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Design, synthesis, and antifungal activity of inhibitors of brassilexin detoxification in the plant pathogenic fungus *Leptosphaeria maculans*

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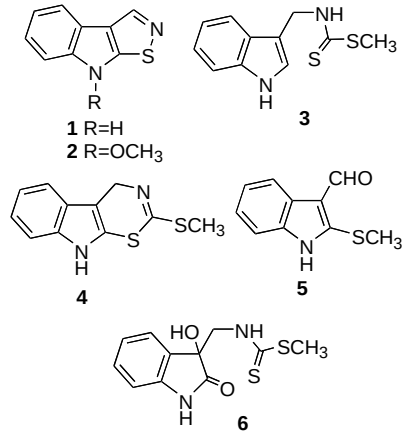
Abstract—Potential inhibitors of *Leptosphaeria maculans* mediated detoxification of the phytoalexin brassilexin were designed and synthesized based on the planar heteroaromatic structure of isothiazolo[5,4-*b*]indole. Screening of these compounds for inhibition of brassilexin detoxification in cultures of *L. maculans* indicated that 4-(2-chlorophenyl)isothiazole had the largest effect on the rate of brassilexin detoxification. However, the most antifungal compound among the potential inhibitors, isothiazolo[5,4-*b*]quinoline, did not appear to affect the metabolism of brassilexin noticeably, suggesting that growth inhibition is not sufficient to slow down the rate of brassilexin detoxification. Furthermore, it was determined that 4-arylisothiazoles as well as isothiazolo[5,4-*b*]thianaphthene displayed antifungal activity against *L. maculans*.

1. Introduction

The detoxification of phytoalexins by fungal plant pathogens is of great concern as it can have a substantially negative impact on the overall plant fitness.[1] Phytoalexins are secondary metabolites produced de novo by plants in response to diverse forms of stress, including fungal infection. Brassilexin (**1**) is one of the most potent antifungal phytoalexins produced by crucifer plants, namely those of the genus *Brassica*.[2,3] The fungal pathogen *Leptosphaeria maculans* [(Desm.) Ces. et de Not., asexual stage *Phoma lingam* (Tode ex Fr.) Desm.] is able to overcome by detoxification some important defenses of brassicas, including brassilexin (**1**),[4] sinalexin (**2**)[4], brassinin (**3**), cyclobrassinin (**4**), brassicanal A (**5**), and dioxibrassinin (**6**).[1] Nonetheless, the strong in vitro growth inhibitory activity of brassilexin (**1**) against *L. maculans* suggests that an increase of its concentration in the plant, through, for example, inhibition of the detoxification pathway, could slow down or prevent the spread of the pathogen. This prospect suggests that potential inhibitors of brassilexin detoxification could be used to protect the plant against the fungal invader. Toward this end, a recent investigation of the metabolism of brassilexin (**1**) in *L. maculans* demonstrated that the first step of its detoxification involved reduction of the isothiazole ring to 3-aminomethyleneindole-2-thione (**7**), followed by hydrolytic and oxidative reactions to yield 3-formylindolyl-2-sulfonic acid (**9**) via aldehyde **8** (Scheme 1). Because the enamine **7** showed a substantially lower antifungal activity than brassilexin (**1**), these findings suggested that potentially effective inhibitors or modulators of brassilexin detoxification must slow down or stop the reductive bioconversion of brassilexin (**1**) into 3-aminomethyleneindole-2-thione (**7**).



Keywords: Brassilexin; Detoxification inhibitor; *Leptosphaeria maculans*; *Phoma lingam*; Phytoalexin.
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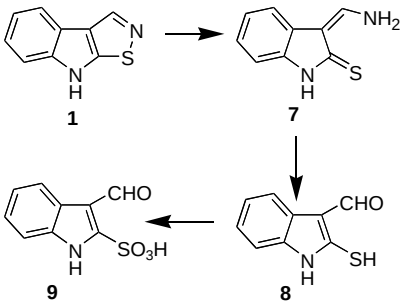


Scheme 1. Metabolism of brassilexin (**1**) in *Leptosphaeria maculans* (*Phoma lingam*).

Considering the chemical structures of brassilexin (**1**, isothiazolo[5,4-*b*]indole) and its first detoxification intermediate, 3-aminomethyleneindole-2-thione (**7**), it was of interest to evaluate the role of the indole–isothiazole and isothiazole ring systems on the antifungal activity and metabolism of **1**. Although previous studies with *L. maculans* indicate that the indole ring is neither metabolized nor essential for antifungal activity,[1] the indole–isothiazole ring system has not been examined. Non-degradable isothiazoles might be effective inhibitors of brassilexin detoxifying enzyme(s) produced in *L. maculans*. Hence, the indole–isothiazole fused-ring system of **1** was replaced with quinoline–isothiazole (**10**), benzothiophene–isothiazole (**11**), indole–thiophene (**12**), and benzo-isothiazole (**13**) to yield compounds **10**–**13**, respectively (Fig. 1). In addition, isothiazoles resulting from disconnecting the 'a' bond of the indole ring and replacing the NH group *ortho* to the isothiazole with various substituents (H, Cl, CH_3, NH_2, NO_2, OH, and OCH_3) were designed (Fig. 1). The resulting 2-substituted-4-phenylisothiazoles **14**–**20**, naphthylisothiazoles **21** and **22** were synthesized and their effects on the detoxification of brassilexin were examined. Here, we describe results of these studies and show that 4-(2-chlorophenyl)isothiazole (**15**) has the largest effect on the rate of brassilexin detoxification. However, the most antifungal compound among the potential inhibitors, isothiazolo[5,4-*b*]quinoline (**10**), did not appear to affect the metabolism of brassilexin noticeably. It is worth noting that the isothiazole ring is a component of a great variety of compounds with pharmacological activity, including antibacterial, antiviral, and insecticidal, but no antifungal activity appears to have been described to date.[5]

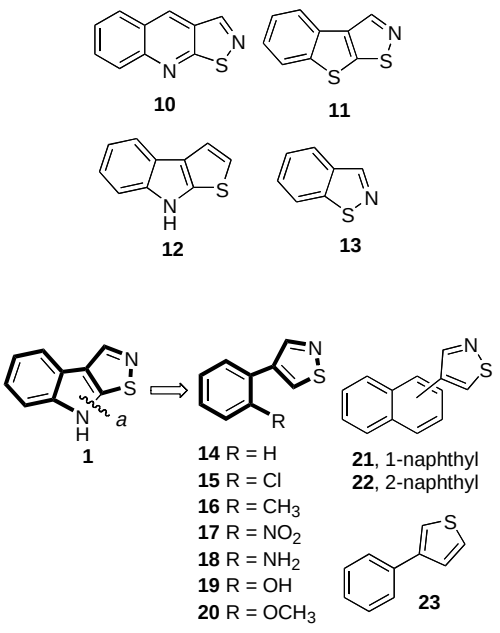


Figure 1. Chemical structures of potential inhibitors of brassilexin (**1**) detoxification.

