Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information (ESI)

The first naturally occurring aromatic isothiocyanates, rapalexins A and B, are cruciferous phytoalexins

M. Soledade C. Pedras,* Qing-An Zheng and Ravi S. Gadagi

Department of Chemistry, University of Saskatchewan, 110 Science Place, Saskatoon, SK, S7N 5C9, Canada.

Fax: 1-306-9664730

Tel: 1-306-966-4772

E-mail: *s.pedras@usask.ca*

Experimental

General.

All solvents were HPLC grade and used as such, except for THF and Et_2O (dried over sodium) and chloroform (glass redistilled). Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Flash column chromatography (FCC): silica gel, mesh size 230-400, 60 Å. Organic extracts were dried over Na_2SO_4 and the solvents were removed using a rotary evaporator. Melting points are uncorrected.

Analytical HPLC was performed with a liquid chromatograph (Agilent 1100 series HPLC system, Agilent Technologies, USA) equipped with a quaternary pump, an automatic injector, a photodiode array detector, a degasser, and a Hypersil ODS column (5 μm particle size silica, 200 mm × 4.6 i.d.), equipped with an on-line filter. HPLC retention times were obtained under the following conditions: mobile phase, H_2O-CH_3CN (75%:25%) to CH_3CN (100%) for 35 min, linear gradient and a flow rate of 1.0 ml/min. For LC-ESI, an Agilent 1100 series HPLC system equipped with an autosampler, binary pump, degasser, a photodiode array detector connected directly to a mass detector (Agilent G2440A MSD-Trap-XCT ion trap mass spectrometer) with an ESI source was used. Chromatographic separation was carried out at room temperature using an Eclipse XSB C-18 column (5 μm particle size silica, 150 mm × 4.6 mm I.D.). The mobile phase consisted of a gradient of 0.2% formic acid in water (A) and 0.2% formic acid in acetonitrile (B) (75% A to 75% B in 35 min, to 100% B in 5 min) and a flow rate of 1.0 ml/min. The ion mode was set as positive and negative. The interface and MSD parameters were as follows: nebuliser pressure, 70.0 psi (N_2); dry gas, N_2 (12.0 l/min); dry gas temperature, 350 °C; spray capillary voltage 3500 V; skimmer voltage, 40.0 V; ion transfer capillary exit, 100 V; scan range, *m*/*z* 100-500. Ultrahigh pure He was used as

the collision gas. Mass spectral (MS) [high resolution (HR), electron impact (EI)] data were obtained on a mass spectrometer using a solid probe.

NMR spectra were recorded on Bruker 500 MHz Avance spectrometers. For ^{1}H NMR spectra (500 MHz) the chemical shifts (δ) are reported in parts per million (ppm) relative to TMS. The δ values were referenced to $CDCl_3$ ($CHCl_3$ at 7.28 ppm), CD_3CN (CD_2HCN at 1.94 ppm), CD_3OD (CD_2HOD at 4.87 ppm). For ^{13}C NMR (125.8 MHz) the chemical shifts (δ) were referenced to $CDCl_3$ (77.4 ppm), CD_3OD (49.0 ppm). The multiplicities of the ^{13}C signals refer to the number of attached protons: s = C, d = CH, t = CH_2, q = CH_3. Fourier transform infrared (FTIR) data were recorded on a spectrometer and spectra were measured by the diffuse reflectance method on samples dispersed in KBr. Ultraviolet (UV) spectra were recorded on a spectrophotometer using a 1 cm path length quartz cell.

Plant material

Albugo candida race 7V used in this study was obtained from Agriculture and Agri-Food Canada, Saskatoon Research Centre. The isolates were multiplied by inoculation on cotyledons and/or leaves of *B. rapa* cv. Torch (race 7V) as reported by Liu and Rimmer (1993).[1]

Seeds were sown 0.5 cm deep in individual pots in peat-lite at day/night temperature 20/16 ºC, 16 h photoperiod with 250 μmol s^{-1} m^{-2}. Seven-day-old or fully expanded cotyledons and 14-day-old leaves were used for inoculation.

