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Probing the Phytopathogenic Stem Rot Fungus with Phytoalexins and Analogues: Unprecedented Glucosylation of Camalexin and 6-Methoxycamalexin

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Abstract—The remarkable metabolism of the cruciferous phytoalexins camalexin and 6-methoxycamalexin by the stem rot phytopathogen *Sclerotinia sclerotiorum* is reported. The biotransformations yielded camalexins glucosylated at *N*-1 or C-6 of the indole ring, with substantially lower antifungal activity than camalexins. A camalexin analogue with the positions *N*-1 and C-6 blocked was metabolized but at a much slower rate than the natural phytoalexins. The chemistry involved in the metabolism of natural camalexins and two new analogues, as well as their novel metabolites and respective antifungal activities is described.

Introduction

Camalexins are unique phytoalexins biosynthesized by a few wild crucifers.[1] Phytoalexins, induced plant chemical defenses, can selectively inhibit plant pathogens and plant pathogens can react differently to phytoalexins. In instances where phytopathogens can circumvent phytoalexins through metabolic detoxification, an environmentally attractive strategy to control such plant pathogens could use selective inhibitors of the fungal detoxifying enzymes.[2] Towards this end, we have been investigating the metabolism of cruciferous phytoalexins by economically important phytopathogenic fungi.[1] Our previous work showed that camalexin (**1**) could be detoxified by the fungus *Rhizoctonia solani* Kuhn (Scheme 1) to 5-hydroxycamalexin (**2**), which was further transformed into metabolites **3** and **4**, substantially less toxic to the pathogen than camalexin (**1**).[3,4] However, we have also shown that not all plant pathogenic fungi or bacteria can transform camalexin (**1**).[5]

In continuation of those studies, we examined the metabolism of naturally occurring camalexins **1** and **1a** by the stem rot fungus *Sclerotinia sclerotiorum* (Lib.) de Bary. *S. sclerotiorum* causes stem rot disease in a vast range of plants and is considered a worldwide problem.[6] An evaluation of the biological activity of diverse phytoalexins against *S. sclerotiorum* indicated that camalexin (**1**) could inhibit completely the growth of the pathogen.

Subsequently, we established that metabolism and detoxification of camalexins **1** and **1a** occurs via glycosylation, an unusual detoxification reaction in plant pathogenic fungi.[7] To probe the selectivity of these enzymatic reactions we designed camalexin analogues based on the structure of the detoxification products. The results of these studies are now reported for the first time.[8]

Results and Discussion

Camalexins **1**–**1c** were synthesized from the respective indoles as described in the Experimental and previously reported.[9] Initial experiments established the minimum inhibitory concentration (5×10^{-4} M) of camalexin (**1**) and 6-methoxycamalexin (**1a**) to *S. sclerotiorum*. Subsequently, camalexins **1** and **1a** were incubated separately with *S. sclerotiorum*, culture samples were extracted and analyzed. HPLC analysis of the extracts of fungal cultures incubated with camalexins **1** and **1a** indicated that both were completely metabolized in ca. 48 h. After 24 h incubation of *S. sclerotiorum* with camalexin (**1**), products **5** and **5a** were isolated, whereas **5, 5a** and **5b** were isolated after incubation with 6-methoxycamalexin (**1a**)

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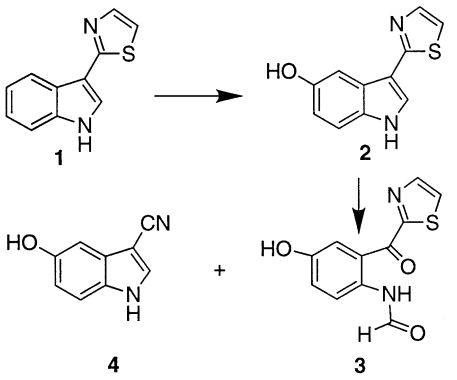
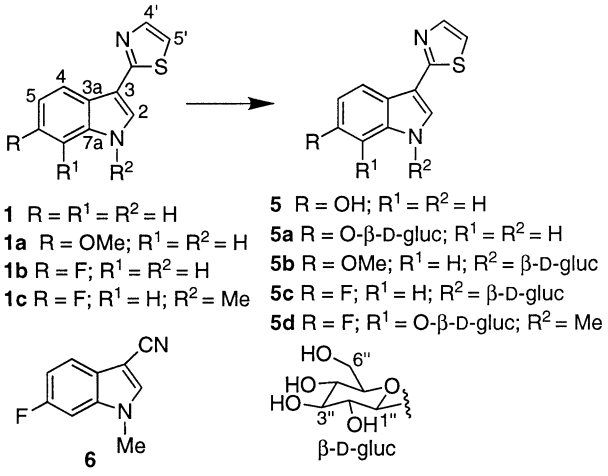


Scheme 1. Detoxification pathway of camalexin (**1**) in the phytopathogen *Rhizoctonia solani*.

