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# Methoxycamalexins and related compounds: Syntheses, antifungal activity and inhibition of brassinin oxidase

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ABSTRACT

The phytoalexin camalexin is a competitive inhibitor of brassinin oxidase, an enzyme that detoxifies the phytoalexin brassinin and is produced by an economically important plant pathogen. For this reason, the camalexin scaffold has guided the design of inhibitors of brassinin detoxification. To further understand the structure–activity relationships of camalexin related compounds, the syntheses of monomethoxy and dimethoxycamalexins were undertaken. Four monomethoxy camalexins together with 4,6-dimethoxy and 5,7-dimethoxy camalexins were prepared from the corresponding methoxyindoles using the Ayer's method. The dimethoxy derivatives were prepared from the corresponding dimethoxyindole-3-thiocarboxamides using the Hantzsch reaction; however, this method did not work for the syntheses of 4,6-dimethoxy and 5,7-dimethoxycamalexins due to the lower reactivities of the corresponding indole-3-thiocarboxamides. The antifungal activity and brassinin oxidase inhibitory activity of all methoxycamalexins and ten camalexin related compounds were investigated. Among the 20 compounds evaluated, monomethoxycamalexins were stronger antifungals than the dimethoxy derivatives. However, remarkably, 5,6-dimethoxycamalexin, 6,7-dimethoxycamalexin and 5-methoxycamalexin displayed the strongest inhibitory activity against brassinin oxidase, while 4,5-dimethoxycamalexin displayed no inhibitory effect. Altogether the structure–activity relationships of camalexin related compounds suggest that the targets for fungal growth inhibition and brassinin oxidase inhibition are unrelated and emphasize that brassinin oxidase inhibitors do not need to be antifungal.

## 1. Introduction

Twenty-seven years after its discovery,[1] camalexin (**1a**) continues to be the most popular cruciferous phytoalexin[2,3] with more than 360 articles retrieved through the SciFinder database (accessed June 2018 using the keyword camalexin). Crucifer plants (family Brassicaceae) comprise a broad range of economically important species, from worldwide cultivated crops such as the oilseeds canola and rapeseed to vegetables like cauliflower and cabbage, or condiments such as wasabi and mustard. Notwithstanding its unique chemical structure, 3-(2-thiazolyl)-1*H*-indole, the fascination with camalexin (**1a**) is mostly due to the popularity of one of the producing plant species, the widely investigated model species *Arabidopsis thaliana* (L.) Heynh. Both camalexin (**1a**) and 6-methoxycamalexin (**1e**) were first isolated from the crucifer wild species *Camelina sativa* (L.) and *Capsella bursa-pastoris* (L.). together with *N*-methylcamalexin (**1b**) (Fig. 1).[1,2] Consistent with the critical role that phytoalexins play in disease resistance mechanisms,[2,3] it has been shown in several instances that the disease resistance of *A. thaliana*[4] to various fungal pathogens depends on the production of camalexin (**1a**).[5–7] Phytoalexins are plant defense metabolites biosynthesized *de novo* under stress conditions to protect plants against microbial pathogens.[8]

Importantly, camalexin (**1a**) is resistant to degradation by specific plant fungal pathogens[3] and is one of the most potent phytoalexins produced by crucifers. Phytoalexins that resist degradation by plant pathogens provide the producing plants with higher disease resistance levels. For example, the resistance and susceptibility of *A. thaliana* to the phytopathogenic fungi *Alternaria brassicicola* ((Schwein.) Wiltshire) and *Botrytis cinerea* (Pers. Fr., teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel), respectively, correlate with the rates of metabolism of camalexin (**1a**) by each pathogen (6–8 days vs. 12 h, respectively).[9] That is, the transformation and detoxification of phytoalexins by plant pathogens makes plants more susceptible to infection. To prevent the detrimental degradation of phytoalexins by fungal pathogens, paldoxins (**p**hyto**al**exin **d**et**ox**ifying **in**hibitors) were proposed for crop protectants. Such chemicals are designed to inhibit phytoalexin detoxifying enzymes and thus are selective in that their mechanism of action will only affect some fungal species.[10,11]