Inoculation of Brassica rapa

The inoculum was prepared according to the method of Liu et al. (1996).[2] In brief, zoosporangia (10 mg) were added to sterile distilled water (50 ml in a 100 ml Erlenmeyer flask), the flask was swirled to suspend zoosporangia followed by incubation at 16°C for 3 h (no shaking). The

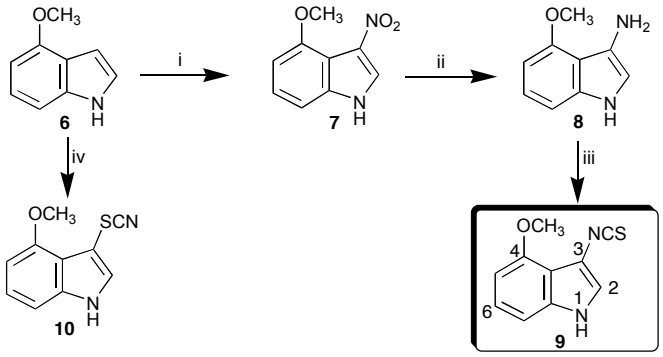
emerging motile zoospore suspension was kept on ice to maintain zoospore motility. The number of zoospores were counted under a light microscope (40×) using a haemocytometer. Prior to placing the cover glass on the spore haemocytometer, a Q-tip soaked in formalin was held near the drop of zoospores for a few seconds, to stop zoospores from moving before counting was initiated.

Two-week-old leaves of *B. rapa* cv. Torch were inoculated with a zoospore suspension of *A. candida* 7V. Ten-µl droplets of zoospore inocula were placed on leaf surfaces (6-8 droplets per leaf) using a micropipette and plants were incubated in a growth chamber in the dark for 24 h at 16 ºC, at 100% relative humidity. After 24 h, the chamber environment was changed to normal condition as described before.

After incubation for seven days in a growth chamber, infected leaves (1.3 kg) were collected, frozen in liquid nitrogen, ground, and extracted with methanol. The solvent was concentrated under reduced pressure and the residue was dissolved in dichloromethane. The dichloromethane layer was separated by filtration and concentrated. The dichloromethane residue (11.5 g) was fractionated repeatedly by FCC on silica gel and finally by preparative TLC to yield compounds **9** (rapalexin A, 0.5 mg) and **18** (rapalexin B, 1.4 mg).