(Scheme 2).[10] The chemical structure of each metabolite was deduced from comparison of their spectroscopic data with those of camalexins **1** and **1a**, as follows.

Relative to camalexin ($C_{11}H_8N_2S$), metabolite **5** contained an additional oxygen atom ($C_{11}H_8N_2OS$), as determined by HRMS-EI. Comparison of the ^{1}H NMR spectrum (Table 1) of **5** with that of camalexin (**1**) indicated the presence of an indolic substituent, since only a three-proton spin system was displayed in addition to H-2 and two thiazole protons. The ^{13}C NMR spectrum



Scheme 2. Detoxification pathway of camalexins **1**–**1c** in the phytopathogen *Sclerotinia sclerotiorum*.

(Table 2) displayed 11 carbon signals, one of which was characteristic of a deshielded sp^2 C atom (154.1 ppm). These spectroscopic features suggested that metabolite **5** contained an OH group attached to either C-5 or C-6. That the OH group was attached to C-6 rather than C-5 was finally deduced from comparison of HMBC and HMQC spectral data with that of camalexin (**1**) and 5-hydroxycamalexin.[3] In addition, methylation of **5** with diazomethane yielded **1a**, thus confirming the structure of **5** unambiguously.

The molecular formulas of metabolites **5a** ($C_{17}H_{19}N_2O_6S$) and **5b** ($C_{18}H_{21}N_2O_6S$) obtained by HRMS-FAB indicated the presence of a hexose, which was corroborated by NMR data (Tables 1 and 2). The ^{1}H NMR spectra of **5a** and **5b** (Table 1) displayed resonances indicative of a β-D-glucopyranosyl substituent on the indole moiety; analysis of the HMQC and HMBC spectral data confirmed that this substituent was located at C-6 in **5a** (C–H long-range correlation between anomeric H and C-6) and at *N*-1 in **5b** (C–H long-range correlation between anomeric H and C-2 and C-7a).

To establish the sequence of biotransformation steps of camalexin (**1**) and 6-methoxycamalexin (**1a**), compound **5** was administered to cultures of *S. sclerotiorum*. The cultures were incubated and samples extracted and analyzed by TLC and HPLC. As expected, **5a** was detected in the cultures incubated with **5** after 12 h; complete metabolism of **5** to **5a** occurred in ca. 24 h. These results indicated that **1** and **1a** were transformed to 6-*O*-β-D-glucopyranosylcamalexin (**5a**) via 6-hydroxycamalexin (**5**). In addition, in 6-methoxycamalexin (**1a**) was partly converted to **5b**, a minor metabolite; that is, *S. sclerotiorum* converted 6-methoxycamalexin (**1a**) via two pathways, with the major product **5a** resulting from demethylation of the methoxy group at C-6, followed by glucosylation; the minor product **5b** resulted from direct *N*-glucosylation. The results of these experiments are summarized in Table 3.[11]

Subsequently, it was anticipated that, if the fungal detoxifying enzyme(s) was specific, blocking the C-6 of camalexin would direct glucosylation to *N*-1, and blocking both *N*-1 and C-6 sites would slow down if not stop this metabolism. Thus, camalexins **1b** and **1c** were

Table 1. ^{1}H NMR chemical shifts (ppm) and multiplicities (*J* in Hz)[a] for compounds **1b**–**6** in CD_3CN