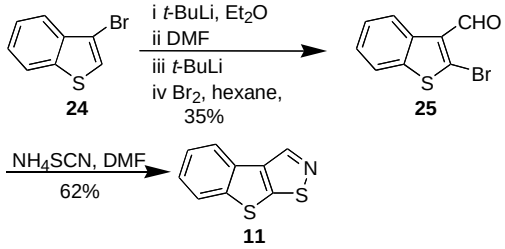
2. Results

2.1. Syntheses

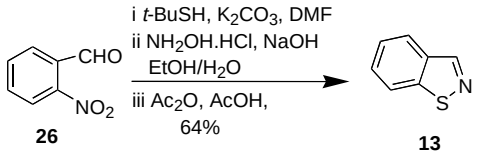
Compounds **10**[6,7] and **12**[8] were prepared similar to previously described procedures, and 3-phenylthiophene (**23**) was commercially available. New conditions for the syntheses of compounds **11** and **13**–**20** are reported, and the syntheses of compounds **21** and **22** are described here for the first time.

Isothiazolo[5,4-*b*]thianaphthene (**11**) was previously prepared from 2-methylsulfanylthianaphthene-3-carboxaldehyde oxime by heating in polyphosphoric acid (PPA).[9] Since the yield was low (18%), a new synthesis of **11** was developed using less harsh conditions. 3-Bromothianaphthene (**24**) was treated with *t*-BuLi and the corresponding 3-lithiothianaphthene was quenched with DMF, followed by addition of another equivalent of *t*-BuLi. A solution of bromine in hexane was added to the reaction mixture followed by quenching with HCl to afford 2-bromothianaphthene-3-carboxaldehyde (**25**) in 35% yield, based on 3-bromothianaphthene (**24**). Heating **25** with ammonium thiocyanate[10] in DMF afforded compound **11** in 62% yield (Scheme 2).[11]

1,2-Benzoisothiazole (**13**) was prepared by cyclization of 2-*t*-butylsulfanylbenzaldehyde oxime, obtained from 2-nitrobenzaldehyde (**26**),[12] using acetic acid anhydride in acetic acid to promote ring closure (Scheme 3). PPA[13] was previously used, however, we found that isolation of **13** becomes rather difficult under these conditions (repeated column chromatography), affording a lower yield than that obtained when the reaction is carried out using acetic acid anhydride in acetic acid.

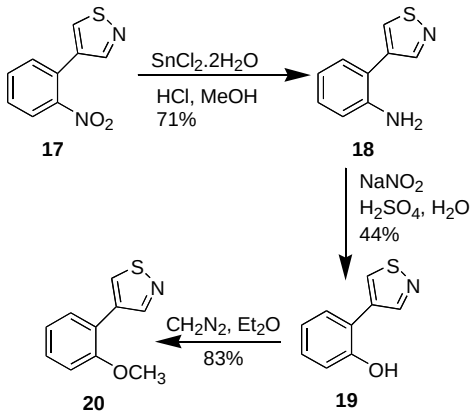


Scheme 2. Synthesis of isothiazolo[5,4-*b*]thianaphthene (**11**).



Scheme 3. Synthesis of 1,2-benzoisothiazole (**13**).

The syntheses of 4-arylisothiazoles **14**–**17**, **21**, and **22**, followed a modified multi-step synthesis,[14] employing aryl substituted acetic acids **27**–**32** as starting materials. Thus, aryl-acetic acids **27**–**32** underwent double Vilsmeier–Haack reaction, followed by basic hydrolysis in boiling sodium hydroxide/ethanol/water[15] and treatment with thionyl chloride in dichloromethane to yield chloroacroleins **33**–**38** (Scheme 4). Subsequent heating of the chloroacroleins **33**–**38** with ammonium thiocyanate in DMF[10] afforded 4-arylisothiazoles **14**–**17**, **21**, and **22** in low to moderate yields (25–54%, Scheme 4). When 2-nitrophenylacetic acid (**30**) was used, only very low yields (less than 5%) of chloroacrolein **36** were obtained. The Vilsmeyer–Haack reaction appeared to be problematic as black tar was formed even at room temperature. The problem was solved using commercially available 2-nitrophenyl malondialdehyde which yielded, upon reaction with thionyl chloride, chloroacrolein **36** in 97% yield. 4-(2-Aminophenyl)isothiazole (**18**, Scheme 5) was prepared from **17** using $SnCl_2{\cdot}2H_2O$ in acidic methanol[16] in 71% yield. Diazotization of **18** and subsequent hydrolysis[16] afforded 4-(2-hydroxyphenyl)isothiazole (**19**, Scheme 5) in 44% yield based on **17**. Methylation of **19** using ethereal diazomethane afforded 4-(2-methoxyphenyl)isothiazole (**20**, Scheme 5) in 83% yield.



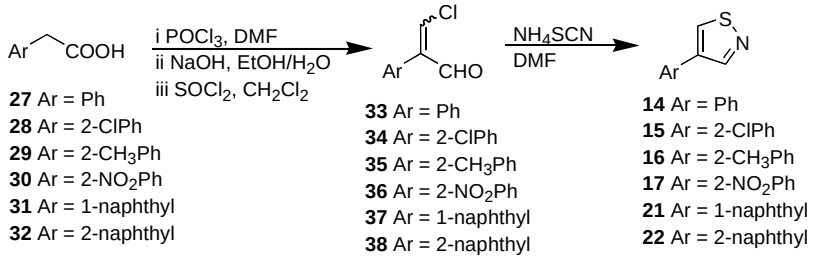
Scheme 5. Synthesis of compounds **18**–**20**.

2.2. Antifungal bioassays

The antifungal activity of compounds **10**–**23** against *L. maculans* was evaluated using a mycelial radial growth assay, as described in the Experimental section. Results of these assays (Table 1) showed that isothiazolo[5,4-*b*]thianaphthene (**11**) inhibited completely the mycelial growth of *L. maculans* at 5.0×10^{-4} M, whereas isothiazolo[5,4-*b*]quinoline (**10**) showed complete inhibition of mycelial growth even at 2.0×10^{-4} M. Antifungal activity (40–100%, 5.0×10^{-4} M) was observed with 4-arylisothiazoles **14**–**21**. The most antifungal compound among the tested 4-arylisothiazoles appeared to be 4-(2-hydroxyphenyl)isothiazole (**19**), where complete inhibition of mycelial growth was observed at 5.0×10^{-4} M. 1,2-Benzoisothiazole (**13**) exhibited weak antifungal activity (22%, 5.0×10^{-4} M). Compounds **12**, **22**, and **23** were not sufficiently soluble in potato dextrose media to reach the concentration of 5.0×10^{-4} M, however using the concentration 2.0×10^{-4} M, moderate **12** (52%), **22** (53%) to weak **23** (27%) antifungal activity was observed. This is the first time that isothiazolo[5,4-*b*]thianaphthene (**11**), isothiazolo[5,4-*b*]quinoline (**10**), and 4-arylisothiazoles **14**–**21** are shown to be antifungal.

