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Toward the control of *Leptosphaeria maculans*: Design, syntheses, biological activity, and metabolism of potential detoxification inhibitors of the crucifer phytoalexin brassinin

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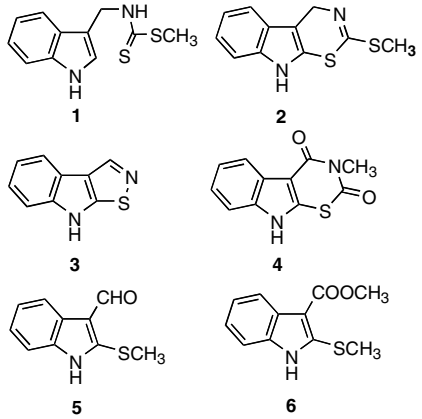
Abstract—Brassinin (**1**), a crucial plant defense produced by crucifers, is detoxified by the phytopathogenic fungus *Leptosphaeria maculans* (*Phoma lingam*) to indole-3-carboxaldehyde using a putative brassinin oxidase. Potential inhibitors of brassinin detoxification were designed by replacement of its dithiocarbamate group (toxophore) with carbamate, dithiocarbonate, urea, thiourea, sulfamide, sulfonamide, dithiocarbazate, amide, and ester functional groups. In addition, the indolyl moiety was substituted for naphthalenyl and phenyl. The syntheses and chemical characterization of these potential detoxification inhibitors, along with their antifungal and cytotoxic activity, as well as screening using cultures of *L. maculans* are reported. Overall, three types of interaction were observed in cultures of *L. maculans* co-incubated with the potential inhibitors and brassinin: (1) a decrease on the rate of brassinin detoxification due to the strong inhibitory activity of the compound on fungal growth, (2) a decrease on the rate of brassinin detoxification due to the inhibitory activity of the compound on the putative brassinin oxidase, and (3) a low to no detectable effect on the rate of brassinin detoxification. A noticeable decrease in the rate of brassinin detoxification was observed in the presence of *N*′-methylbrassinin, methyl *N*-methyl-*N*-(naphthalen-2-ylmethyl) dithiocarbamate, tryptophol dithiocarbonate, and methyl 3-phenyldithiocarbazate. Tryptophol dithiocarbonate appeared to be the best inhibitor among the designed compounds, representing the first inhibitor of brassinin detoxification and potentially the first selective protecting agent of oilseed crucifers against *L. maculans* infestation.

1. Introduction

Phytoalexins are low molecular weight secondary metabolites produced de novo by plants in response to various types of stress, including microbial attack.[1] The crucifer phytoalexin brassinin (**1**) is of great interest in the interaction of crucifers with their fungal pathogens due to both its biological activity and intermediacy in the biosynthetic pathway of other relevant phytoalexins (e.g., cyclobrassinin (**2**), brassilexin (**3**), rutalexin (**4**), brassicanal A (**5**), and brassicanate A (**6**)).[2] Crucifers are cultivated around the world and comprise an extremely valuable group of oilseeds and vegetables. Pathogens of crucifers, similar to many plant pathogens, can metabolize phytoalexins to less toxic compounds, a detrimental process that deprives the plant of important induced chemical defenses.[3] These detoxification reactions are catalyzed by detoxifying enzymes, which are likely to have evolved during the multiple life cycles of pathogens. Such phytoalexin detoxifying enzymes can be of sufficient importance to determine virulence in a fungal pathogen.[3]



Keywords: Brassinin; *Leptosphaeria maculans*; Phytoalexins; Phoma lingam; Dithiocarbamate; *Brassica*; Detoxifying enzyme; Brassinin oxidase; Antifungal.
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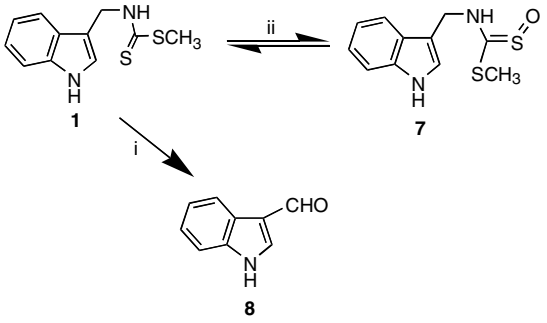
The blackleg fungus [*Leptosphaeria maculans* (Desm.) Ces. et de Not., asexual stage *Phoma lingam* (Tode ex Fr) Desm.], an important pathogen of crucifer oilseeds (rapeseed, canola, and mustard), can detoxify several phytoalexins including brassinin (**1**) (Scheme 1),[4] cyclobrassinin (**2**), brassilexin (**3**), and brassicanal A (**5**) via different pathways.[3] Considering that brassinin (**1**) is a biosynthetic precursor of many other phytoalexins, this metabolic detoxification can make plants particularly vulnerable to further pathogen colonization. Because fungal pathogens appear to use specific enzymes in the detoxification of phytoalexins,[3] the inhibition of such enzymes may be a suitable strategy to control *L. maculans*.[2] That is, enhancement of plant self-defenses through the use of selective detoxification inhibitors, that is, paldoxins,[2b] could lead to a selective and environmentally safer control of *L. maculans*. Selective inhibitors are less likely to affect non-targeted organisms and thus are expected to have lower impact on the cultivated ecosystem, that is, be environmentally 'neutral.' Toward this end, we report the design, syntheses, biological activity, screening, and evaluation of potential brassinin detoxification inhibitors.



Scheme 1. Detoxification of brassinin (**1**) in the phytopathogenic fungus *Leptosphaeria maculans*; (i) in mycelial cultures or cell-free extracts (brassinin oxidase);[4] (ii) in mycelial cultures.[3]

2. Results

The chemical structure of brassinin (**1**) contains a dithiocarbamate group (toxophore, substructure A) and an indole nucleus (substructure B) linked by a methylene bridge located at the C-3 of indole (Fig. 1). Brassinin (**1**) detoxification in mycelial cultures of *L. maculans* involves the formation of brassinin sulfoxide (**7**) and indole-3-carboxaldehyde (**8**) (Scheme 1) which is oxidized slowly to indole-3-carboxylic acid.[3] However, it is not understood whether sulfoxide **7** is an intermediate in the detoxification pathway, since it was not detected in cell-free extracts containing brassinin