H #	1b	1c	5	5a	5b	5c	5d	6
H-1	9.74 broad s	—	9.44 broad s	9.67 broad s	—	—	—	—
H-2	7.89 s	7.68 s	7.73 d (2)	7.82 d (3)	7.94 s	8.04 s	7.75 s	7.83 s
H-4	8.25 dd (9, 5.5)	8.17 dd (9, 5.5)	8.04 d (8.5)	8.13 d (9)	8.14 d (9)	8.30 dd (9, 5.5	8.01 dd (9, 4.5)	7.66 dd (9, 5.5)
H-5	7.03 ddd (10, 9, 2.5)	6.98 ddd (10, 9, 2.5)	6.77 dd (8.5, 2)	6.89 dd (9, 2)	6.92 dd (9, 2)	7.10 ddd (10, 9, 2.5)	7.06 dd (12, 9)	7.09 ddd (10, 9, 2.5)
H-7	7.23 dd 10, 2.5)	7.09 dd (10, 2.5)	6.89 d (2)	7.22 d (2)	7.12 d (2)	7.27 dd (10, 2.5)	—	7.30 dd (10, 2.5)
H-4′	7.78 d (3.5)	7.74 d (3.5)	7.75 d (3.5)	7.76 d (3.5)	7.78 d (3.5)	7.81 d (3.5)	7.76 d (3.5)	—
H-5′	7.34 d (3.5)	7.26 d (3.5)	7.31 d (3.5)	7.33 d (3.5)	7.35 d (3.5)	7.39 d (3.5)	7.33 d (3.5)	—
H-1″	—	—	—	4.94 d (7)	5.50 d (9)	5.44 d (9)	5.10 d (7.5)	—
Other		3.68 s, *N*-Me		3.90–3.25, m, H-2″-H-6″	3.87 s, OMe 3.90–3.25, m, H-2″-H-6″	3.90–3.25, m, H-2″-H-6″	4.07 s, *N*-Me 3.70–3.15, m, H-2″-H-6″	3.80 s, *N*-Me

[a]Coupling constants (*J*) are reported to the nearest 0.5 Hz.

Table 2. ^{13}C NMR chemical shifts (ppm) of compounds **1b**–**1c** (CD_3CN), **5a** (DMSO-d_6), **5**, **5b**–**5d** (CD_3CN), **6**(CD_3OH)[a]

C #	1b	1c	5	5a	5b	5c	5d	6
C-2	127.3	133.1	124.6	126.5	125.2	127.1	132.5	137.8
C-3	112.4	113.4	112.0	113.1	112.4	112.5	110.4	84.7
C-3a	122.7	124.6	118.9	120.5	119.8	122.5	124.3	124.4
C-4	122.3, d $^3j_{C\text{-}F}=10$	124.8, d $^3j_{C\text{-}F}=10$	121.6	121.4	121.9	122.6, d $^3j_{C\text{-}F}=10$	117.1, d $^3j_{C\text{-}F}=9$	120.2, d $^3j_{C\text{-}F}=10$
C-5	110.4, d $^2j_{C\text{-}F}=25$	112.1, d $^2j_{C\text{-}F}=25$	111.4	111.4	111.9	110.3, d $^2j_{C\text{-}F}=25$	111.0, d $^2j_{C\text{-}F}=22$	110.8, d $^2j_{C\text{-}F}=26$
C-6	161.7, d $j_{C\text{-}F}=238$	162.9, d $j_{C\text{-}F}=237$	154.1	155.1	157.6	160.5, d $j_{C\text{-}F}=238$	151.7, d $j_{C\text{-}F}=237$	161.0, d, $j_{C\text{-}F}=240$
C-7	99.2, d $^2j_{C\text{-}F}=26$	99.4, d $^2j_{C\text{-}F}=27$	97.4	100.0	94.9	98.2, d $^2j_{C\text{-}F}=28$	130.0, d $^2j_{C\text{-}F}=28$	97.4, d $^2j_{C\text{-}F}=27$
C-7a	138.5, d $^3j_{C\text{-}F}=12$	140.4 $^3j_{C\text{-}F}=12$	137.6	137.9	138.6	138.6, d $^3j_{C\text{-}F}=13$	130.9, d $^3j_{C\text{-}F}=13$	137.0, d $^3j_{C\text{-}F}=12$
C-2′	165.6	165.6	165.6	163.8	163.0	162.8	162.9	—
C-4′	143.2	145.5	142.9	143.4	143.1	143.2	143.1	—
C-5′	117.5	118.8	116.2	116.9	116.7	117.1	116.5	—
C-1″	—	—	—	102.6	85.2	85.7	104.4	—
C-2″	—	—	—	74.3[b]	72.7[b]	72.7[b]	74.7[b]	—
C-3″	—	—	—	77.5[b]	77.8[b]	77.6[b]	76.7[b]	—
C-4″	—	—	—	70.6[b]	70.4[b]	70.3[b]	70.4[b]	—
C-5″	—	—	—	77.9[b]	79.3[b]	79.5[b]	77.1[b]	—
C-6″	—	—	—	61.6[b]	61.9[b]	61.9[b]	61.5[b]	—
Other	—	35.6, *N*-$\underline{C}H_3$	—	—	55.7, O$\underline{C}H_3$	—	36.9, *N*-$\underline{C}H_3$	32.8, *N*-$\underline{C}H_3$ 115.5, $\underline{C}N$