2.3. Screening of potential inhibitors

Compounds **10**–**23** were screened for potential inhibition of the transformation of brassilexin (**1**) to 3-aminomethyleneindole-2-thione (**7**) in cultures of *L. maculans* over a 96 h period. The potential inhibitors (at 1.0×10^{-4} and 2.0×10^{-4} M) were added to shake cultures of *L. maculans* (48-h-old mycelia in minimal media), cultures were incubated for 10 min (to allow adsorption/transport of compounds into cells), and brassilexin (**1**, 1.0×10^{-4} M) was then added to cultures followed by additional incubation. Control cultures (48-h-old mycelia in minimal media) prepared similarly but containing only brassilexin (**1**) were incubated in parallel. The stability of brassilexin and compounds **10**–**23** was determined by incubation in minimal media under similar conditions. Samples were withdrawn from cultures immediately after addition of brassilexin and at 24, 48, and 96 h, were extracted and the extracts were analyzed by HPLC (photodiode array detector), to determine the concentration of brassilexin (**1**) remaining in the cultures at different times. Brassilexin (**1**) was stable in minimal medium for at least 10 days; compounds **10**–**13**, and **18**–**20** were stable in minimal media but **14**–



Scheme 4. Synthesis of 4-arylisothiazoles **14**–**17**, **21**, and **22**.

Table 1. Inhibitory activity (%)[a] of compounds **10**–**23** against *Leptosphaeria maculans* (procedure is described in Section 4.3)

Compound	1.0×10^{-4} M	2.0×10^{-4} M	5.0×10^{-4} M
Brassilexin (**1**)	8 ± 1	62 ± 1	C.I.[b]
Isothiazolo[5,4-*b*]quinoline (**10**)	60 ± 13	C.I.[b]	C.I.[b]
Isothiazolo[5,4-*b*]thianaphthene (**11**)	49 ± 7	60 ± 5	C.I.[b]
Thieno[2,3-*b*]indole (**12**)	33 ± 8	52 ± 12	N.S.[c]
1,2-Benzoisothiazole (**13**)	N.I.[d]	14 ± 2	22 ± 7
4-Phenylisothiazole (**14**)	32 ± 4	46 ± 6	69 ± 9
4-(2-Chlorophenyl)isothiazole (**15**)	42 ± 5	56 ± 7	79 ± 4
4-(2-Tolyl)isothiazole (**16**)	31 ± 6	51 ± 9	65 ± 8
4-(2-Nitrophenyl)isothiazole (**17**)	35 ± 5	44 ± 6	67 ± 10
4-(2-Aminophenyl)isothiazole (**18**)	18 ± 5	25 ± 6	40 ± 3
4-(2-Hydroxyphenyl)isothiazole (**19**)	38 ± 5	44 ± 3	C.I.[b]
4-(2-Methoxyphenyl)isothiazole (**20**)	46 ± 7	59 ± 5	82 ± 3
4-(1-Naphthyl)isothiazole (**21**)	37 ± 4	60 ± 8	73 ± 4
4-(2-Naphthyl)isothiazole (**22**)	41 ± 7	53 ± 4	N.S.[c]
3-Phenylthiophene (**23**)	N.I.[d]	27 ± 5	N.S.[c]

[a] % inhibition = 100 − [(growth on treated/growth on control) × 100)] ± SD; results are the means of at least three separate experiments.
[b] C.I. = complete inhibition.
[c] N.S. = not soluble.
[d] N.I. = no inhibition.

16 decomposed slowly (ca. 50% or more remaining in solution after 96 h) to undetermined products; 3-phenylthiophene (**23**) decomposed completely in 24 h, thus was not used in further metabolism experiments. As shown in Table 2, a few of the test compounds slowed down the transformation of brassilexin (**1**) relative to control cultures. For example, in the case of 4-arylisothiazoles **15** and **20** (2.0×10^{-4} M) 22% and 31%, respectively, of brassilexin remained in the cultures after 48 h of incubation, whereas almost complete transformation of brassilexin (**1**) was observed in 24 h (<5% remaining) in control cultures (containing only brassilexin). Furthermore, brassilexin was present in the cultures incubated with compounds **11**, **15**, and **20** (2.0×10^{-4} M) even after 96 h of incubation. However, a few of these compounds were metabolized by the fungus (Table 2), slowly in the case of isothiazolo[5,4-*b*]thianaphthene (**11**, 22% remaining after 96 h) and arylisothiazole **17** (33% remaining after 96 h), and faster in the case of 4-(2-hydroxyphenyl)isothiazole (**19**), which was metabolized completely in 48 h (no products of metabolism could be detected). Because 4-naphthylisothiazoles **21** and **22** were not sufficiently soluble in minimal media their stability in fungal culture or in minimal media could not be determined at the highest concentration.

Because compounds **11** and **15**–**20** appeared to have a greater effect of on the rate of detoxification of brassilexin (**1**) to enamine **7** in cultures of *L. maculans* (Table 2), additional time–course analyses were carried out. Thus, brassilexin (**1**) was added to cultures after 30 min of incubation with inhibitors **11** and **15**–**20** at 1.0×10^{-4} M and 3.0×10^{-4} M (instead of 10 min, as reported in the first study to allow additional time for inter/intracellular transport) and samples were withdrawn at 3-h intervals. Extraction of the culture samples and HPLC analyses of the extracts allowed the determination of the amounts of brassilexin (**1**) remaining in the cultures at different times. Progress curves were constructed depicting the decrease of brassilexin (**1**) concentration in cultures incubated with compounds **11** and **15**–**20** plus brassilexin (**1**) and cultures incubated with brassilexin (**1**) only (Fig. 2 and Figs. 3–7 in Supplementary data). 4-(2-Chlorophenyl)isothiazole (**15**) was the most active inhibitor of brassilexin detoxification among the tested compounds (Fig. 2). When **15** was present at 3.0×10^{-4} M, 48% of brassilexin (**1**) remained in culture after three hours of incubation, whereas with **15** at 10^{-4} M, 34% of brassilexin remained in culture, compared to 24% in control flasks containing only brassilexin (**1**). The remaining progress curves indicated that 4-(2-nitrophenyl)isothiazole (**17**) was the next best inhibitor followed by isothiazolo[5,4-*b*]thianaphthene (**11**) (Fig. 3 in Supplementary data). For example, when **17** was present at 3.0×10^{-4} M, 40% of brassilexin (**1**) remained in culture after 3 h of incubation, whereas with **17** at 1.0×10^{-4} M, 28% of brassilexin remained in culture, compared to 24% in control flasks containing only brassilexin (**1**). The remaining compounds **16**, and **18**–**20** did not affect substantially the detoxification of brassilexin over a 12-h period relative to control cultures containing only brassilexin (**1**) (Figs. 3–7 in Supplementary data).