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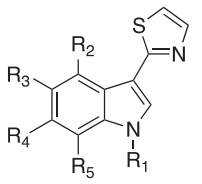



**1b** $R_1$=Me; $R_2$=$R_3$=$R_4$=$R_5$=H
**1c** $R_2$= OMe; $R_1$=$R_3$=$R_4$=$R_5$=H
**1d** $R_3$= OMe; $R_1$=$R_2$=$R_4$=$R_5$=H
**1e** $R_4$= OMe; $R_1$=$R_2$=$R_3$=$R_5$=H
**1f** $R_5$= OMe; $R_1$=$R_2$=$R_3$=$R_4$=H
**1g** $R_2$=$R_3$=OMe; $R_1$=$R_4$=$R_5$=H
**1h** $R_2$=$R_4$=OMe; $R_1$=$R_3$=$R_5$=H
**1i** $R_2$=$R_5$=OMe; $R_1$=$R_3$=$R_4$=H
**1j** $R_3$=$R_4$=OMe; $R_1$=$R_2$=$R_5$=H
**1k** $R_3$=$R_5$=OMe; $R_1$=$R_2$=$R_4$=H
**1l** $R_4$=$R_5$=OMe; $R_1$=$R_2$=$R_3$=H

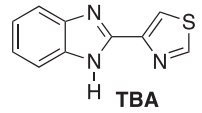



**Fig. 1.** Structures of camalexin (**1a**), 1-methylcamalexin (**1b**), methoxycamalexins **1c–1l** and commercial fungicide thiabendazole (**TBA**).

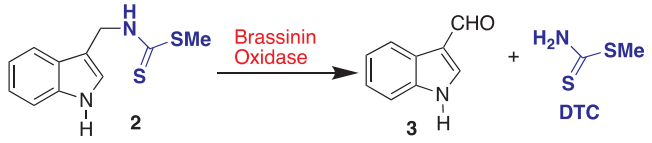



**Fig. 2.** Detoxification of the phytoalexin brassinin (**2**) to indole-3-carboxaldehyde (**3**) and **DTC**[13] by brassinin oxidase (BO) in the phytopathogenic fungus *Leptosphaeria maculans*.

Brassinin oxidase (BO), produced by the plant pathogen *Leptosphaeria maculans* [(Desm.) Ces. et Not., asexual stage *Phoma lingam* (Tode ex Fr.) Desm.)], is an inducible enzyme that degrades the phytoalexin brassinin (**2**) to the non-toxic metabolite indole-3-carboxaldehyde (**3**) and *S*-methyl thiocarbamate (DTC, Fig. 2).[12,13] Because camalexin (**1a**) is a competitive inhibitor of BO (i.e. inhibits brassinin detoxification)[12] and is not transformed by *L. maculans*, the camalexin scaffold has been employed to design paldoxins for crop protection against *L. maculans*.[3,14,15]