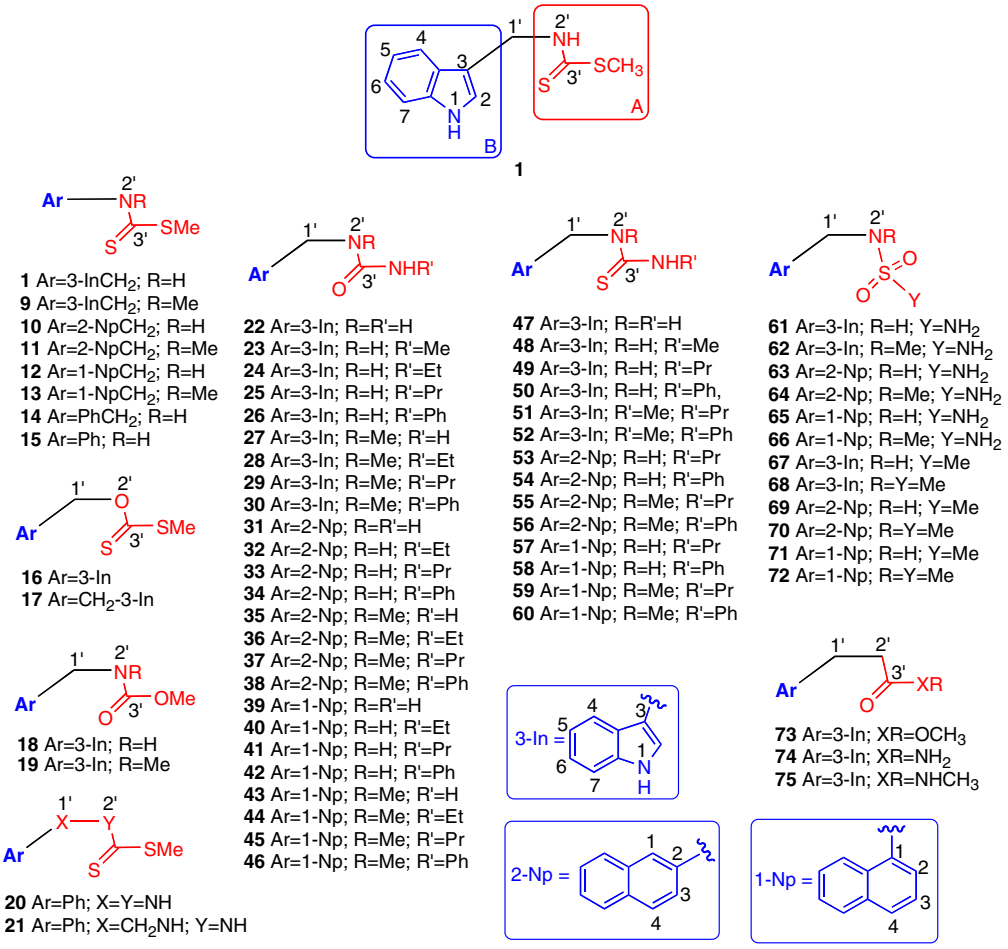


Figure 1. Potential inhibitors **9**–**75** of brassinin (**1**) detoxification.

oxidase activity.[4] Notwithstanding the details of the detoxification mechanism, it is clear that enzymatic detoxification of brassinin (**1**) by a putative brassinin oxidase[4] takes place only on the side chain of brassinin (**1**), while the indolyl component remains unaffected. For this reason, to design selective inhibitors of brassinin detoxification, the side chain of brassinin (**1**) was of crucial importance. Alternatively, replacement of the indolyl moiety with naphthalenyl (e.g., **10**–**13**) or phenyl (e.g., **14** and **15**) would lead to structures in which the contribution of the indolyl part (more polar) of brassinin (**1**) to metabolism and toxicity could be directly evaluated. In the case of naphthalene derivatives, both C-1 and C-2 were chosen as points of substitution because previous work showed that a fungal pathogen transformed these compounds differently.[5] In addition, since dithiocarbamates have strong biological activity,[6] the design of compounds with a wider range of bioactivities was planned as well.

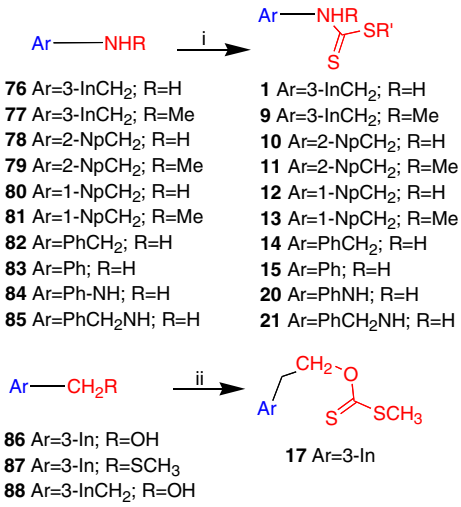
In most cases, isosteric replacement[7] of the heteroatoms of the dithiocarbamate group of brassinin (**1**) led to incorporation of carbamate, urea, thiourea, sulfamide, sulfonamide, dithiocarbazate, amide, and ester functional groups into the structure of potential inhibitors (**16**–**75**). Besides the potential role as detoxification inhibitors, this wide range of functional groups was designed to provide further insights into the detoxification mechanism of brassinin as well as structure–activity correlations. Accordingly, replacement of both sulfur atoms of brassinin (**1**) with oxygen atoms afforded carbamates **18** and **19**, whereas replacement of the thiocarbonyl with carbonyl and the SCH_3 with NH_2 or NHR (R = alkyl or phenyl) gave rise to unsymmetrical urea derivatives **22**–**46** containing indolyl or naphthalenyl (less polar) moieties. Similarly, keeping the thiocarbonyl intact and replacing the SCH_3 group with NH_2 or NHR (R = alkyl or phenyl) resulted in unsymmetrical thioureas **47**–**60**, whereas dithiocarbazates **20** and **21** could be obtained by replacing the (H)N-2′ with (H)N–NH (hydrazine). Sulfamides **61**–**66** and sulfonamides **67**–**72** were obtained by replacing the thiocarbonyl group with a sulfone moiety and the SCH_3 with NH_2, $NHCH_3$ (sulfamides), and CH_3 (sulfonamides) containing indolyl or naphthalenyl moieties as well. Amides **74** and **75** and ester **73** were obtained by replacing the (H)N-2′ of brassinin (**1**) with CH_2, the thiocarbonyl with a carbonyl, and SCH_3 with either NH_2/$NHCH_3$ (amide) or OCH_3 (ester), respectively. Overall, it was anticipated that some of these new compounds (Fig. 1) would hinder brassinin (**1**) detoxification by inhibiting the putative brassinin oxidase.