[a]Chemical shifts of camalexins **1b** and **1c** change with concentration; reported values are for $c=10$ mg/mL.
[b]Signals in the same column may be interchanged.

Table 3. Products of metabolism of camalexins **1a**–**1c** and compound **5** at 1×10^{-4} M by *Sclerotinia sclerotiorum*

Compound added to fungal cultures[a]	Products (%)[b] of metabolism after incubation for 24 and 48 h
Camalexin (**1**)	Complete biotransformation to **5** (28%) and **5a** (22%) with no camalexin recovered after 24 h
6-Methoxycamalexin (**1a**)	Complete biotransformation to **5** (18%), **5a** (20%) and **5b** (12%) with no camalexin recovered after 24 h
6-Fluorocamalexin (**1b**)	Complete biotransformation to **5c** (52%) with no camalexin recovered after 48 h
6-Fluoro-1-methylcamalexin (**1c**)	Biotransformation to **5d** (17%) and **6** (17%) with 35% camalexin recovered after 48 h
6-Hydroxycamalexin (**5**)	Complete biotransformation to **5a** in 24 h
Camalexin (**1**) + 6-fluorocamalexin (**1b**)	Biotransformation to **5a** (80%) and no **5c** in 24 h, **5a** (91%) and **5c** (5%) in 48 h[c]

[a]Compounds were dissolved in DMSO, added to 6-day-old cultures and incubated at 24 ± 2 °C.
[b]Percentage yields of products represents isolated amounts; due to losses during extraction and separation, yields of glucosyl derivatives **5a**–**5d** determined by HPLC analysis are 25–30% higher than isolated yields.
[c]Percentage yields of products represents HPLC determined yields.

synthesized and separately administered to cultures of *S. sclerotiorum*. After incubation and isolation of the resulting products, metabolites **5c** and **5d** were obtained; while **1b** was completely metabolized to **5c**, **1c** was transformed to **5d** (17%) and **6** (17%) (Table 3). The chemical structures of **5c** and **5d** were deduced from analysis of NMR spectroscopic data (Tables 1 and 2), and HMQC and HMBC spectral data (substituent located at *N*-1 in **5c** displayed C–H long-range correlation between anomeric H and C-2 and C-7a; substituent located C-7 in **5d** displayed C–H long-range correlation between anomeric H and C-7). The chemical structure of **6** was deduced from spectroscopic data and confirmed by synthesis as described in the Experimental.

The antifungal activity of camalexins **1**–**1c** and metabolites **5a**–**5d** to *S. sclerotiorum* was compared (Table 4). After 7 days of incubation, the mycelium of control plates incubated with *S. sclerotiorum* covered 100% of the plates, while plates containing **1** or **1c** (5×10^{-4} M) showed no mycelial growth, and plates containing **1a** or **1b** (5×10^{-4} M) showed slower growth than controls. By contrast, plates containing **5a**–**5d** were similar to control plates. These results demonstrated for the first time that metabolism of camalexins **1**–**1c** in *S. sclerotiorum* leads to products having no detectable antifungal activity, thus this metabolism is a detoxification process.

Results of the metabolism of camalexin (**1**) suggest that the first step for camalexin detoxification involves oxidation of C-6 to the 6-hydroxy derivative **5**, whereas in the case of **1a**, the first step involves oxidative demethylation to **5**. The second metabolic step involves glucosylation of **5** to **5a**; however, a minor pathway for detoxification of **1a** leads to glucosylation of *N*-1. Next, to probe the specificity of the oxygenase(s) involved in the first transformation step of **1** and **1a**, in additional experiments, camalexin (**1**) and 6-fluorocamalexin (**1b**) were administered together to fungal cultures, and the cultures incubated and analyzed as previously described. Camalexin (**1**) was metabolized to **5** and **5a** almost completely (ca. 95%) in 48 h, whereas analogue **1b** was metabolized only after camalexin. On the other hand, although analogue **1c** was metabolized much slower than camalexin (ca. 24 h versus 7 days) it did not affect the rate of camalexin transformation when co-incubated. These results indicate that there is competition between **1** and **1b** but no competition between substrates **1** and **1c**. Nonetheless, a better understanding of the mechanisms involved in these biotransformations will require kinetic studies with the cell-free cultures and purified enzymes, which are of pivotal importance in the design of detoxification inhibitors of camalexins.