Synthesis

Rapalexin A (9) and thiocyanate 10



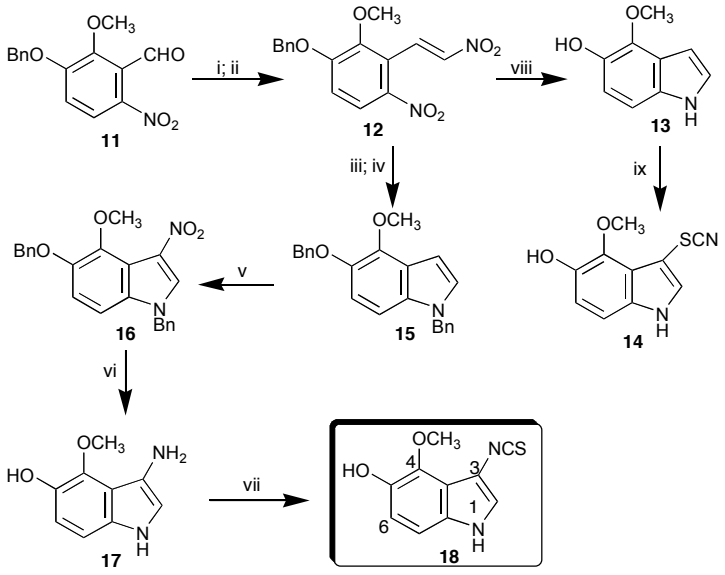
4-Methoxy-3-nitroindole (7). A solution of benzoyl chloride (120 μl, 1.02 mmol) was added dropwise to a solution of silver nitrate (167 mg, 0.990 mmol) in acetonitrile (1 ml) at 0 ºC with stirring for 10 min.[3] This solution was added to a solution of 4-methoxyindole[4] (120 mg, 0.82 mmol) in acetonitrile (10 ml) at -20 ºC with vigorous stirring. After stirring at -20 ºC for 60 min, the reaction was allowed to proceed at room temperature for 120 min. The reaction mixture was diluted with water (20 ml) and extracted with EtOAc (3×20 ml). The combined organic layer was washed with sodium carbonate (10% aqueous solution, 10 ml). The organic solvent was dried and concentrated under reduced pressure. The residue was fractionated by FCC on silica gel (hexane- EtOAc, 10:1) to yield 4-methoxy-3-nitroindole (**7**) as a yellow powder (47.0 mg) in 30 % yield. Yellow crystals (CH_2Cl_2), mp > 155 ºC (decomposed). HPLC t_R 10.3 min. ^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 9.85 (brs, N-H), 8.13 (d, *J* = 3.4 Hz, 1H), 7.26 (t, *J* = 8.1 Hz, 1H), 7.06 (d, *J* = 8.1 Hz, 1H), 6.78 (d, *J* = 8.1 Hz, 1H), 3.99 (s, 3H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 153.9 (s), 137.3 (s), 132.1 (s), 128.0 (d), 126.3 (d), 110.3 (s), 105.7 (d), 105.3 (d), 56.6 (q). HREI-MS: calc. for $C_9H_8N_2O_3$ (M^+) *m/z* 192.0534, found 192.0533. EI-MS *m/z* (% relative int.): 192 (100), 163 (15), 149 (22), 116 (66), 119 (16), 89 (18). υ_{max} (KBr)/cm^{-1}: 3314, 3148, 2911, 1592, 1515, 1368, 1352, 1319, 1256, 1306, 819.

Rapalexin A (4-methoxyindole-3-isothiocyanate, 9). Platinum (IV) oxide (25.0 mg) was added to a solution of 4-methoxy-3-nitroindole (**7**, 25 mg, 0.13 mmol) in 95% EtOH (5 ml) and the mixture was hydrogenated at balloon pressure with vigorous stirring. After the reaction was complete, the mixture was filtered, the organic layer was concentrated to yield a mixture containing 4-methoxyindole-3-amine (**8**) which was used immediately in the next step. The reaction mixture in EtOH (1 ml) was added dropwise to a mixture of CH_2Cl_2 (3 ml), thiophosgene (10 μl), and calcium carbonate (10 mg, 0.10 mmol) in water (5 ml) at room temperature during 5 min with vigorous stirring.[5] Stirring was continued for an additional 5 min, the mixture was diluted with water (10 ml) and extracted with CH_2Cl_2 (3×10 ml). The organic solvent was dried, concentrated and the product

was separated by preparative TLC to yield colourless crystals (5.4 mg) in 20 % yield over two steps, mp 77-78 ºC (CH_2Cl_2). HPLC t_R 24.6 min. ^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.98 (brs, N-H), 7.18 (t, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 2.2 Hz, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 6.59 (d, *J* = 7.9 Hz, 1H), 4.00 (s, 3H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 154.3 (s), 136.9 (s), 136.1 (s), 125.3 (d), 118.5 (d), 114.4 (s), 108.1 (s), 105.1 (d), 101.2 (d), 55.7 (q). HREI-MS: calc. for $C_{10}H_8N_2OS$ (M^+) *m/z* 204.0368, found 204.0370; ESI-MS (negative): 203.1, 188.8. EI-MS *m/z* (% relative int.): 204 (100), 189 (58), 161 (40), 95 (14), 81 (12). υ_{max} (KBr)/cm^{-1}: 3373, 2922, 2850, 2138, 2080, 1714, 1594, 1511, 1461, 1273, 1085, 730. λ_{max} (MeOH)/nm: 223 (log ε, 4.53), 292 (4.08).