2.1. Syntheses of potential inhibitors and metabolism

Among the designed potential inhibitors **9**–**75**, compounds **9**,[8] **10**,[5] **12**,[5] **14**,[9] **15**,[9] **18**,[10] **20**,[11] **21**,[12] **22**,[13] **26**,[14] **48**,[15] **72**,[16] **73**,[17] **74**,[18] and **75**[19] were reported previously. Of the designed sulfonamides **67**–**72**, *N*-methyl-*N*-(naphthalen-1-ylmethyl)methanesulfonamide (**72**) has been used to study aldose reductase inhibitors, but its synthesis was not described.[16] Amines such as indol-3-ylmethylamine (**76**), *N*′-methylindol-3-ylmethylamine (**77**), naphthalen-2-ylmethylamine (**78**), *N*-methylnaphthalen-2-ylmethylamine (**79**), naphthalen-1-ylmethylamine (**80**), and *N*-methylnaphthalen-1-ylmethylamine (**81**) were used in the syntheses of most of the designed compounds. These key starting materials were synthesized in good yields using known methodology.[20–22]

2.1.1. Syntheses of dithiocarbamates, dithiocarbonates, carbamates, dithiocarbazates, ureas, and thioureas (1, 9–15, and 17–60). The syntheses of brassinin (**1**),[2] *N*′-methylbrassinin (**9**), and dithiocarbamates **10**–**15**[5] were carried out as reported earlier for brassinin by first allowing the respective amines **76**–**83** to react with CS_2 in pyridine and triethylamine followed by quenching with MeI and acidic work-up (H_2SO_4). As expected, attempts to transform indol-3-ylmethanol (**86**) to methyl indol-3-ylmethyldithiocarbonate (**16**) under experimental conditions similar to those used for dithiocarbamates were not successful as indol-3-ylmethanol (**86**) was found to be stable under those conditions. When triethylamine was replaced with a stronger base such as NaH for deprotonation of –(O)–H,[23] followed by addition of CS_2, thioether **87** (30% yield) along with recovered starting material (**86**, 40% yield) was obtained. Heating 3-indolylmethanol (**86**) to 70 °C in the presence of NaH in THF resulted in extensive decomposition of the starting material. Using a reagent such as 1-(methyldithiocarbonyl)imidazole,[24] a reagent developed for the mild conversion of alcohols to S–Me dithiocarbonates, also failed to give the desired compound **16** and instead yielded thioether **87** (60% yield). By contrast, deprotonation of tryptophol (**88**), the CH_2 homolog of 3-indolylmethanol (**86**), with NaH in THF at 0 °C followed by addition of CS_2 and quenching with MeI afforded the corresponding dithiocarbonate **17** (15 min) in excellent yield (95% yield). Methyl 3-phenyldithiocarbazate (**20**) and methyl 3-benzyldithiocarbazate (**21**) were synthesized from phenylhydrazine and benzylhydrazine, respectively (Scheme 2).[25]

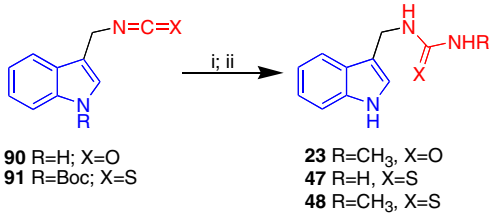
The syntheses of *N*-(indol-3-yl)methylurea (**22**),[13] *N*-indol-3-ylmethyl-*N*′-phenylurea (**26**),[14] and *N*-(naphthalen-1-ylmethyl)-*N*′-phenylurea (**44**)[26] were carried out as previously described. Although urea **22** was prepared quantitatively from gramine (**89**) and excess urea, a similar attempt to make *N*-indol-3-ylmethyl-*N*′-methylurea (**23**) by reacting gramine (**89**) and methylurea was unsuccessful. Compound **23** could be obtained in good yield by first reacting amine **76** with $COCl_2$ to yield isocyanate **90**, which was then reacted with CH_3NH_2 to give urea **23** (Scheme 3). Other unsymmetrical ureas (**24**–**46**) were prepared in good yields by the reaction of primary or secondary amines with alkyl isocyanates (Scheme 3).[27] Similar to ureas **24**–**46**, most of the unsymmetrical thioureas **49**–**60** (Scheme 3) were synthesized by reacting the corresponding primary or secondary amine with an isothiocyanate (RNCS, R = Pr or Ph, Scheme 3).[27] Methyl *N*-(indol-3-ylmethyl)-*N*-methylcarbamate (**19**) was obtained in 85% yield by treatment of *N*-(indol-3-ylmethyl)-*N*-methylamine (**77**) with ethylchloroformate in the presence of *N*,*O*-bis(trimethylsilyl)acetamide (Scheme 3).[10]



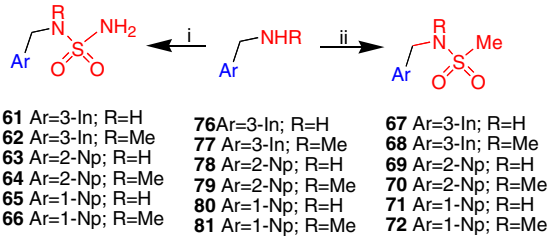
Scheme 2. Syntheses of dithiocarbamates **1**, **9**–**15**, and dithiocarbonate **17**, and dithiocarbazates **20** and **21**. Reagents: (i) for **9**–**15**, CS_2, pyridine/Et_3N; MeI; for **20** and **21**, CS_2, KOH, EtOH, MeI; (ii) NaH, THF, MeI.

Attempts to synthesize indol-3-ylmethylthiourea (**47**) by condensing the salt of **76** with KSCN in THF[28] were unsuccessful. Thus, the syntheses of **47** and **48** involved rather lengthy procedures starting with protection of N-1 with Boc,[21] followed by isothiocyanate formation, which was then reacted with ammonia/methylamine to give *N*-Boc-indolyl thioureas. The Boc group was hydrolyzed using excess sodium methoxide to give **47** and **48** in moderate yields (Scheme 4).[21]

2.1.2. Syntheses of sulfamides and sulfonamides (61–72). The sulfamide functional group was conveniently introduced into amines **76**–**81** upon treatment with sulfamide at 100 °C in aqueous solution. After heating



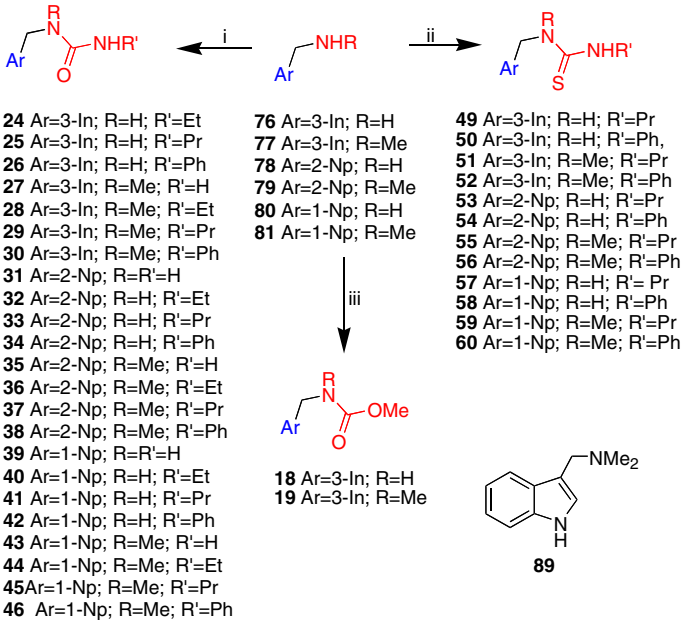
Scheme 4. Syntheses of urea **23** and thioureas **47** and **48**. Reagents: (i) RNH_2 (R = H, Me), CH_2Cl_2; (ii) NaOMe, MeOH.