3. Discussion and conclusion

Potential inhibitors of *L. maculans* mediated transformation of brassilexin were designed and synthesized based on the planar heteroaromatic structure of brassilexin (**1**). It was determined that 4-arylisothiazoles **15**–**20** as well as isothiazolo[5,4-*b*]thianaphthene (**11**) displayed antifungal activity against *L. maculans* (Table 1). Quinoline **10** and thianaphthene **11**, both containing an isothiazole moiety, displayed the strongest growth inhibitory activity, whereas 3-phenylthiophene (**23**) was the weakest inhibitor. Although 4-phenylisothiazole (**14**) caused substantial growth inhibition, two of the weakest inhibitors contained an isothiazole moiety as well, suggesting that additional structural features other than the isothiazole ring are necessary to inhibit the growth of *L. maculans*. Furthermore, the 2-substituent

Table 2. Concentrations of brassilexin (**1**) remaining in cultures of *Leptosphaeria maculans* incubated with compounds **10**–**22** over a 96-h period (procedure is described in Sections 4.4)

Compound	Brassilexin (**1**) remaining in the cultures (molar %)[a] incubated with compounds at	
	1.0×10^{-4} M	2.0×10^{-4} M
Control	24 h, <5%	—
Isothiazolo[5,4-*b*]quinoline (**10**)	24 h, <5% 48 h, N.D.[b]	24 h, <5% 48 h, N.D.[b]
Isothiazolo[5,4-*b*]thianaphthene (**11**)[c]	24 h, 16 ± 3% 48 h, 19 ± 1% 96 h, 8 ± 5%	24 h, 23 ± 2% 48 h, 27 ± 1% 96 h, 19 ± 1%
Thieno[2,3-*b*]indole (**12**)	24 h, <5% 48 h, N.D.[b]	24 h, <5% 48 h, N.D.[b]
1,2-Benzoisothiazole (**13**)	24 h, <5% 48 h, N.D.[b]	24 h, <5% 48 h, N.D.[b]
4-Phenylisothiazole (**14**)[c]	24 h, <5% 48 h, N.D.[b]	24 h, <5% 48 h, N.D.[b]
4-(2-Chlorophenyl)isothiazole (**15**)[d]	24 h, 23 ± 1% 48 h, 23 ± 1% 96 h, N.D.[b]	24 h, 23 ± 5% 48 h, 22 ± 5% 96 h, 12 ± 10%
4-(2-Tolyl)isothiazole (**16**)[c]	24 h, 11 ± 2% 48 h, < 5%% 96 h, N.D.[b]	24 h, 17 ± 2% 48 h, 9 ± 1% 96 h, N.D.[b]
4-(2-Nitrophenyl)isothiazole (**17**)[c]	24 h, 11 ± 3% 48 h, < 5% 96 h, N.D.[b]	24 h, 17 ± 2% 48 h, 9 ± 1% 96 h, N.D.[b]
4-(2-Aminophenyl)isothiazole (**18**)	24 h, 6 ± 1% 48 h, N.D.[b]	24 h, 8 ± 1% 48 h, N.D.[b]
4-(2-Hydroxyphenyl)isothiazole (**19**)[e]	24 h, 8 ± 2% 48 h, N.D.[b] 96 h, N.D.[b]	24 h, 13 ± 1% 48 h, < 5% 96 h, N.D.[b]
4-(2-Methoxyphenyl)isothiazole (**20**)	24 h, 20 ± 1% 48 h, < 5% 96 h, N.D.[b]	24 h, 28 ± 1% 48 h, 31 ± 3% 96 h, 20 ± 8%
4-(1-Naphthyl)isothiazole (**21**)	24 h, 31 ± 8% 48 h, < 5% 96 h, N.D.[b]	24 h, 32 ± 5% 48 h, < 5% 96 h, N.D.[b]
4-(2-Naphthyl)isothiazole (**22**)	Not soluble	Not soluble

[a] Percentages were determined using a calibration curve and are averages of at least two independent experiments conducted in duplicate ±standard deviation.
[b] N.D. = not detected.
[c] Compound is metabolized by the fungus (20–40% remaining after 96 h of incubation).
[d] Compound is metabolized by the fungus in 96 h.
[e] Compound is metabolized completely by the fungus after 48 h of incubation.

of the phenyl group of compounds **14**–**20** appeared to affect substantially the inhibitory activity, as shown for compounds **15**–**20** (strong inhibitors) and **14** (weaker inhibitor). 4-Arylisothiazoles were shown previously to exhibit a wide range of biological activities,[5] however, to the best of our knowledge, no reports on the antifungal activity of compounds containing a 4-arylisothiazole moiety have been published so far. This is the first time that the antifungal activity of 4-arylisothiazoles and isothiazolo[5,4-*b*]thianaphthene (**11**) has been examined. Thus, these lead structures could guide the search for new antifungal compounds useful for both agrochemical and pharmaceutical industries.

Screening of compounds **10**–**22** for inhibition of brassilexin detoxification in cultures of *L. maculans* indicated that isothiazolo[5,4-*b*]thianaphthene (**11**) and 4-arylisothiazoles **15**–**20** slowed down the metabolism of brassilexin (**1**). Further screening suggested that compound **15** had the largest effect on the rate of brassilexin metabolism among the tested compounds (Fig. 2 and Figs. 3–7 in Supplementary data). That is, **15**, although slowly metabolized by the fungus, appeared to inhibit the putative brassilexin reductase responsible for the detoxification of brassilexin (**1**) to 3-aminomethyleneindole-2-thione (**7**, Scheme 1). Because the most antifungal compound among the potential inhibitors

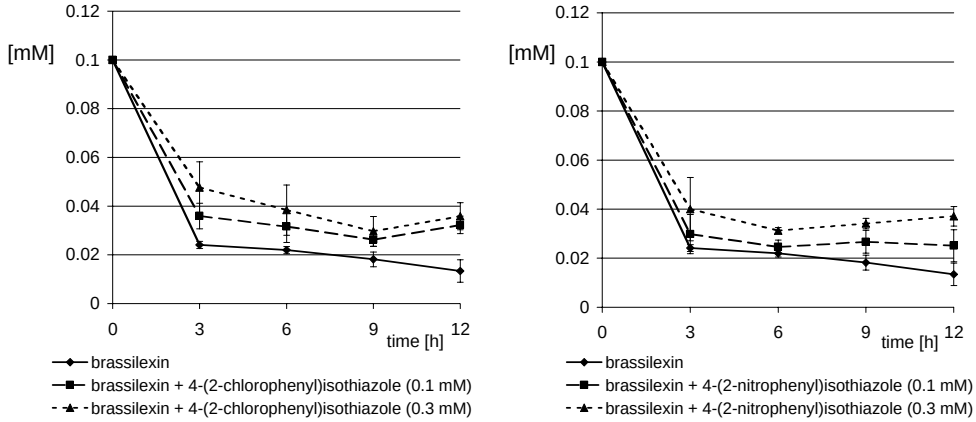


Figure 2. Metabolism of brassilexin (1.0×10^{-4} M) in the presence of 4-(2-chlorophenyl)isothiazole (**15**) and 4-(2-nitrophenyl)isothiazole (**17**). All points are averages of three independent experiments ± standard deviation.

(isothiazolo[5,4-*b*]quinoline (**10**)), did not affect the metabolism of brassilexin noticeably (Table 2), we suggest that the inhibitory effect of **15** on brassilexin detoxification is likely due to its direct interaction with the detoxification enzyme(s). Hence, it is concluded that the isothiazole ring may be useful as a lead structure to further improve the design of this type of inhibitors. Nonetheless, only co-incubation of the potential inhibitors and brassilexin (**1**) with the putative brassilexin reductase, which still remains to be detected and isolated, could confirm this hypothesis. It is expected that isolation of the putative brassilexin reductase involved in this detoxification reaction will assist the biorational design of crop protection agents selective against *L. maculans*.

4. Experimental

4.1. General experimental procedures

All chemicals were purchased from Sigma–Aldrich Canada Ltd., Oakville, ON. All solvents were HPLC grade and used as such, except for CH_2Cl_2 and $CHCl_3$ that were redistilled. Organic extracts were dried over anhydrous Na_2SO_4 and solvents removed under reduced pressure in a rotary evaporator.