4-Methoxyindole-3-thiocyanate (10). 4-Methoxyindole (6.4 mg, 0.044 mmol) in MeOH was added dropwise to a stirred solution of ammonium thiocyanate (27.5 mg, 0.36 mmol) and iodine (15.0 mg, 0.060 mmol) in MeOH (2 ml).[6] After 60 min, the reaction mixture was diluted with water (10 ml) and extracted with CH_2Cl_2 (3×10 ml). The combined organic layer was dried and concentrated under reduced pressure. The residue was separated by preparative TLC (hexane-acetone, 2:1) to yield colourless crystals of **10** (6.5 mg) in 75% yield, mp 123-125 ºC. HPLC: t_R 16.7 min. ^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.54 (brs, NH), 7.38 (d, *J* = 2.7 Hz, 1H), 7.22 (t, *J* = 8.1 Hz, 1H), 7.03 (d, *J* = 8.1 Hz, 1H), 6.65 (d, *J* = 8.1 Hz, 1H), 4.02 (s, 3H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 154.6 (s), 138.3 (s), 128.9 (d), 125.4 (d), 117.4 (s), 113.1 (s), 105.3 (d), 102.2 (d), 93.3 (s), 56.0 (q). HREI-MS: calc. for $C_{10}H_8N_2OS$ (M^+) *m/z* 204.0357, found 204.0365. EIMS *m/z*, (% relative int.): 204 (100), 189 (58), 161 (22). ESI-MS (negative): 203.4, 189.3. υ_{max} (KBr)/cm^{-1}: 3352, 3114, 2956, 2836, 2153, 1616, 1587, 1511, 1357, 1320, 1252, 1086, 778, 732. λ_{max} (MeOH)/nm: 218 (log ε, 4.51), 270 (3.85).

Rapalexin B (18) and thiocyanate 14



3-Benzyloxy-2-methoxy-6,β-dinitrostyrene (12). 3-Benzyloxy-2-methoxy-6-nitrobenzaldehyde[7] (**11**, 426.5 mg, 1.49 mmol) was stirred under an argon atmosphere at 0 ºC with *N*-methylmorpholine (15 ml), KF (61.4 mg, 0.90 mmol), 18-crown-6 (20.4 mg, 0.077 mmol), and nitromethane (400 μl, 7.27 mmol) for 30 min and then allowed to warm up to room temperature and stirred for 12 h. The mixture was poured into acetic anhydride (4 ml) containing sodium acetate (75 mg) and warmed to 60 ºC. After 60 min, the reaction was poured over ice-water (100 ml) and stirred until a fine powder formed. The solid was collected and separated by FCC (hexane-acetone, 3:1) to yield **12** (490.9 mg) in 88% yield. Yellow crystals (CH_2Cl_2), mp 95-96 ºC. HPLC t_R 30.1 min. ^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.24 (d, *J* = 13.5 Hz, 1H), 7.94 (d, *J* = 9.1 Hz, 1H), 7.73 (d, *J* = 13.5 Hz, 1H), 7.12 (d, *J* = 9.1 Hz, 1H), 7.41-7.46 (m, 5H), 5.27 (s, 2H), 3.91 (s, 3H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 156.8 (s), 148.8 (s), 142.6 (d), 135.3 (s), 130.3 (d), 129.4 (d, 2C), 129.2 (d), 127.9 (d, 3C), 122.7 (d), 121.2 (s), 114.3 (d), 71.9 (t), 61.4 (q). HREI-MS: calc. for $C_{16}H_{14}N_2O_6$ (M^+) *m/z* 330.0851, found 330.0846. EIMS *m/z*, (% relative int.): 330 (M^+) (4), 91 (100). υ_{max} (KBr)/cm^{-1}:

3094, 2940, 1640, 1600, 1569, 1514, 1472, 1452, 1343, 1318, 1277, 1232, 1087, 1069, 1021, 961, 934.