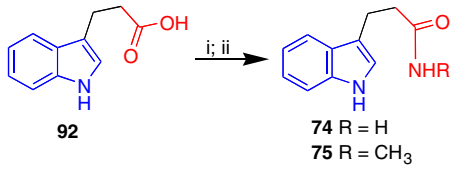
Scheme 5. Syntheses of sulfamides **61**–**66** and sulfonamides **67**–**72**. Reagents and conditions: (i) $NH_2SO_2NH_2$, H_2O, 100 °C; (ii) $ClSO_2Me$, THF, rt.

for 4 h and then cooling to rt, the precipitates of desired sulfamides **61**–**66** were obtained in moderate yields (Scheme 5).[29] Sulfonamides **67**–**72** were synthesized in good yields by reacting primary and secondary amines **76**–**81** with methane sulfonyl chloride in THF (Scheme 5).[30]

2.1.3. Syntheses of amides and ester (73–75). Amide **73** has been previously synthesized starting from 3-(indol-3-yl)propanoic acid (**92**) and 3-(indol-3-yl)propanenitrile



Scheme 3. Syntheses of ureas **24**–**46**, thioureas **49**–**60**, and carbamates **18**–**19**. Reagents: (i) RNCO, CH_2Cl_2; (ii) RNCS, CH_2Cl_2; (iii) ClCOOMe, *N*,*O*-bis(trimethylsilyl)acetamide.



Scheme 6. Syntheses of amides **74** and **75**. Reagents and conditions: (i) ClCOOEt, THF, Et_3N, 0 °C; (ii) NH_3 for **74**; $MeNH_2$ for **75**.

(**93**) in good yields.[31,32] However, the synthesis of **73** and **74** was carried out using a milder procedure in which acid **92** was first reacted with ethylchloroformate in THF and triethylamine, then ammonia/methyl amine was bubbled through the solution for 30 min (Scheme 6).[33] Ester **75** was prepared from acid **92** as published previously.[31]

2.2. Biological activity of potential inhibitors and metabolism

The antifungal activity against *L. maculans* and cytotoxic activity to *Artemia salina* (brine shrimp) of the synthesized compounds (**1** and **9**–**75**) were determined using established bioassay methods, as follows.

2.2.1. Antifungal activity. To establish the antifungal activity, bioassays were carried out using a mycelial radial growth assay, as described in Section 4. The results summarized in Table 1 indicate that *N*′-methylbrassinin (**9**), naphthalen-2-ylmethylurea (**31**), and *N*-(indol-3-ylmethyl)-*N*-methyl-*N*-phenylthiourea (**52**) were the most potent antifungal compounds among all tested compounds. All three compounds were completely inhibitory of mycelial growth at 0.2 mM and showed a substantial inhibition even at 0.1 mM (>70%, 0.1 mM). Interestingly, *N*′-methylbrassinin (**9**) was found to be almost twice as antifungal as the naturally occurring dithiocarbamate **1**, suggesting that replacement of the side chain (N)–H of brassinin with a CH_3 group increased the toxicity toward *L. maculans*. Among other dithiocarbamates, naphthalene-based compounds **10**–**13** were significantly less inhibitory than brassinin (**1**) and showed no pronounced effect when the (N)–H was replaced with a CH_3 group. Dithiocarbonate **17** showed substantially lower antifungal activity (41%, 0.5 mM) than brassinin (**1**), whereas dithiocarbazate exhibited somewhat similar antifungal activity. In the case of ureas **22**–**46**, indole-based compounds **22**–**30** showed an increase in the activity with the increase of the bulkiness of the alkyl substituent. For example, *N*-(indol-3-ylmethyl)-*N*-propylurea (**25**) was twice as toxic as *N*-(indol-3-ylmethyl)urea (**22**). However, introduction of an additional CH_3 group on the N of the urea moiety (**27**–**30**) led to lower toxicity. Naphthalenyl ureas **31**–**46** were significantly more toxic than indolyl ureas **22**–**30** and showed a decrease in the antifungal activity with the increase in bulkiness of the alkyl substituent on the urea moiety. Methylated ureas **35**–**38** and **43**–**46** were found to be substantially less toxic then their demethylated analogs **31**–**34** and **39**–**42**. While indol-3-ylmethylthiourea (**47**) showed no toxicity to *L. maculans* at the tested concentrations, other indolylthioureas (**48**–**60**) were found to have antifungal activity, showing an increase in the activity with the increase in the size of alkyl group substituent on the thiourea moiety. The activity of *N*-(indol-3-ylmethyl)-*N*-methyl-*N*-propylthiourea (**51**) increased markedly with the replacement of (N)–H with a CH_3 group on thiourea moiety. The naphthalenylthioureas **53**–**60** showed comparable antifungal activity. Compounds containing sulfamide or sulfonamide functional groups (**61**–**72**) were found to have mild antifungal activity, whereas carbamates **18** and **19**, amides **73** and **74**, and ester **75** showed little or no toxicity against *L. maculans* at the tested concentrations. In general, those compounds having substitution at C-2 were more active than the compounds with substitution at C-1 of the naphthalene ring (Table 1).