Although glucosylation is a very common detoxification mechanism among plants, it is less usual in

Table 4. Percentage of inhibition[a] of *Sclerotinia sclerotiorum* incubated with camalexins **1**–**1c** (after 7 days, constant light)

Compound assayed against fungal cultures	Concentration (M)	% of Inhibition ± SD (average diameter of mycelium growth, mm)
Control	—	0 ± 0.5 (35)
Camalexin (**1**)	5×10^{-4}	100 ± 0.5 (8)[b]
	5×10^{-5}	0 ± 0.5 (35)
6-Methoxycamalexin (**1a**)	5×10^{-4}	41 ± 0.5 (24)
	5×10^{-5}	0 ± 0.5 (35)
6-Fluorocamalexin (**1b**)	5×10^{-4}	70 ± 0.5 (16)
	5×10^{-5}	0 ± 0.5 (35)
6-Fluoro-1-methylcamalexin (**1c**)	5×10^{-4}	100 ± 0.5 (8)[b]
	5×10^{-5}	0 ± 0.5 (35)

[a]The percentage of inhibition was calculated using the formula: % inhibition = 100−[(growth on treated/growth in control) × 100]; results are the mean of at least three independent experiments conducted in triplicate.
[b]The size of mycelium plug used to initiate cultures was 8 mm.

microorganisms.[12] To the best of our knowledge this is the first example of phytoalexin glucosylation by a plant pathogen. Microorganisms able to transform mycotoxins to glucosyl derivatives may have potential applications in treating contaminated cereals[13] or as models of mammalian metabolites.[14] Here we demonstrate that *S. sclerotiorum* can detoxify natural camalexins and analogues through unusual glycosylation of the indole ring. In this context, because *N*-1 glucosylation of indoles are often low yield and time consuming reactions, the fungus *S. sclerotiorum* might be a potential biotransformation agent in some of these synthetic preparations.[15]

Experimental

General experimental procedures

All chemicals were purchased from Sigma-Aldrich Canada Ltd., Oakville, ON, Canada. All solvents were HPLC grade and used as such, except for CH_2Cl_2 and $CHCl_3$ which were redistilled. Remaining conditions as previously reported.[4]

HPLC analysis was carried out with a high performance liquid chromatograph equipped with quaternary pump, automatic injector, and diode array detector (wavelength range 190–600 nm), degasser, and a Hypersil ODS column (5 μm particle size silica, 4.6 id × 200 mm), equipped with an in-line filter. Mobile phase: 75% H_2O-25% CH_3CN to 100% CH_3CN, for 35 min, linear gradient, and a flow rate 1.0 mL/min. Specific rotations, $[\alpha]_D$ were determined at ambient temperature on a Perkin–Elmer 141 polarimeter using a 1 mL, 10 cm path length cell; the units are 10^{-1} deg cm^2 g^{-1} and the concentrations (*c*) are reported in g/100 mL.

Fungal cultures and bioassays

S. sclerotiorum was obtained from C. Lefol, AAFC, Saskatoon, Canada. The fungal isolates were grown on potato dextrose agar (PDA) plates at 24 ± 2 °C, under constant light for 7 days. The antifungal activity of compounds **1**–**1c** and **5a**–**5d** was determined using the following mycelial radial growth bioassay. Solutions of each compound in DMSO (5×10^{-2} M) were used to prepare assay solutions in minimal media (5×10^{-4}, 5×10^{-5} and 1×10^{-5} M); control solutions contained 1% DMSO in minimal media.[2] Sterile tissue culture plates (6-well, 35 mm diameter) containing test solutions and control solution (2 mL per well) were inoculated with mycelium plugs placed upside down on the center of each plate (8 mm cut from 3-day-old PDA plates of *S. sclerotiorum*, clone # 33) and incubated under constant light for 7 days. All bioassay experiments were carried out in triplicate, at least three times.