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–100% CH_3CN, for 35 min, linear gradient, and a flow rate 1.0 mL/min. UV spectra were recorded on Varian-Cary spectrophotometer in MeOH. Fourier transform IR spectra were obtained on a Bio-Rad FTS-40 spectrometer in KBr. NMR spectra were recorded on Bruker Avance 500 spectrometers; δ values were referenced as follows: for ^1H (500 MHz), $CDCl_3$, 7.27 ppm; for ^{13}C (125 MHz), $CDCl_3$, 77.23 ppm. Mass spectra (MS) were obtained on a VG 70 SE mass spectrometer using a solids probe or on a Q Star XL, Applied Biosystems; fragments with relative intensity lower than 10% are not reported.

4.2. Fungal cultures

Fungal cultures of *L. maculans*/*P. lingam* virulent isolate BJ 125 were obtained from the IBCN collection, Agriculture and Agri-Food Canada Research Station, Saskatoon SK. Cultures were handled as described previously.[17]

4.3. Antifungal bioassays

Antifungal bioassays against *L. maculans* were carried out as follows: a DMSO solution of the compound to be tested (final concentration 5.0×10^{-4}, 2.0×10^{-4}, and 1.0×10^{-4} M, final DMSO concentration 1%) was added to potato dextrose agar medium at ca. 50 °C, mixed quickly and poured onto six-well plates (2.5 mL). An agar plug (8 mm diameter) cut from edges of 7-day-old solid cultures was placed upside down on the center of each plate and the plates were incubated at 24 ± 2 °C under constant light for 5 days. The diameter of the mycelia (in millimeter) was then measured and compared with control plates containing only DMSO. Each assay was conducted in triplicate and repeated at least three times.

4.4. Fungal metabolism

4.4.1. Time–course studies of metabolism. Solutions of potential inhibitors **10**–**23** in acetonitrile (0.25 mL) were added to liquid shake cultures (100 mL of minimal media in 250 mL Erlenmeyer flasks, final concentrations 1.0×10^{-4}, 2.0×10^{-4}, or 3.0×10^{-4} M, Table 2 and Fig. 2 and Figs. 3–7 in Supplementary data) of *L. maculans* (BJ-125, 44-h-old, 10^8/100 mL spores). After 10 (Table 2) or 30 (Figs. 2–7) min of incubation (shaker at 130 rpm, at 24 ± 2 °C), brassilexin (**1**) dissolved in acetonitrile (0.25 mL) was added and cultures (final concentration 1.0×10^{-4} M) were further incubated for various periods of time. Samples (2.5 mL) were withdrawn and either frozen or immediately extracted with EtOAc (2 × 5 mL). The organic phases were concentrated and analyzed by HPLC. Experiments

were performed in duplicate (Table 2) or triplicate (Figs. 2–7); control flasks containing mycelia in minimal media or compounds in minimal media were incubated under similar conditions.

4.5. Synthesis

4.5.1. Isothiazolo[5,4-*b*]thianaphthene (11). A solution of *t*-BuLi in pentane (2 mL, 2.2 mmol) was added dropwise to a solution of 3-bromothianaphthene (**24**, 426 mg, 2 mmol) in dry diethyl ether at −78 °C (Ar atmosphere). The mixture was stirred for 30 min at −78 °C and DMF (170 μL, 2.2 mmol) was then added, the cooling bath was removed, and the reaction mixture was stirred for further 30 min at room temperature. The reaction mixture was cooled to −30 °C, another portion of *t*-BuLi in pentane (2 mL, 2.2 mmol) was added dropwise, the cooling bath was removed and the mixture was stirred for 30 min at room temperature. The reaction mixture was cooled to −78 °C, and a solution of bromine (140 μl, 2.6 mmol) in hexane (2 mL) was added in one portion. The temperature was allowed to gradually raise to 0 °C (2 h), the reaction mixture was diluted with 1 M HCl (50 mL) and extracted with EtOAc. The combined organic extract was dried, the solvent was evaporated, and the residue was subjected to FCC (flash chromatography) (15 g silica gel, hexane), to afford 2-bromothianaphthene-3-carboxaldehyde (**25**) as a colorless solid (170 mg, 35%), mp: 59–60 °C (lit.[11] 74–76 °C, ligroin). HPLC: t_R 25.6 min; ^{1}H NMR δ (500 MHz, $CDCl_3$): 10.24 (s, 1 H), 8.71 (d, J = 8 Hz, 1 H), 7.75 (d, J = 8 Hz, 1 H), 7.48 (dd, J = 8, 8 Hz, 1 H), 7.45 (dd, J = 8, 8 Hz, 1 H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 186.1 (s), 138.9 (s), 135.7 (s), 133.7 (s), 131.5 (s), 126.7 (d), 126.4 (d), 124.1 (d), 121.4 (d); HREIMS *m*/*z*: measured 241.9225, calcd for C_9H_5BrOS: 241.9224; EI-MS *m*/*z* (relative int): 242 ([M+2]$^+$, 100), 240 (M$^+$, 100), 213 (16), 211 (15), 132 (40), 89 (29). FTIR ν_{max} (cm^{-1}) 3039, 1672, 1458, 1422, 1388, 751.

NH_4SCN (187 mg, 2.5 mmol) was added to a solution of aldehyde **25** (148 mg, 0.61 mmol) in DMF (1 mL) and the mixture was stirred for 4 h at 70 °C (caution: in hood, NaOH trap for HCN). After cooling to room temperature, the reaction mixture was diluted with brine (50 mL) and extracted with diethyl ether. The combined organic extract was washed with brine (50 mL) and dried, the solvent was evaporated, and the residue was subjected to FCC (20 g silica gel, dichloromethane/hexane, 1:1) to afford isothiazolo[5,4-*b*]thianaphthene (**11**) as a slightly orange oil, which was crystallized using dichloromethane/hexane solution. Yield: 72 mg (62%), mp: 79–80 °C (lit.[9] 82–84 °C, benzene/hexane). HPLC: t_R 21.2 min; ^{1}H NMR δ (500 MHz, $CDCl_3$): 8.94 (s, 1 H), 8.05 (d, J = 8 Hz, 1 H), 7.83 (d, J = 8 Hz, 1 H), 7.50 (dd, J = 8, 8 Hz, 1 H), 7.43 (dd, J = 8, 8 Hz, 1 H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 158.8 (s), 148.9 (d), 146.2 (s), 142.6 (s), 130.3 (s), 125.7 (d), 125.6 (d), 123.7 (d), 122.3 (d); HREIMS *m*/*z*: measured 190.9862, calcd for $C_9H_5NS_2$: 190.9863; EI-MS *m*/*z* (relative int): 191 (M$^+$, 100), 159 (20), 120 (26). FTIR ν_{max} (cm^{-1}) 3055, 1495, 1392, 1351, 1261, 762, 499. UV λ_{max} nm (MeOH, ε): 226 (42,800).