2.2.2. Cytotoxic activity. Assays using brine shrimp larvae (*A. salina*) were carried out for a preliminary evaluation of cytotoxicity. Shrimp eggs were allowed to hatch for 24 h, incubated with brassinin or designed compounds at 0.5, 0.2, and 0.1 mM, and the number of surviving shrimps was counted after 24 h. A control experiment was carried out similarly without adding any compound. The results summarized in Table 1 show that compounds containing a dithiocarbamate group (**1** and **9**–**13**) were lethal at all the tested concentrations, whereas carbamates (**18** and **19**) were found to be moderately toxic. Among the ureas **22**–**46**, naphthalen-2-ylmethylurea (**31**) and naphthalen-1-ylmethylurea (**39**) were found to be most potent. A decrease in lethality was observed with the increase in the size of substituents on the urea fragment of these compounds. For instance, *N*-(indol-3-ylmethyl)-*N*-methyl-*N*-propylurea (**29**) and *N*-methyl-*N*-(naphthalen-2-ylmethyl)-*N*-propylurea (**37**) had no toxicity. Compounds having urea functional group placed at the naphthalen-2-ylmethyl position were found to be more active than the 1-substituted isomers. Only *N*-(indol-3-ylmethyl)-*N*-methylthiourea (**23**) was lethal at 0.5 and 0.2 mM, among thioureas **47**–**60**. Other compounds containing thiourea functionality were notably less toxic. Sulfamides, sulfonamides, and amides were very mild or non-cytotoxic at the tested concentrations.

2.3. Screening of potential inhibitors and metabolism

The metabolism of brassinin (**1**) and potential detoxification inhibitors **9**–**15** and **17**–**75** was investigated in cultures of *L. maculans* over a period of 96 h. Initial experiments were carried out to determine the time required by *L. maculans* to completely metabolize brassinin (**1**) at three different concentrations (0.1, 0.2, and 0.3 mM). Next, the screening of potential inhibitors of brassinin detoxification was performed by co-incubating each compound with brassinin (**1**). Mycelial cultures (48-h-old) in a chemically defined medium[34] were incubated with the potential inhibitors at 0.1 and 0.2 mM for 10 min (to allow absorption/transport of compounds into cells) before adding brassinin (**1**, 0.1 mM). Control cultures prepared similarly but containing only brassinin (**1**) or the potential inhibitor were incubated in parallel. The stability of brassinin (**1**) and compounds **9**–**15** and **17**–**75** was determined by incubations in culture media under similar conditions. Samples were withdrawn from

Table 1. Biological activity of brassinin (**1**) and potential detoxification inhibitors **9**–**15** and **17**–**75** against *Leptosphaeria maculans* and *Artemia salina*

Compound	Biological activity	Percentage of activity		
		0.5 mM	0.2 mM	0.1 mM
Brassinin (**1**)	Antifungal activity[a]	100 ± 0	45 ± 6	0
	Cytotoxic activity[b]	100 ± 0	100 ± 0	100 ± 0
N′-Methylbrassinin (**9**)	Antifungal activity	100 ± 0	100 ± 0	74 ± 2
	Cytotoxic activity	100 ± 0	100 ± 0	100 ± 0
Methyl naphthalen-2-ylmethyldithiocarbamate (**10**)	Antifungal activity	40 ± 8	32 ± 7	0
	Cytotoxic activity	100 ± 0	100 ± 0	100 ± 0
Methyl *N*-methylnaphthalen-2-ylmethyldithiocarbamate (**11**)	Antifungal activity	40 ± 6	28 ± 6	0
	Cytotoxic activity	100 ± 0	100 ± 0	100 ± 0
Methyl naphthalen-1-ylmethyldithiocarbamate (**12**)	Antifungal activity	44 ± 7	32 ± 4	23 ± 6
	Cytotoxic activity	100 ± 0	100 ± 0	100 ± 0
Methyl *N*-methylnaphthalen-1-ylmethyldithiocarbamate (**13**)	Antifungal activity	35 ± 3	20 ± 2	0
	Cytotoxic activity	100 ± 0	100 ± 0	100 ± 0
Methyl *N*-benzyldithiocarbamate (**14**)	Antifungal activity	100 ± 0	52 ± 2	0
	Cytotoxic activity	100 ± 0	100 ± 0	100 ± 0
Methyl *N*-phenyldithiocarbamate (**15**)	Antifungal activity	62 ± 4	20 ± 3	0
	Cytotoxic activity	100 ± 0	100 ± 0	100 ± 0
Tryptophol dithiocarbonate (**17**)	Antifungal activity	41 ± 7	30 ± 5	20 ± 7
	Cytotoxic activity	100 ± 0	84 ± 10	24 ± 6
Methyl indol-3-ylmethylcarbamate (**18**)	Antifungal activity	17 ± 3	0	0
	Cytotoxic activity	64 ± 6	25 ± 10	0
Methyl 2′-methylindol-3-ylmethylcarbamate (**19**)	Antifungal activity	33 ± 5	0	0
	Cytotoxic activity	82 ± 6	52 ± 10	10 ± 0
Methyl phenyldithiocarbazate (**20**)	Antifungal activity	100 ± 0	76 ± 4	32 ± 2
	Cytotoxic activity	100 ± 0	96 ± 4	66 ± 6
Methyl benzyldithiocarbazate (**21**)	Antifungal activity	84 ± 5	26 ± 6	0
	Cytotoxic activity	100 ± 0	82 ± 7	56 ± 6
Indol-3-ylmethylurea (**22**)	Antifungal activity	62 ± 4	33 ± 6	0
	Cytotoxic activity	61 ± 6	25 ± 10	0
N-(Indol-3-ylmethyl)-*N*′-methylurea (**23**)	Antifungal activity	74 ± 4	40 ± 6	0
	Cytotoxic activity	82 ± 12	64 ± 6	57 ± 6
N-(Indol-3-ylmethyl)-*N*′-ethylurea (**24**)	Antifungal activity	84 ± 3	44 ± 6	21 ± 5
	Cytotoxic activity	61 ± 12	25 ± 5	0
N-(Indol-3-ylmethyl)-*N*′-propylurea (**25**)	Antifungal activity	0	58 ± 3	43 ± 5
	Cytotoxic activity	0	0	0
N-(Indol-3-ylmethyl)-*N*′-phenylurea (**26**)	Antifungal activity	75 ± 2	36 ± 2	22 ± 3
	Cytotoxic activity	17 ± 6	0	0
N-(Indol-3-ylmethyl)-*N*′-methylurea (**27**)	Antifungal activity	70 ± 4	53 ± 3	0
	Cytotoxic activity	86 ± 6	61 ± 6	48 ± 10
N-(Indol-3-ylmethyl)-*N*-methyl-*N*′-ethylurea (**28**)	Antifungal activity	48 ± 2	31 ± 5	0
	Cytotoxic activity	75 ± 6	50 ± 6	39 ± 6
N-(Indol-3-ylmethyl)-*N*-methyl-*N*′-propylurea (**29**)	Antifungal activity	17 ± 3	0	0
	Cytotoxic activity	0	0	0
N-(Indol-3-ylmethyl)-*N*-methyl-*N*′-phenylurea (**30**)	Antifungal activity	61 ± 4	37 ± 2	30 ± 3
	Cytotoxic activity	39 ± 0	17 ± 6	0
Naphthalen-2-ylmethylurea (**31**)	Antifungal activity	100 ± 0	100 ± 0	81 ± 3
	Cytotoxic activity	100 ± 0	68 ± 11	29 ± 12
N′-Ethyl-*N*-(naphthalen-2-ylmethyl)urea (**32**)	Antifungal activity	64 ± 3	44 ± 5	23 ± 3
	Cytotoxic activity	68 ± 6	25 ±10	0
N-(Naphthalen-2-ylmethyl)-*N*′-propylurea (**33**)	Antifungal activity	74 ± 5	48 ± 6	24 ± 3
	Cytotoxic activity	48 ± 0	14 ± 6	0
N′-Phenyl-*N*-(naphthalen-2-ylmethyl)urea (**34**)	Antifungal activity	61 ± 3	60 ± 2	58 ± 2
	Cytotoxic activity	36 ± 6	0	0