Time-course study and metabolism of camalexins 1–1c and 6-hydroxycamalexin (5). Erlenmeyer flasks (250 mL) containing 100 mL of minimal media were employed. Each flask was inoculated with sclerotia of *S. sclerotiorum* clone # 33 and was incubated at 25 ± 2 °C on a shaker at 120 rpm under constant light. After six days a solution of each compound in DMSO was added to fungal cultures (1×10^{-4} or 2×10^{-4} M) and to uninoculated medium (control); DMSO (200 μL) was added to control cultures. Samples (10 mL each) were taken from the flasks immediately after adding the compounds. Subsequently, 15 mL samples were taken every 24 h for 4 days and final samples after 7 days. The samples were either immediately extracted or frozen for later extraction. Each sample was first extracted with EtOAc; the resulting aqueous layer was acidified (to pH 2 with dil HCl) and extracted with EtOAc. Finally the acidic aqueous layer was made alkaline (to pH 10 with 28% aqueous ammonia) and extracted with chloroform. After concentration of the solvent, the extracts were analyzed by HPLC. Metabolite **5** in DMSO (1×10^{-4} M) was subjected to further biotransformation by *S. sclerotiorum* clone # 33. The biotransformation was monitored by taking samples after 3 h, 6 h, 12 h, 24 h, 48 h, and 7 days. The samples were worked up and analyzed by HPLC.

Isolation of metabolites 5–5d and 6. To obtain larger amounts of extract to isolate the products of metabolism of each camalexin, experiments were carried out with 1-L batches, as described above for time-course studies. Only the chromatograms of the EtOAc extracts of the neutral broth showed peaks not present in chromatograms of extracts of control cultures. Thus, the EtOAc extracts were fractionated by FCC on silica gel (gradient elution: CH_2Cl_2–MeOH, 100:0–85:15), and each fraction was analyzed by HPLC. The metabolites were isolated by preparative TLC (silica gel, CH_2Cl_2–

MeOH, 90:10, multiple development) and/or reverse-phase preparative TLC (RP C-18 silica gel, H_2O–CH_3CN, 60:40).

Synthesis of camalexins 1–1c. Camalexin (**1**) was synthesized as previously reported.[9] 6-Methoxycamalexin (**1a**, 94 mg, 67% yield based on recovered starting material) was synthesized similarly but replacing indole with 6-methoxy indole (100 mg, 0.68 mmol). 6-Fluorocamalexin (**1b**, 131 mg, 59% yield based on recovered starting material) was synthesized similarly but replacing indole with 6-fluoroindole (150 mg, 1.1 mmol). 6-Fluoro-1-methylcamalexin (**1c**, 112 mg, 96%) was synthesized from 6-fluorocamalexin (**1b**, 110 mg, 0.51 mmol) by treatment with MeI/NaH.