5-Hydroxy-4-methoxyindole (13). A mixture of 3-benzyloxy-2-methoxy-6,β-dinitrostyrene (**12**, 40 mg, 0.06 mmol), Pd/C (10% on carbon, 10 mg), glacial acetic acid (200 μl), and methanol (5 ml) was hydrogenated under balloon pressure with vigorous stirring.[8] After 60 min, the mixture was filtered and the solids were washed with EtOAc. The combined organic layer was dried and concentrated under reduced pressure to yield a dark oil. The oil was further separated by FCC (hexane-EtOAc, 3:1) to yield 5-hydroxy-4-methoxyindole (**13**, 5.7 mg) in 29% yield as a colourless oil. HPLC t_R 5.9 min. ^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.09 (brs, 1H), 7.18 (brs, 1H), 7.03 (d, J = 8.6 Hz, 1H), 6.91 (d, J = 8.6 Hz, 1H), 6.61 (brs, 1H), 5.45 (brs, 1H), 4.13 (s, 3H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 141.0 (s), 138.3 (s), 132.8 (s), 124.8 (d), 120.4 (s), 111.9 (d), 106.6 (d), 99.7 (d), 60.7 (q). HREI-MS calc. for $C_9H_9NO_2S$ (M^+) m/z 163.0633, found 163.0640. EIMS m/z, (relative int.): 163 (100), 148 (97), 120 (32), 91 (21). υ_{max} (KBr)/cm^{-1}: 3406, 2932, 2885, 1625, 1581, 1496, 1444, 1347, 1235, 1173, 1052, 988, 882, 771, 726.

5-Hydroxy-4-methoxyindole-3-thiocyanate (14). 5-Hydroxy-4-methoxyindole (**13**, 6.0 mg, 0.04 mmol) in MeOH (1 ml) was added to a stirred solution of ammonium thiocyanate (30.0 mg, 0.40 mmol) and iodine (20.0 mg, 0.08 mmol) in MeOH (2 ml) at 0 ºC. The reaction was allowed to proceed for 10 min, the mixture was diluted with water (10 ml) and extracted with EtOAc (20 ml). The organic layer was washed with sodium thiosulphate solution (10 % aqueous solution, 20 ml) and water (20 ml), dried and concentrated under reduced pressure. The residue was separated by FCC to yield 5-hydroxy-4-methoxyindole-3-thiocyanate (**14**, 4.0 mg) in 49 % yield. Colourless crystals, mp 125-126 ºC. HPLC t_R 8.90 min. ^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.54 (brs, 1H), 7.51 (d, J = 3.8 Hz, 1H), 7.13 (d, J = 8.7 Hz, 1H), 7.02 (d, J = 8.7 Hz, 1H), 5.53 (brs, 1H), 4.09 (s, 3H). ^{1}H-NMR (500.1 MHz, CD_3OD): δ 7.56 (s, 1H), 7.08 (d, J = 8.65 Hz, 1H), 6.88 (d, J = 8.65 Hz, 1H), 4.01 (s, 3H). ^{13}C-

NMR (125.8 MHz, $CDCl_3$): δ 144.2 (s), 138.9 (s), 132.7 (s), 132.5 (d), 114.5 (d), 112.9 (s), 112.6 (s), 109.0 (d), 63.6 (q). ^{13}C-NMR (125.8 MHz, CD_3OD): δ 143.9 (s), 139.3 (s), 132.2 (s), 132.1 (d), 121.6 (s), 114.8 (d), 113.3 (s), 108.4 (d), 88.3 (s), 61.3 (q). HREI-MS: calc. for $C_{10}H_8N_2O_2S$ (M^+) *m/z* 220.0306, found 220.0313. EIMS *m/z* (relative int.): 220 (100), 205 (67), 193 (16), 150 (82). λ_{max} (MeOH)/nm: 216 (log ε, 4.45), 266 (3.77), 297 (3.65). υ_{max} (KBr)/cm^{-1}: 3352, 2932, 2846, 2147, 1593, 1509, 1313, 1255, 1189, 1087, 1058, 925.