4.5.2. 1,2-Benzoisothiazole (13). 2-*t*-Butylsulfanylbenzaldehyde oxime[12] (419 mg, 2 mmol) was dissolved in acetic acid (0.8 mL) and acetic acid anhydride (0.4 mL), and the mixture was heated with stirring for 20 min at 100 °C. The reaction mixture was cooled to room temperature, diluted with water (30 mL) and extracted with dichloromethane. The combined organic extract was washed with 0.5 M solution of NaOH (10 mL), and dried, the solvent was evaporated and the residue was separated by FCC (silica gel, 60 g, hexane/diethyl ether, 5:1) to afford 1,2-benzoisothiazole (**13**, 220 mg, 64%, based on 2-nitrobenzaldehyde (**26**)) as a colorless solid, mp: 28–29 °C, (lit.[13] 32–33 °C). HPLC: t_R 14.1 min; ^{1}H NMR δ (500 MHz, $CDCl_3$): 8.94 (s, 1 H), 8.09 (d, J = 8 Hz, 1 H), 7.99 (d, J = 8 Hz, 1 H), 7.56 (dd, J = 8, 8 Hz, 1 H), 7.47 (dd, J = 8, 8 Hz, 1 H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 155.2 (d), 151.9 (s), 136.3 (s), 128.0 (d), 125.1 (d), 124.3 (d), 119.8 (d); HREIMS *m*/*z*: measured 135.0148, calcd for C_7H_5NS: 135.0143; EI-MS *m*/*z* (rel int): 135 (M$^+$, 100). FTIR ν_{max} (cm^{-1}) 3060, 1594, 1481, 1250, 1211, 884, 750, 570, 489. UV λ_{max} nm (MeOH, ε): 204 (31,700), 223 (19,400), 254 (3500), 298 (4100).

4.5.3. 4-Arylisothiazoles 14–16, 21, 22. NH_4SCN (304 mg, 4 mmol) was added to a solution of chloroacroleins **33**–**35**, **37**, and **38** (1 mmol) in DMF (1.5 mL), and the reaction mixtures were heated for 16 h at 70 °C with stirring (caution: in hood, NaOH trap for HCN). The reaction mixture was diluted with brine (20 mL) and extracted with ethyl acetate. The combined extract was dried, the solvent evaporated, and the residue subjected to FCC (silica gel) as follows: **33**, chloroform/hexane (1:4); **34**, dichloromethane/hexane (1:1); **35**, dichloromethane/hexane (1:1); **37**, acetone/hexane (1:8); **38**, diethyl ether/hexane (1:5).

4.5.4. 4-(2-Nitrophenyl)isothiazole (17). $SOCl_2$ (1.3 mL, 18 mmol) was added to a suspension of 2-nitrophenyl malondialdehyde (579 mg, 3 mmol) in dichloromethane (15 mL), and the mixture was stirred for 1 h at room temperature. The solvent was evaporated and the residue was subjected to FCC (silica gel, 15 g, dichloromethane/hexane, 2:1). Evaporation of the solvent afforded unstable chloroacrolein **36** as a colorless oil (616 mg, 97%).[18] NH_4SCN (886 mg, 11.6 mmol) was added to compound **36** dissolved in DMF (5 mL) and the reaction mixture was stirred for 16 h at 70 °C (caution: in hood, NaOH trap for HCN). The reaction mixture was cooled to room temperature, diluted with brine (70 mL), and extracted with EtOAc, the combined organic extract was dried, and the solvent evaporated. The residue was subjected to FCC (silica gel, 50 g, hexane/acetone, 5:1) to afford 4-(2-nitrophenyl)isothiazole (**17**) as a yellow solid (222 mg, 37%).

4.5.5. 4-(2-Aminophenyl)isothiazole (18) and 4-(2-hydroxyphenyl)isothiazole (19). Compound **18**[16] was obtained 71% yield, compound **19**[16] in 44% yield, based on 4-(2-nitrophenyl)isothiazole (**17**). No spectroscopic data was available, thus compounds were fully characterized.

4.5.6. 4-(2-Methoxyphenyl)isothiazole (20).[19] 4-(2-Hydroxyphenyl)isothiazole (**19**, 44 mg, 0.25 mmol) was dissolved in ethereal diazomethane solution (5 mL) and the mixture was stirred for 8 h at room temperature. The excess diazomethane was quenched with acetic acid, the solvent was evaporated, and the residue was subjected to FCC (silica gel, 10 g, hexane/acetone, 5:1) to afford 4-(2-methoxyphenyl)isothiazole (**20**) as a colorless oil (40 mg, 83%) HPLC: t_R 18.6 min; ^{1}H NMR δ (500 MHz, $CDCl_3$): 8.87 (s, 1 H), 8.86 (s, 1 H), 7.53 (d, J = 7.5 Hz, 1 H), 7.36 (dd, J = 7.5, 7.5 Hz, 1 H), 7.04 (m, 2 H), 3.91 (s, 3H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 158.1 (d), 156.6 (s), 144.9 (d), 136.0 (s), 129.8 (d), 129.5 (d), 122.0 (s), 121.2 (d), 111.7 (d), 55.8 (q); HREIMS *m/z*: measured 191.0407, calcd for $C_{10}H_9NOS$: 191.0405; EI-MS *m/z* (relative int): 191 (M^+, 100), 176 (30), 148 (12), 121 (20), 77 (12). FTIR ν_{max} (cm^{-1}) 2934, 1589, 1463, 1337, 1245, 1120, 1025, 751. UV λ_{max} nm (MeOH, ε): 204 (45,800), 286 (10,400).

4.5.7. 2-Aryl-3-chloroacroleins 33–35, 37, and 38. DMF (0.7 mL) was added dropwise to $POCl_3$ (0.7 mL, 7.5 mmol) and cooled to 0 °C. Aryl acetic acids (**27**–**29**, **31**, and **32**, 2.5 mmol) were then added and the mixture was stirred at 85 °C for 90 min (acid **31**), 2 h (acids **27**–**29**) or 2.5 h (acid **32**). The reaction mixtures were cooled to room temperature and cracked ice was added to adjust the volume to ca. 30 mL. The reaction mixtures were extracted with dichloromethane, the combined extracts were dried, and the solvents were evaporated to leave crude 2-phenyl-3-*N*,*N*-dimethylaminoacroleins as yellow oils. NaOH (25% aq soln, 5 mL) was added to crude 2-phenyl-3-*N*,*N*-dimethylaminoacroleins in EtOH (3.8 mL) and the reaction mixtures were refluxed with stirring for 30 min (acids **28**, **32**), 45 min (acid **31**) or 1 h (acids **27**, **29**). Ethanol was removed in vacuum, the residue was diluted to ca. 30 mL by addition of cracked ice and made acidic (pH < 3) using aqueous HCl (1:1). The resulting mixtures were extracted with diethyl ether, the combined extracts were dried, and the solvents were evaporated to leave crude enolized 2-aryl malondialdehydes as yellow oils. $SOCl_2$ (3.5 mL, 48 mmol) was added to crude 2-aryl malondialdehydes in CH_2Cl_2 (5 mL) and cooled to 0 °C and the reaction mixtures were stirred at 0 °C for 10 min (acid **29**), 20 min (acids **28**, **31**) or 1 h (acids **27**, **32**). The solvents were evaporated and the residues were subjected to FCC (silica gel, 20 g, dichloromethane/hexane, 1:1, **33**–**35**, **38**; or silica gel 50 g, dichloromethane/hexane, 2:1, **37**). 2-Aryl-3-chloroacroleins[18] (**33**, 310 mg, 75%; **34** 267 mg, 53%; **35**, 106 mg, 23%; **37**, 187 mg, 35%; **38**, 475 mg, 88%) were immediately used in the next step. All yields are based on starting aryl acetic acids.