Synthesis of 6-fluoroindole-3-carbonitrile (6). Phosphorus oxychloride (50 μL, 0.55 mmol, freshly distilled) was added dropwise with stirring to DMF (160 mg, 1.12 mL) under argon atmosphere. 6-Fluoroindole (67.5 mg, 0.5 mmol) in DMF (40 mg, 30 μL) was then added dropwise with continuous stirring, at room temperature. The reaction mixture was kept at 35 °C for 45 min and then poured into crushed ice. Aqueous NaOH (96 mg in 50 mL, 2.4 mmol) was added slowly to the reaction mixture until it became alkaline. The solution was boiled for 1 min, cooled to room temperature and then extracted with EtOAc (50 mL, 2×), the organic phases were combined, washed with water, dried (Na_2SO_4) and concentrated under vacuum to give 6-fluoroindole-3-carboxaldehyde (70.1 mg, 86%, $R_t = 8.5$ min). ^{1}H NMR (300 MHz, CD_3CN) δ 10.03 (br s, 1H, D_2O exchangeable), 9.95 (s, 1H), 8.11 (dd, $J = 9$, 5.5 Hz, 1H), 7.98 (s, 1H), 7.26 (dd, $J = 10$, 2.5 Hz, 1H), 7.04 (ddd, $J = 10$, 9, 2.5 Hz, 1H); ^{13}C NMR (75 MHz, CD_3CN) δ 188.1, 163.3 (d, $J = 238$ Hz), 140.9, 140.1 (d, $J = 13$ Hz), 125.3 (d, $J = 10$ Hz), 123.9, 121.6, 113.6 (d, $J = 24$ Hz), 101.5 (d, $J = 27$ Hz); HRMS-EI *m/z*: measured 163.0430 (M^+, calcd 163.0433 for C_9H_6NOF); FTIR (cm^{-1}) 3131, 2922, 2862, 1630, 1535, 1453, 1158, 1074, 951, 823; UV (λ_{max}, CH_3CN) 221 (log ε 4.5), 247 (log ε 4.2), 295 (log ε 4.2), 318 (log ε 4.2). 6-Fluoroindole-3-carboxaldehyde (40 mg, 0.25 mmol) was dissolved in anhydrous THF under argon atmosphere. Sodium hydride (8.2 mg, 0.34 mmol) was added with constant stirring at 0 °C followed by methyl iodide (32 μL, 0.34 mmol) and the reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was poured into ice-cold water and then extracted with EtOAc (50 mL, 2×). The extract was dried (Na_2SO_4) and the solvent removed under reduced pressure to give 6-fluoro-1-methylindole-3-carboxaldehyde (42.4 mg, 96%, $R_t = 11.4$ min). ^{1}H NMR (300 MHz, CD_3OD) δ 9.82 (s, 1H), 8.15 (dd, $J = 9$, 5.5 Hz, 1H), 8.06 (s, 1H), 7.27 (dd, $J = 10$, 2.5 Hz, 1H), 7.05 (ddd, $J = 10$, 9, 2.5 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 186.8, 162.2 (d, $J = 240$ Hz), 143.7, 140.1 (d, $J = 12$ Hz), 123.8 (d, $J = 9$ Hz), 122.9, 119.2, 112.2 (d, $J = 24$ Hz), 98.3 (d, $J = 24$ Hz), 34.1; HRMS-EI mass *m/z*: measured 177.0587 (M^+, calcd 177.0590 for $C_{10}H_8NOF$,). Pyridine (10 μL in 0.5 mL $CHCl_3$) was added to a refluxing mixture of $HONH_2 \cdot HCl$ (8.6 mg, 0.12 mmol) and 6-fluoro-1-methylindole-3-carboxaldehyde (20 mg, 0.11 mmol) in $CHCl_3$–EtOH (7:3, v/v, 3 mL).[4] After 3 h, SeO_2 (13.7 mg, 0.12 mmol) was added to the reaction mixture and refluxing proceeded for 3 h. The reaction mixture was allowed to cool, anhydrous $MgSO_4$ (2 mg) was added and stirring was continued for 10 min at room temperature. The mixture was then filtered and the solvent was removed under reduced pressure. The residue was separated by prep. TLC (CH_2Cl_2, developed three times) to give **6** as off-white powder (9.1 mg, 74%, $R_t = 15.9$ min) and unreacted 6-fluoro-1-methylindole-3-carboxaldehyde (6.8 mg).

Camalexin (1). $R_t = 16.5$ min (HPLC, broad peak); remaining data in supporting information.

6-Methoxycamalexin (1a). $R_t = 23.4$ min (HPLC, broad peak); remaining data in supporting information.

6-Fluorocamalexin (1b). $R_t = 19.3$ min (HPLC, broad peak); ^{1} H NMR in Table 1; ^{13}C NMR in Table 2; HRMS-EI *m/z*: measured 218.0315 (M^+, calcd 218.0314 for $C_{11}H_7N_2FS$,); MS-EI *m/z* (relative intensity): 218 (100); FTIR (cm^{-1}) 3162, 1624, 1555, 1451, 1134, 839; UV (λ_{max}, CH_3CN) 218 (log ε 4.4), 287 (log ε 4.1), 314 (log ε 4.1).

6-Fluoro-1-methylcamalexin (1c). $R_t = 23.6$ min (HPLC, broad peak); ^{1} H NMR in Table 1; ^{13}C NMR in Table 2; HRMS-EI *m/z*: measured 232.0473 (M^+, calcd 232.0471 for $C_{12}H_9N_2FS$); MS-EI *m/z* (relative intensity): 232 (100), 174 (49), 173 (27); FTIR (cm^{-1}) 2922, 2853, 1462, 943; UV (λ_{max}, CH_3CN) 224 (log ε 4.4), 290 (log ε 4.1), 322 (log ε 4.3).