1-Benzyl-5-benzyloxy-4-methoxyindole (15). Benzyl bromide (60 μl, 0.51 mmol) was added to a solution of 5-benzyloxy-4-methoxyindole (130 mg, 0.51 mmol) and NaH (80 mg, 2.0 mmol) in dry THF (10 ml) with vigorous stirring. After the reaction was complete, the reaction mixture was quenched with water (30 ml) and the mixture was extracted with EtOAc (3×30 ml). The organic layer was dried and concentrated, the residue was separated by FCC (hexane-EtOAc, 20:1) to yield **15** (167 mg) as colourless oil in 93% yield. HPLC t_R 35.1 min. HREI-MS: calc. for $C_{23}H_{21}NO_2$ (M^+) *m/z* 343.1572, found 343.1569. EIMS (*m/z*, relative int.): 343 (18), 252 (100), 91 (98). ^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 7.50 (d, *J* = 7.2 Hz, 2H), 7.39 (t, *J* =7.2 Hz, 2H), 7.33-7.27 (m, 4H), 7.14 (d, *J* = 7.2 Hz, 2H), 7.08 (d, *J* = 3.1 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 1H), 6.88 (d, *J* = 8.8 Hz, 1H), 6.65 (d, *J* = 3.1 Hz, 1H), 5.27 (s, 2H), 5.14 (s, 2H), 4.11 (s, 3H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 144.1 (s), 143.6 (s), 138.6 (s), 137.8 (s), 134.7 (s), 129.2 (d), 128.8 (d, 2C), 128.7 (d), 128.1 (s), 128.0 (d, 2C), 128.0 (d), 127.3 (d, 2C), 123.5 (s), 114.8 (d), 105.1 (d), 99.3 (d), 74.4 (t), 61.3 (q), 50.7 (t). υ_{max} (KBr)/cm^{-1}: 3405, 2927, 2854, 1605, 1566, 1510, 1492, 1276, 1252, 1053, 733. HREI-MS: calc. for $C_{23}H_{21}NO_2$ (M^+) *m/z* 343.1569, found 343.1572. EIMS *m/z* (relative int.): 343 (18), 252 (100), 91 (90).

1-Benzyl-5-benzyloxy-4-methoxy-3-nitroindole (16). Method A. A solution of benzoyl chloride (70 μl, 0.60 mmol) was added dropwise to a solution of silver nitrate (92.7 mg, 0.55 mmol) in acetonitrile (2 ml) at -20 ºC with vigorous stirring for 10 min.[9] Then, this solution was added

dropwise to a solution of 1-benzyl-5-benzyloxy-4-methoxyindole (**15**, 157 mg, 0.46 mmol) in acetonitrile (20 ml) with vigorous stirring at -20 ºC. After 5 h, the reaction mixture was diluted with water (20 ml) and extracted with EtOAc (3×20 ml). The combined organic layer was washed with sodium carbonate (10% aqueous solution, 20 ml), the organic layer was dried and concentrated under reduced pressure. The residue was fractionated by FCC (hexane:EtOAc, 10:1) to yield 1-benzyl-5-benzyloxy-4-methoxy-3-nitroindole (**16**, 36.7 mg) as yellow powder in 20% yield. **Method B.** A solution of HNO_3 (70%, 200 μl, 3.13 mmol) was added slowly to a solution of acetic anhydride (1.0 ml, 11 mmol) at 10 °C with rapid stirring and the reaction was allowed to proceed at 10 °C for 10 min. The freshly prepared acetyl nitrate solution (12 μl) was added to a solution of 1-benzyl-5-benzyloxy-4-methoxyindole (8.2 mg, 0.024 mmol) in acetic anhydride (200 μl) at -20 °C with vigorous stirring. After 10 min, the reaction mixture was quenched with ice-water (10 ml) and extracted with EtOAc (2×10 ml). The combined organic layer was washed with water (2×10 ml), was dried and concentrated under reduced pressure. The residue (20.3 mg) was separated by preparative TLC (hexane-EtOAc, 3:1) to yield **16** (0.8 mg) in 10% yield. HPLC t_R 32.2 min. mp 103-105 ºC. ^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.09 (s, 1H), 7.49 (d, J = 7.2 Hz, 2H), 7.33-7.43 (m, 6H), 7.22 (d, J = 6.5 Hz, 2H), 7.06 (d, J = 8.9 Hz, 1H), 7.03 (d, J = 8.9 Hz, 1H), 5.29 (s, 2H), 5.19 (s, 2H), 4.07 (s, 3H). ^{13}C-NMR (125.8 MHz, $CDCl_3$): δ 149.5 (s), 144.0 (s), 137.8 (s), 134.6 (s), 133.4 (s), 132.8 (d), 130.2 (s), 129.7 (d, 2C), 129.2 (d), 128.9 (d, 2C), 128.3 (d), 127.9 (d, 2C), 127.8 (d, 2C), 116.3 (d), 116.2 (s), 106.9 (d), 73.6 (t), 62.8 (q), 51.7 (t). HREI-MS: calc. for $C_{23}H_{20}N_2O_4$ (M^+) m/z 388.1423, found 388.1414. EIMS m/z (% relative int.): 388 (M^+) (14), 298 (12), 297 (59), 91 (100). υ_{max} (KBr)/cm^{-1}: 3122, 2932, 1575, 1527, 1492, 1442, 1358, 1269, 1108, 1039, 795, 747.