(*continued on next page*)

Table 1 (*continued*)

Compound	Biological activity	Percentage of activity		
		0.5 mM	0.2 mM	0.1 mM
N-Methyl-*N*-(naphthalen-2-ylmethyl)urea (**35**)	Antifungal activity	100 ± 0	66 ± 6	24 ± 2
	Cytotoxic activity	100 ± 0	64 ± 6	32 ±6
N′-Ethyl-*N*-methyl-*N*-(naphthalen-2-ylmethyl)urea (**36**)	Antifungal activity	60 ± 4	41 ± 5	18 ± 4
	Cytotoxic activity	71 ± 10	25 ± 6	0
N-Methyl-*N*-(naphthalen-2-ylmethyl)-*N*′-propylurea (**37**)	Antifungal activity	76 ± 6	38 ± 3	21 ± 4
	Cytotoxic activity	0	0	0
N-Methyl-*N*-(naphthalen-2-ylmethyl)-*N*′-phenylurea (**38**) (low solubility)	Antifungal activity	64 ± 4	30 ± 3	15 ± 2
	Cytotoxic activity	14 ± 5	0	0
Naphthalen-1-ylmethylurea (**39**)	Antifungal activity	100 ± 0	46 ± 4	0
	Cytotoxic activity	0	72 ± 11	36 ± 6
N′-Ethyl-*N*-(naphthalen-1-ylmethyl)urea (**40**)	Antifungal activity	68 ± 4	26 ± 6	0
	Cytotoxic activity	68 ± 10	22 ± 6	0
N-(Naphthalen-1-ylmethyl)-*N*′-propylurea (**41**)	Antifungal activity	54 ± 6	36 ± 4	0
	Cytotoxic activity	0	0	0
N-(Naphthalen-1-ylmethyl)-*N*′-phenylurea (**42**) (low solubility)	Antifungal activity	60 ± 4	34 ± 2	0
	Cytotoxic activity	0	0	0
N-Methyl-*N*-(naphthalen-1-ylmethyl)urea (**43**)	Antifungal activity	100 ± 0	76 ± 6	28 ± 2
	Cytotoxic activity	100	46 ± 6	78 ± 0
N′-Ethyl-*N*-methyl-*N*-(naphthalen-1-ylmethyl)urea (**44**)	Antifungal activity	50 ± 5	31 ± 4	0
	Cytotoxic activity	64 ± 6	32 ± 10	0
N-Methyl-*N*-(naphthalen-1-ylmethyl)-*N*′-propylurea (**45**)	Antifungal activity	53 ± 4	21 ± 3	0
	Cytotoxic activity	14 ± 6	0	0
N-Methyl-*N*-(naphthalen-1-ylmethyl)-*N*′-phenylurea (**46**) (low solubility)	Antifungal activity	54 ± 4	18 ± 6	0
	Cytotoxic activity	32 ± 6	0	0
Indol-3-ylmethylthiourea (**47**)	Antifungal activity	0	0	0
	Cytotoxic activity	79 ± 0	29 ± 12	0
N-(Indol-3-ylmethyl)-*N*′-methylthiourea (**48**)	Antifungal activity	73 ± 3	38 ± 2	0
	Cytotoxic activity	100	100	75 ± 6
N-(Indol-3-ylmethyl)-*N*′-propylthiourea (**49**)	Antifungal activity	64 ± 4	29 ± 3	0
	Cytotoxic activity	78 ± 6	24 ± 6	0
N-(Indol-3-ylmethyl)-*N*′-phenylthiourea (**50**)	Antifungal activity	83 ± 7	81 ± 5	64 ± 3
	Cytotoxic activity	42 ± 10	14 ± 0	0
N-(Indol-3-ylmethyl)-*N*-methyl-*N*′-propylthiourea (**51**)	Antifungal activity	35 ± 3	30 ± 4	0
	Cytotoxic activity	38 ± 6	14 ± 6	0
N-(Indol-3-ylmethyl)-*N*-methyl-*N*′-phenylthiourea (**52**)	Antifungal activity	100 ± 0	100 ± 0	73 ± 3
	Cytotoxic activity	0	0	0
N-(Naphthalen-2-ylmethyl)-*N*′-propylthiourea (**53**)	Antifungal activity	100 ± 0	62 ± 1	0
	Cytotoxic activity	0	0	0
N-(Naphthalen-2-ylmethyl)-*N*′-phenylthiourea (**54**)	Antifungal activity	81 ± 4	39 ± 4	0
	Cytotoxic activity	14 ± 6	0	0
N-Methyl-*N*-(naphthalen-2-ylmethyl)-*N*′-propylthiourea (**55**)	Antifungal activity	89 ± 6	32 ± 7	0
	Cytotoxic activity	0	0	0
N-Methyl-*N*-(naphthalen-2-ylmethyl)-*N*′-phenylthiourea (**56**)	Antifungal activity	68 ± 6	30 ± 4	0
	Cytotoxic activity	0	0	0
N-(Naphthalen-1-ylmethyl)-*N*′-propylthiourea (**57**)	Antifungal activity	90 ± 4	34 ± 4	0
	Cytotoxic activity	0	0	0
N-(Naphthalen-1-ylmethyl)-*N*′-phenylthiourea (**58**)	Antifungal activity	86 ± 3	46 ± 6	0
	Cytotoxic activity	0	0	0
N-Methyl-*N*-(naphthalen-1-ylmethyl)-*N*′-propylthiourea (**59**)	Antifungal activity	76 ± 4	31 ± 5	0
	Cytotoxic activity	14 ± 6	0	0

Table 1 (*continued*)