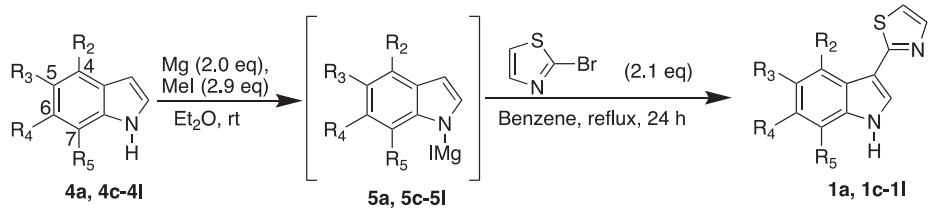
Subsequently, it was discovered that the inhibitory effect of camalexins on BO activity was influenced by the substituents on the benzene moiety and indolyl nitrogen atom.[14] For example, 5-methoxycamalexin (**1d**) was determined to be the best BO inhibitor (ca. 72% at 300 μM), followed by 6-methoxycamalexin (**1e**, ca. 63% at 300 μM) that was better than camalexin (**1a**, 53%, 300 μM). By contrast, *N*-methylcamalexin (**1b**) showed no inhibitory effect on BO activity. Furthermore, camalexins **1a**, **1d** (5-methoxy) and **1e** (6-methoxy) displayed similar antifungal activity against *L. maculans* causing 100% mycelial growth inhibition at 0.50 mM, whereas the commercial fungicide thiabendazole (**TBA**) was a much stronger growth inhibitor (100% inhibition at 0.10 mM), but a much weaker inhibitor of BO activity (25%, 300 μM). To further understand the functional structure-activity relationships of camalexins, it was of great interest to establish and compare the antifungal and BO inhibitory activities of the monomethoxy (4-, 5-, 6-, 7-) and dimethoxy (4,5-, 4,6-, 4,7-, 5,6-, 5,7-, 6,7-) derivatives of camalexin (**1c–1l**). During this work, due to the different reactivities of the starting materials, it became apparent that different methodologies had to be employed to synthesize the desired methoxycamalexins. The syntheses and reactivity of methoxyindoles **4c–4l** and dimethoxyindole-3-thiocarboxamides **8g–8l**, starting materials that provided the desired methoxycamalexins, the structure-activity correlations among methoxycamalexins **1c–1l**, camalexin derivatives **9a–9d**[9] and thiazolyl isomers **10–13**,[9] as well as a more economical protocol to determine BO activities are described in this report.

## 2. Results and discussion

### 2.1. Chemical synthesis of methoxycamalexins

A few methods have been reported to prepare camalexin (**1a**)[16] and derivatives, with the simplest and shortest route being reported first by Ayer et al. in 1992.[17] This one-pot preparation involved the reaction of two equivalents of indolyl magnesium iodide with one equivalent of 2-bromothiazole in benzene heated at reflux. Although it was then indicated that the ratio of 2:1 of indolyl-MgI/bromothiazol was important

**Table 1**
Preparation of camalexins **1a**, **1c–1f**, **1h**, **1k** and **1l** using Ayer's method.[17]




**a** $R_2$=$R_3$=$R_4$=$R_5$=H;
**c** $R_2$= OMe; $R_3$=$R_4$=$R_5$=H;
**d** $R_3$= OMe; $R_2$=$R_4$=$R_5$=H;
**e** $R_4$= OMe; $R_2$=$R_3$=$R_5$=H;
**f** $R_5$= OMe; $R_2$=$R_3$=$R_4$=H;
**g** $R_2$=$R_3$=OMe; $R_4$=$R_5$=H;
**h** $R_2$=$R_4$=OMe; $R_3$=$R_5$=H;
**i** $R_2$=$R_5$=OMe; $R_3$=$R_4$=H;
**j** $R_3$=$R_4$=OMe; $R_2$=$R_5$=H;
**k** $R_3$=$R_5$=OMe; $R_2$=$R_4$=H;
**l** $R_4$=$R_5$=OMe; $R_2$=$R_3$=H

| Starting material | Recovered starting material (amount) | Product – % yield (isolated amount) | Side products (isolated amount) |
|---|---|---|---|
| **4a** | None | **1a**, 81% (55 mg) | None |
| **4c** (4-OMe) | 25% (10 mg) | **1c**, 43% (27 mg) | 3 mg |
| **4d** (5-OMe) | 13% (5 mg) | **1d**, 65% (41 mg) | 8 mg |
| **4e** (6-OMe) | 30% (11 mg) | **1e**, 65% (41 mg) | None |
| **4f** (7-OMe) | 37% (15 mg) | **1f**, 58% (36 mg) | 5 mg |
| **4g** (4,5-dimethoxy) | 50% (20 mg) | None | 30 mg |
| **4h** (4,6-dimethoxy) | None | **1h**, 50% (29 mg) | 8 mg |
| **4i** (4,7-dimethoxy) | 100% | No reaction | None |
| **4j** (5,6-dimethoxy) | 100% | No reaction | None |
| **4k** (5,7-dimethoxy) | None | **1k**, 37% (22 mg) | 20 mg |
| **4l** (6,7-dimethoxy) | 35% (9 mg) | **1l**, traces | 47 mg |