4.5.8. Isothiazolo[5,4-*b*]quinoline (10). This compound was prepared following a literature procedure.[6,7] The overall process afforded a slightly yellow solid, isothiazolo[5,4-*b*]quinoline (**10**, 80 mg, 15% yield based on quinoline). Mp: 168–170 °C, acetone/hexane (lit.[7] 169–170 °C, ethyl acetate). HPLC: t_R 13.6 min; ^{1}H NMR δ (500 MHz, $CDCl_3$): 9.13 (s, 1H), 8.90 (s, 1H), 8.21 (d, J = 8 Hz, 1H), 8.07 (d, J = 8 Hz, 1H), 7.91 (ddd, J = 8, 8, 1 Hz, 1H), 7.64 (ddd, J = 8, 8, 1 Hz, 1H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 170.5 (s), 154.6 (d), 148.9 (s), 133.1 (d), 132.3 (d), 129.5 (d), 129.0 (d), 127.3 (s), 126.4 (d), 125.2 (d); HREIMS *m/z*: measured 186.0250, calculated for $C_{10}H_6N_2S$: 186.0252; EI-MS *m/z* (rel int): 186 (M^+, 100), 153 (14), 142 (11). FTIR ν_{max} (cm^{-1}) 3046, 1619, 1598, 1548, 1321, 1131, 927, 755. UV λ_{max} nm (MeOH, ε): 222 (16,100), 251 (59,200), 309 (4000), 323 (4900), 348 (2700).

4.5.9. Thieno[2,3-*b*]indole (12).[8] Yield 29%, based on *N*-Boc-2-chloroindole-3-carboxaldehyde.[20] For ^{1}H NMR and ^{13}C NMR.[21] HREIMS *m/z*: measured 173.0297, calcd for $C_{10}H_7NS$: 173.0299; EI-MS *m/z* (rel int): 173 (M^+, 100), 100 (18). FTIR ν_{max} (cm^{-1}) 3393, 1437, 1379, 745, 706, 494. UV λ_{max} nm (MeOH, ε): 231 (47,100), 266 (8800).

4.5.10. 4-Phenylisothiazole (14). Yield 54%, slightly yellow solid, mp: 32–33 °C, (lit.[22] 35–36 °C; 1it.[23] 33–37 °C). HPLC: t_R 17.6 min; ^{1}H NMR δ (500 MHz, $CDCl_3$): 8.80 (s, 1H), 8.73 (s, 1H), 7.63 (dd, J = 7.5, 7.5 Hz, 2H), 7.47 (dd, J = 7.5, 7.5 Hz, 2H), 7.38 (dd, J = 7.5, 7.5 Hz, 1H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 156.3 (d), 142.8 (d), 140.3 (s), 132.8 (s), 129.3 (d, 2 × C), 128.2 (d), 127.1 (d, 2× C). HREIMS *m/z*: measured 161.0303, calcd for C_9H_7NS: 161.0299; EI-MS *m/z* (rel int): 161 (M^+, 100), 134 (25). FTIR ν_{max} (cm^{-1}) 3033, 1603, 1487, 1350, 1236, 860, 754. UV λ_{max} nm (MeOH, ε): 203 (33,900), 243 (7300), 269 (8800).

4.5.11. 4-(2-Chlorophenyl)isothiazole (15).[19] Yield 30%, slightly yellow oil. HPLC: t_R 25.5 min; ^{1}H NMR δ (500 MHz, $CDCl_3$): 8.81 (s, 1H), 8.72 (s, 1H), 7.52 (m, 1H), 7.45 (m, 1H), 7.35 (m, 2H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 158.0 (d), 146.5 (d), 136.9 (s), 132.9 (s), 132.0 (s), 131.2 (d), 130.6 (d), 129.5 (d), 127.3 (d); HREIMS (*m/z*: measured 194.9914, calcd for C_9H_6ClNS: 194.9909); EI-MS *m/z* (rel int): 197 ($[M+2]^+$, 37), 195 (M^+, 100), 168 (24), 133 (11), 89 (10). FTIR ν_{max} (cm^{-1}) 3064, 1691, 1531, 1470, 1228, 1074, 1038, 753. UV λ_{max} nm (MeOH, ε): 203 (50,000), 261 (7700).

4.5.12. 4-(2-Tolyl)isothiazole (16).[19] Yield 25%, slightly yellow oil. HPLC: t_R 21.9 min; ^{1}H NMR δ (500 MHz, $CDCl_3$): 8.58 (s, 1H), 8.56 (s, 1H), 7.32 (m, 3H), 7.29 (m, 1H), 2.35 (s, 3H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 158.1 (d), 145.2 (d), 139.5 (s), 136.1 (s), 132.9 (s), 130.9 (d), 130.0 (d), 128.4 (d), 126.3 (d), 21.1 (q); HREIMS *m/z*: measured 175.0459, calcd for $C_{10}H_9NS$: 175.0456; MS-EI *m/z* (rel int): 175 (M^+, 29), 149 (100), 105 (40), 57 (27). FTIR ν_{max} (cm^{-1}) 3062, 1602, 1531, 1480, 1227, 1117, 914, 755. UV λ_{max} nm (MeOH, ε): 202 (29,500), 260 (5100).

4.5.13. 4-(2-Nitrophenyl)isothiazole (17). Mp: 43–45 °C (lit.[16] 59.5–62.5 °C, benzene/cyclohexane, containing 10% of 4-(4-nitrophenyl)isothiazole). HPLC: t_R 15.9 min; ^{1}H NMR δ (500 MHz, $CDCl_3$): 8.65 (s, 1H), 8.53 (s, 1H), 7.95 (dd, J = 8, 1 Hz, 1H), 7.67 (ddd, J = 8, 8, 1 Hz, 1H), 7.57 (ddd, J = 8, 8, 1 Hz, 1H), 7.48 (dd, J = 8, 1 Hz, 1H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 156.9 (d), 149.2 (s), 146.2 (d), 134.7 (s), 132.9 (d),

132.2 (d), 129.4 (d), 127.7 (s), 124.7 (d); HREIMS (*m/z*: measured 206.0144, calcd for $C_9H_6N_2O_2S$: 206.0150); EI-MS *m/z* (rel int): 206 (M^+, 100), 159 (21), 133 (17), 119 (25), 89 (44), 63 (16). FTIR ν_{max} (cm^{-1}) 2916, 1609, 1525, 1348, 849, 783, 745. UV λ_{max} nm (MeOH, ε): 202 (16,500), 253 (8900).

4.5.14. 4-(2-Aminophenyl)isothiazole (18). Mp: 46–48 °C. HPLC: t_R 10.6 min; ^1H NMR δ (500 MHz, $CDCl_3$): 8.74 (s, 1H), 8.69 (s, 1H), 7.21 (m, 2H), 6.85 (m, 2H), 3.85 (br s, 2H, D_2O exch.); ^{13}C NMR δ (125 MHz, $CDCl_3$): 158.1 (d), 145.0 (d), 144.2 (s), 137.2 (s), 130.5 (d), 129.6 (d), 119.2 (d), 118.9 (s), 116.3 (d); HREIMS *m/z*: measured 176.0406, calcd for $C_9H_8N_2S$: 176.0408; EI-MS *m/z* (rel int): 176 (M^+, 100), 159 (10), 143 (42), 117 (26). FTIR ν_{max} (cm^{-1}) 3346, 3084, 1618, 1489, 1299, 750, 654. UV λ_{max} nm (MeOH, ε): 209 (25,200), 244 (10,700), 302 (2300).