Compound	Biological activity	Percentage of activity		
		0.5 mM	0.2 mM	0.1 mM
N-Methyl-*N*-(naphthalen-1-ylmethyl)-*N*′-phenylthiourea (**60**)	Antifungal activity	60 ± 5	26 ± 3	0
	Cytotoxic activity	0	0	0
Indol-3-ylmethylsulfamide (**61**)	Antifungal activity	57 ± 5	29 ± 2	0
	Cytotoxic activity	43 ± 5	24 ± 7	0
N-(Indol-3-ylmethyl)-*N*-methylsulfamide (**62**)	Antifungal activity	43 ± 5	20 ± 7	0
	Cytotoxic activity	32 ± 6	14 ± 6	0
Naphthalen-2-ylmethylsulfamide (**63**)	Antifungal activity	48 ± 4	37 ± 8	0
	Cytotoxic activity	29 ± 6	0	0
N-Methyl-*N*-(naphthalen-2-ylmethyl)sulfamide (**64**)	Antifungal activity	40 ± 4	24 ± 6	0
	Cytotoxic activity	14 ± 5	0	0
Naphthalen-1-ylmethylsulfamide (**65**)	Antifungal activity	38 ± 4	0	0
	Cytotoxic activity	0	0	0
N-Methyl-*N*-(naphthalen-1-ylmethyl)sulfamide (**66**)	Antifungal activity	32 ± 6	0	0
	Cytotoxic activity	0	0	0
N-(Indol-3-ylmethyl)methanesulfonamide (**67**)	Antifungal activity	56 ± 5	39 ± 4	22 ± 3
	Cytotoxic activity	50 ± 6	29 ± 6	0
N-(Indol-3-ylmethyl)-*N*-methyl methanesulfonamide (**68**)	Antifungal activity	46 ± 4	28 ± 6	0
	Cytotoxic activity	32 ± 6	0	0
N-Methyl-*N*-(naphthalen-2-ylmethyl)methanesulfonamide (**69**)	Antifungal activity	38 ± 3	18 ± 3	0
	Cytotoxic activity	32 ± 12	0	0
N-Methyl-*N*-(naphthalen-2-ylmethyl)methanesulfonamide (**70**)	Antifungal activity	44 ± 3	0	0
	Cytotoxic activity	0	0	0
N-(Naphthalen-1-ylmethyl)methanesulfonamide (**71**)	Antifungal activity	55 ± 3	0	0
	Cytotoxic activity	14 ± 6	0	0
N-Methyl-*N*-(naphthalen-1-ylmethyl)methanesulfonamide (**72**)	Antifungal activity	38 ± 6	0	0
	Cytotoxic activity	0	0	0
Indolyl-3-propanamide (**73**)	Antifungal activity	48 ± 2	26 ± 5	0
	Cytotoxic activity	86 ± 12	0	0
N′-Methyl-indolyl-3-propanamide (**74**)	Antifungal activity	0	0	0
	Cytotoxic activity	64 ± 6	0	0

[a] Percent of inhibition = 100 − [(growth on medium containing compound/growth on control medium) × 100)] ± standard deviation.
[b] Percent of mortality = 100 − [(number of surviving larvae in solution containing compound/number of surviving larvae in control solution) × 100)] ± standard deviation.

cultures immediately after addition of brassinin (**1**) and at 24, 48, and 96 h, and were extracted with ethyl acetate. The organic extracts were analyzed by HPLC (photodiode array detection at 220 nm, brassinin t_R = 18.8 min) to determine the concentration of both brassinin (**1**) (remaining in the cultures at different times) and the indole-3-carboxaldehyde (**8**, t_R = 6.8 min), the product of brassinin biotransformation. Brassinin (**1**) and potential inhibitors **9**–**15** and **17**–**75** were stable in minimal media for at least 8 days. The rate of disappearance of brassinin (**1**) in the presence of the potential inhibitor was compared with that of control cultures (mycelial culture containing only brassinin at 0.1, 0.2, and 0.3 mM) by HPLC analyses of culture extracts. HPLC analyses of culture extracts showed that brassinin (**1**) at 0.1 mM was metabolized completely to indole-3-carboxaldehyde (**8**) in ca. 16 h, while in cultures containing brassinin (**1**) at higher concentrations, 0.2 and 0.3 mM, complete metabolism to **8** took place in ca. 24 and 48 h, respectively. As shown in Table 2, some of the compounds tested slowed down the metabolism of brassinin (**1**) relative to control cultures. That is, *N*′-methylbrassinin (**9**), methyl *N*-methyl-*N*-(naphthalen-2-ylmethyl)dithiocarbamate (**11**), tryptophol dithiocarbonate (**17**), and methyl 3-phenyldithiocarbazate (**20**) affected the rate of detoxification of brassinin (**1**) noticeably (Table 2). In the presence of methylbrassinin (**9**, 0.1 mM), brassinin (**1**, 0.1 mM) was completely metabolized to **8** in ca. 48 h (vs 16 h for brassinin at 0.1 mM), whereas doubling the concentration of **9** slowed down the transformation of brassinin (**1**) to 96 h (vs 24 h for brassinin at 0.2 mM). Importantly, no biotransformation of product of **9** was detected in any of the cultures. Less pronounced but similar results were obtained when the cultures were incubated with methyl *N*-methyl-*N*-(naphthalen-2-ylmethyl)dithiocarbamate (**11**) and brassinin (**1**), as shown in Table 2. On the other hand, methyl, *N*-benzyldithiocarbamate (**14**), and methyl *N*-phenyldithiocarbamate (**15**) did not affect the metabolism of brassinin (**1**).

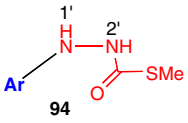
Table 2. Compounds that were metabolized (**9**, **11**, **14**, **17**, **20**, **48**, and **73**–**75** at 0.1 and 0.2 mM) or decreased the rate (**9**, **11**, **17**, and **20** at 0.1 and 0.2 mM) of brassinin detoxification in cultures of *Leptosphaeria maculans* co-incubated with brassinin (**1**, 0.1 mM) for different time periods (h)