6-Hydroxycamalexin (5). $R_t = 11.4$ min (HPLC); ^{1} H NMR in Table 1; ^{13}C NMR in Table 2; HRMS-EI *m/z*: measured 216.0361 (M^+, calcd 216.0357 for $C_{11}H_8N_2OS$); MS-EI *m/z* (relative intensity): 216 (100), 158 (35). FTIR (cm^{-1}) 3380, 2924, 2854, 1534, 1361, 1312, 1085; UV (λ_{max}, CH_3CN) 221 (log ε 4.5), 251 (log ε 4.1), 295 (log ε 4.2), 322 (log ε 4.1).

6-(*O*-β-D-Glucopyranosyl)camalexin (5a). $R_t = 4.2$ min (HPLC) ; $[\alpha]_D = -12.2$ (*c* 0.36, MeOH); ^{1} H NMR in Table 1; ^{13}C NMR in Table 2; HRMS-FAB *m/z*: measured 379.0967 ($[M^+H]^+$, calcd 379.0964 for $C_{17}H_{19}N_2O_6S$); MS-FAB *m/z* (relative intensity): 379 (15), 329 (40), 176 (100); FTIR (cm^{-1}) 3312, 2924, 1549, 1248, 1077; UV (λ_{max}, CH_3CN) 222 (log ε 4.0), 251 (log ε 3.7), 286 (log ε 3.9), 321 (log ε 3.7).

6-Methoxy 1-(β-D-glucopyranosyl)camalexin (5b). $R_t = 7.1$ min (HPLC); $[\alpha]_D = -14.4$ (*c* 0.08, MeOH); ^{1} H NMR in Table 1; ^{13}C NMR in Table 2; HRMS-FAB *m/z*: measured 393.1117 ($[M+H]^+$, calcd 393.1120 for $C_{18}H_{21}N_2O_6S$); MS-FAB *m/z* (relative intensity): 393 (20), 207 (40), 115 (100). FTIR (cm^{-1}) 3335, 2924, 2853, 1557, 1456, 1079; UV (λ_{max}, CH_3CN) 216 (log ε 4.1), 254 (log ε 3.7), 297 (log ε 3.7), 318 (log ε 3.7).

6-Fluoro-1-(β-D-glucopyranosyl)camalexin (5c).
$R_t = 7.4$ min (HPLC); $[\alpha]_D = -5.0$ (*c* 0.09, MeOH); ^{1}H NMR in Table 1; ^{13}C NMR in Table 2; HRMS-FAB *m/z*: measured 381.0921 ($[M+H]^+$, calcd 381.0920 for

$C_{17}H_{17}N_2FSO_5$); MS-FAB *m/z* (relative intensity): 381 (90), 329 (25), 218 (38), 176 (100), 149 (33); FTIR (cm^{-1}) 3342, 2924, 2852, 1553, 1499, 1459, 1127, 1083, 1022, 960, 637. UV (λ_{max}, CH_3CN) 221 (log ε 3.8), 315 (log ε 3.9).

6-Fluoro-1-methyl-7-(*O*-β-D-glucopyranosyl) camalexin (5d). $R_t = 7.2$ min (HPLC); $[\alpha]_D = 12.4$ (*c* 0.06, MeOH); ^{1}H NMR in Table 1; ^{13}C NMR in Table 2; HRMS-FAB *m/z*: measured 411.1028 ($[M+H]^+$ calcd 411.1026 for $C_{18}H_{20}N_2FSO_6$); MS-FAB *m/z* (relative intensity): 411 (10), 329 (22), 176 (100); FTIR (cm^{-1}) 3331, 2924, 1629, 1466, 1381, 1074; UV (λ_{max}, CH_3CN) 218 (log ε 2.9), 326 (log ε 3.3).

6-Fluoro-1-methylindole-3-carbonitrile (6). $R_t = 15.9$ min (HPLC); ^{1}H NMR in Table 1; ^{13}C NMR in Table 2; HRMS-EI *m/z*: measured 174.0596 (M^+, calcd 174.0593 for $C_{10}H_7N_2F$); MS-EI *m/z* (relative intensity): 174 (100), 173 (38); FTIR (cm^{-1}) 2922, 2219, 1739, 1463, 1246, 1110; UV (λ_{max}, CH_3CN) 215 (log ε 4.0), 283 (log ε 3.4).

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