4.5.15. 4-(2-Hydroxyphenyl)isothiazole (19). Mp: 138–140 °C (lit.[16] 136–138 °C, methanol). HPLC: t_R 11.0 min; ^1H NMR δ (500 MHz, $CDCl_3$): 8.96 (s, 1H), 8.92 (s, 1H), 7.50 (dd, J = 7.5, 1.5 Hz, 1H), 7.25 (ddd, J = 7.5, 7.5, 1.5 Hz, 1H), 7.00 (m, 2H), 6.84 (br s, 1H, D_2O exch.); ^{13}C NMR δ (125 MHz, $CDCl_3$): 157.9 (d), 153.4 (s), 145.1 (d), 135.8 (s), 129.9 (d), 129.6 (d), 121.2 (d), 119.9 (s), 116.7 (d); HREIMS *m/z*: measured 177.0245, calcd for C_9H_7NOS: 177.0248; EI-MS *m/z* (rel int): 177 (M^+, 100), 118 (68). FTIR ν_{max} (cm^{-1}) 3131, 1601, 1452, 1337, 1283, 751. UV λ_{max} nm (MeOH, ε): 204 (29,000), 293 (5600).

4.5.16. 4-(1-Naphthyl)isothiazole (21). Yield 34%, slightly yellow solid, mp: 43–45 °C. HPLC: t_R 25.2 min; ^1H NMR δ (500 MHz, $CDCl_3$): 8.75 (s, 1H), 8.72 (s, 1H), 7.94 (m, 3H), 7.52 (m, 4H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 158.7 (d), 146.2 (d), 138.5 (s), 134.0 (s), 131.9 (s), 131.1 (s), 128.9 (d), 128.7 (d), 127.7 (d), 126.9 (d), 126.4 (d), 125.6 (d), 125.3 (d); HREIMS *m/z*: measured 211.0459, calcd for $C_{13}H_9NS$: 211.0456; EI-MS *m/z* (rel int): 211 (M^+, 100), 184 (13), 152 (33), 139 (13). FTIR ν_{max} (cm^{-1}) 3058, 1592, 1506, 1397, 1021, 909, 776, 661. UV λ_{max} nm (MeOH, ε): 204 (48,400), 223 (56,200), 290 (9500).

4.5.17. 4-(2-Naphthyl)isothiazole (22). Yield 37%, slightly yellow solid, mp: 72–73 °C. HPLC: t_R 25.5 min; ^1H NMR δ (500 MHz, $CDCl_3$): 8.94 (s, 1H), 8.83 (s, 1H), 8.08 (s, 1H), 7.91 (m, 3H), 7.72 (dd, J = 8.5, 1.5 Hz, 1H), 7.54 (m, 2H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 156.4 (d), 143.0 (d), 140.1 (s), 133.8 (s), 133.0 (s), 130.1 (s), 129.1 (d), 128.3 (d), 128.0 (d), 126.9 (d), 126.6 (d), 125.8 (d), 125.2 (d); HREIMS *m/z*: measured 211.0450, calcd for $C_{13}H_9NS$: 211.0456; EI-MS *m/z* (rel int): 211 (M^+, 100), 184 (12), 152 (12), 139 (11). FTIR ν_{max} (cm^{-1}) 3065, 1599, 1506, 1236, 863, 823, 749. UV λ_{max} nm (MeOH, ε): 204 (49,600), 241 (41,300), 267 (16,900).

4.5.18. 2-Phenyl-3-chloroacrolein (33). HPLC: t_R 17.6 min; ^1H NMR δ (500 MHz, $CDCl_3$): 9.68 (s, 1H), 7.45 (m, 3H), 7.36 (m, 3H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 189.8 (d), 144.6 (s), 142.7 (d), 130.0 (s), 129.5 (2× d), 129.1 (d), 128.5 (2× d).

4.5.19. 2-(2-Chlorophenyl)-3-chloroacrolein (34). HPLC: t_R 22.9 min; ^1H NMR δ (500 MHz, $CDCl_3$): 9.68 (s, 1H), 7.53 (s, 1H), 7.50 (d, J = 7 Hz, 1H), 7.37 (m, 2H), 7.18 (d, J = 7 Hz, 1H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 188.6 (d), 144.3 (s), 144.1 (d), 133.7 (s), 131.4 (d), 130.8 (d), 130.3 (s), 130.2 (d), 127.3 (d).

4.5.20. 2-(2-Tolyl)-3-chloroacrolein (35). HPLC: t_R 19.3 min; ^1H NMR δ (500 MHz, $CDCl_3$): 9.70 (s, 1H), 7.48 (s, 1H), 7.29 (m, 3H), 7.05 (d, J = 7 Hz, 1H), 2.17 (s, 3H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 189.8 (d), 146.5 (s), 143.9 (d), 136.8 (s), 130.7 (d), 130.6 (s), 129.6 (d), 129.5 (d), 126.3 (d), 19.9 (q).

4.5.21. 2-(2-Nitrophenyl)-3-chloroacrolein (36). HPLC: t_R 15.3 min; ^1H NMR δ (500 MHz, $CDCl_3$): 9.66 (s, 1H), 8.25 (d, J = 7 Hz, 1H), 7.75 (dd, J = 7, 7 Hz, 1H), 7.64 (dd, J = 7, 7 Hz, 1H), 7.50 (s, 1H), 7.35 (d, J = 7 Hz, 1H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 188.2 (d), 148.4 (s), 144.5 (s), 141.9 (d), 134.2 (d), 132.4 (d), 130.6 (d), 126.5 (s), 125.5 (d).

4.5.22. 2-(1-Naphthyl)-3-chloroacrolein (37). HPLC: t_R 22.1 min; ^1H NMR δ (500 MHz, $CDCl_3$): 9.85 (s, 1H), 7.94 (m, 2H), 7.71 (s, 1H), 7.52 (m, 4H), 7.32 (d, J = 7 Hz, 1H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 189.9 (d), 145. 2 (s), 144.5 (d), 134.0 (s), 131.0 (s), 129.9 (d), 129.1 (d), 128.7 (s), 127.9 (d), 127.0 (d), 126.6 (d), 125.7 (d), 125.0 (d).

4.5.23. 2-(2-Naphthyl)-3-chloroacrolein (38). HPLC: t_R 23.8 min; ^1H NMR δ (500 MHz, $CDCl_3$): 9.75 (s, 1H), 7.91 (m, 4H), 7.55 (m, 2H), 7.43 (m, 2H); ^{13}C NMR δ (125 MHz, $CDCl_3$): 190.2 (d), 144. 9 (s), 143.3 (d), 133.7 (s), 133.4 (s), 129.7 (d), 128.8 (d), 128.4 (d), 128.2 (d), 127.7 (s), 127.3 (d), 126.9 (d), 126.8 (d).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.08.053.

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