5-Hydroxy-4-methoxyindol-3-amine (17). Method A. Pd/C (10% on carbon, 5 mg) was added to a solution of 5-benzyloxy-4-methoxy-3-nitroindole (5.0 mg) in AcOH (1 ml) under argon atmosphere. The mixture was hydrogenated at balloon pressure with vigorous stirring for 1 h and

then was filtered under argon atmosphere. The organic layer was concentrated and used for the next step. **Method B.** Pd/C (10% on carbon, 5.8 mg) was added to a solution of 1-benzyl-5-benzyloxy-4-methoxy-3-nitroindole (**15**, 6.2 mg, 0.016 mmol) in AcOH (300 μl) under argon atmosphere. The mixture was hydrogenated at 40 psi for 4 h, was filtered under argon atmosphere, concentrated and used for the next step.

Rapalexin B (5-hydroxy-4-methoxyindole-3-isothiocyanate, 18). Thiophosgene (10 μl) was added to a mixture of CH_2Cl_2 (3 ml), calcium carbonate (10 mg, 0.1 mmol), and amine **17** with vigorous stirring at room temperature. Stirring was continued for an additional 5 min, the reaction mixture was filtered, the solvent was dried and concentrated. The product was separated by preparative TLC (1.1 mg) in 30 % yield over two steps. Colourless oil. HPLC t_R 14.6 min. ^{1}H-NMR (500.1 MHz, CD_3CN): δ 9.31 (brs, 1H), 7.34 (d, J = 2.9 Hz, 1H), 7.09 (d, J = 8.7 Hz, 1H), 6.86 (d, J = 8.7 Hz, 1H), 6.53 (s, 1H), 3.94 (s, 3H). ^{1}H-NMR (500.1 MHz, CD_3OD): δ 7.29 (s, 1H), 7.01 (d, J = 8.7 Hz, 1H), 6.82 (d, J = 8.7 Hz, 1H), 3.97 (s, 3H). ^{13}C-NMR (CD_3OD): δ 143.2 (s), 138.7 (s), 133.7 (s), 131.1 (s), 121.7 (d), 117.8 (s), 114.8 (d), 108.3 (d), 104.5 (s), 61.2 (q). HREI-MS: calc. for $C_{10}H_8N_2O_2S$ (M^+) m/z 220.0306, found 220.0316. EIMS m/z (relative int.): 220 (100), 205 (10), 177 (24), 149 (16). ESI-MS (negative): 219.1, 204.9. λ_{max} (MeOH)/nm: 203 (log ε, 3.67), 214 (3.85), 264 (3.39), 288 (3.38). υ_{max} (KBr)/cm^{-1}: 3379, 3128, 2931, 2835, 2136, 1590, 1508, 1456, 1320, 975, 786.

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