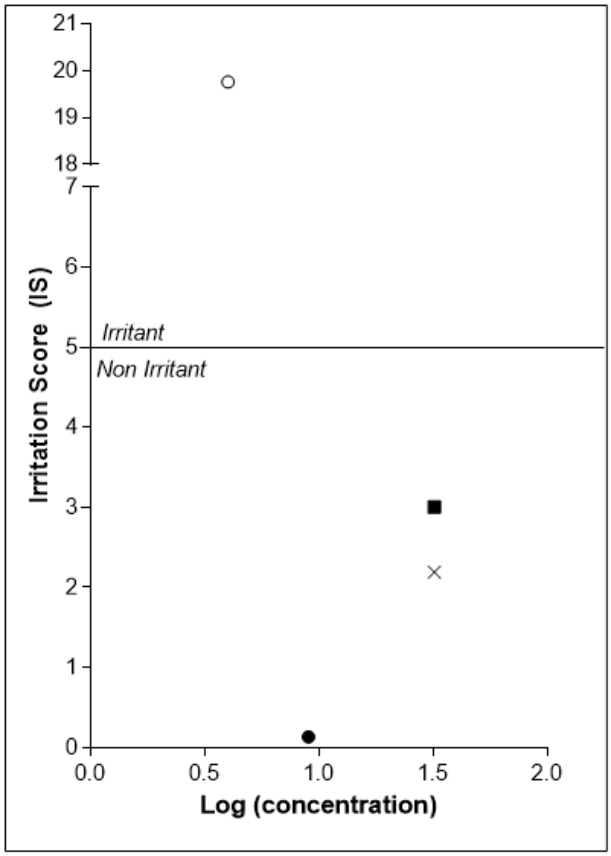


Figure S16. Hen's Egg Test-Chorioallantoic Membrane (HET-CAM for **4a** (32 µg.ml^{-1}) (■), **4b** (32 µg.ml^{-1}) (x), negative control (0.9% NaCl), (●), and positive control (0.1 NaOH) (○). Each point represents one experiment (n = three eggs).

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