Compounds at 0.1 mM and 0.2 mM	Biotransformation product (time required for complete transformation)	Incubation time, remaining brassinin (**1**) molar %[a]	
		0.1 mM	0.2 mM
Control (containing only brassinin (**1**))	Indole-3-carboxaldehyde (**8**)	16 h, <5%	24 h, <5%
N′-Methylbrassinin (**9**)[c]	No transformation	24 h, 25 ± 2	24 h, 94 ± 2
		48 h, n.d.[b]	48 h, 80 ± 4
		72 h, n.d.	72 h, 20 ± 5
		—	96 h, n.d.
Methyl *N*-methyl-*N*-(naphthalen-2-ylmethyl) dithiocarbamate (**11**)[c]	No transformation	24 h, 11 ± 2	24 h, 72 ± 4
		48 h, n.d.	48 h, <5%
Methyl *N*-benzyldithiocarbamate (**14**)	Benzoic acid	24 h, n.d.	24 h, n.d.
Tryptophol dithiocarbonate (**17**)	Tryptophol (120 h) (**88**)	24 h, 83 ± 4	24 h, 97 ± 3
		48 h, 75 ± 4	48 h, 90 ± 4
		72 h, 49 ± 5	72 h, 68 ± 4
		96 h, n.d.	96 h, <5%
Methyl 3-phenyldithiocarbazate (**20**)	Methyl 3-phenylthiocarbazate (48 h) (**94**)	24 h, 19 ± 3	24 h 68 ± 4
		48 h, n.d.	48 h, <5%
N-(Indol-3-ylmethyl)-*N*′-methylthiourea (**48**)	Indole-3-carboxaldehyde (30 h) (**8**)	24 h, n.d.	24 h, n.d.
3-(Indol-3-yl)propanamide (**73**)	3-(Indol-3-yl)propanoic acid (48 h) (**92**)	24 h, n.d.	24 h, n.d.
N′-Methyl-3-(indol-3-yl)propanamide (**74**)	3-(Indol-3-yl)propanoic acid (168 h) (**92**)	24 h, n.d.	24 h, n.d.
Methyl 3-(indol-3-yl)propanoate (**75**)	3-(Indol-3-yl)propanoic acid (6 h) (**92**)	24 h, n.d.	24 h, n.d.

[a] Percentages were determined using calibration curves and are averages of at least two independent experiments conducted in duplicate ± standard deviation.
[b] n.d., not detected.
[c] Compound not metabolized.

Complete detoxification of brassinin (**1**, 0.1 mM) in the presence of tryptophol dithiocarbonate (**17**, 0.1 mM) took place in 96 h (vs 16 h for brassinin alone); similar results were obtained with **17** at 0.2 mM. Tryptophol dithiocarbonate (**17**) was hydrolyzed to tryptophol (**88**) in 120 h (enzymatic transformation). Methyl 3-phenyldithiocarbazate (**20**) slowed down the transformation of brassinin (**1**, 0.1 mM) from 24 to 48 h, although **20** was also metabolized to **94** within 48 h of incubation (Table 2).

Of the remaining synthetic compounds that were screened for the inhibitory activity, only compounds **48** and **73**–**75** were metabolized by *L. maculans*. The thiourea **48** was biotransformed to **8** in 30 h whereas **72**–**75** were metabolized to 3-(indol-3-yl)propanoic acid (**92**) in 48, 168, and 6 h, respectively, without affecting the metabolism of brassinin (**1**). Compounds **10**, **12**–**14**, **18**, **19**, and **21**–**72** were not biotransformed by *L. maculans* and did not appear to affect noticeably the rate of metabolism of brassinin (**1**).



94

3. Discussion and conclusion

Because brassinin (**1**) detoxification by *L. maculans* can deprive plants of crucial natural chemical defenses, it is of great importance to understand and inhibit this process. Selective inhibitors of brassinin detoxification are potential protection agents against blackleg disease of crucifer crops. To design potential inhibitors of brassinin detoxification, the structures of brassinin (**1**) and its metabolic products **7** and **8** were modified to incorporate a wide range of functional groups (**9**–**75**). The designed compounds were synthesized (**9**–**15** and **17**–**75**) and their bioactivities and metabolism in cultures of *L. maculans* were investigated. The antifungal and cytotoxic activity of these compounds indicated that dithiocarbamates, dithiocarbazates, ureas, and thioureas were highly toxic to *L. maculans* and *A. salina*, with dithiocarbamates causing 100% death in larvae of *A. salina* even at 0.1 mM. By contrast, compounds having carbamate, sulfamide, sulfonamide, amide or ester functional groups had either very little or no activity against *L. maculans* or *A. salina*. Interestingly, indolyl and phenyl dithiocarbamates **1**, **9**, **14**, and **15** were more antifungal than their naphthalenyl analogs **10**–**13**, whereas in the case of ureas and thioureas, the naphthalenyl substituted compounds (**31**–**46**) were more active than the indolyl-substituted analogs (**22**–**30**). Among the various naphthalenyl compounds, substitution at C-2 led to higher activity than substitution at C-1 (Table 2). Compounds containing functional groups identical or related to compounds **9**–**75** are known to display pesticidal and/or other biological activities.[35]

Compounds **9**–**15** and **17**–**75** were screened for biotransformation and inhibition of brassinin detoxification in cultures of *L. maculans* by co-incubating these compounds with brassinin (**1**). A noticeable decrease in the rate of brassinin detoxification was observed in the presence of *N*′-methylbrassinin (**9**), dithiocarbamate **11**,

dithiocarbonate **17**, and dithiocarbazate **20**. In agreement with previous results,[3] modification of the aromatic substituent of brassinin (**1**) did not prevent metabolic degradation; however, the presence of the $–CH_2–NH–C{=}S–$ moiety on the side chain was essential for metabolism to occur. That is, replacement of the (N)H with a (N)–CH_3 group or deletion of the methylene bridge prevented the degradation of dithiocarbamates **9**, **11**, and **15**. This result is of great interest as it suggests that the (N)H of the $–NH–C{=}S–$ moiety might be playing an important role in substrate recognition by the putative brassinin oxidase. In addition, this result may explain the substantially higher antifungal activity of *N*′-methylbrassinin (**9**) relative to brassinin (**1**, Table 1) against *L. maculans*. Furthermore, the slower transformation of dithiocarbamates **10** and **13** (to 2- and 1-naphthoic acids, in 48 and 96 h, respectively) than that of brassinin (**1**, 16 h) might be due to either the different polarity of these naphthalenyl derivatives or/and the selectivity of brassinin oxidase. Furthermore, carbamate **17**, ureas **22**–**46**, sulfamides **61**–**66**, and sulfonamides **67**–**72** were not metabolized by *L. maculans*, whereas among thioureas **47**–**60**, only **48** was metabolized (to aldehyde **8** in 30 h). Structurally, **48** is similar to brassinin, having the S(CH_3) atom replaced with NH(CH_3); however, the rate of metabolism of **48** was much slower (30 vs 16 h for brassinin). Taken together, these metabolic results point out the selectivity of brassinin oxidase, although at this point the possibility that somewhat more polar molecules are unable to reach the metabolic cell site cannot be ruled out.

Overall, three types of interaction were observed in cultures of *L. maculans* co-incubated with the compounds under study (**9**–**15** and **17**–**75**) and brassinin (**1**): (1) a decrease on the rate of brassinin detoxification due to the strong inhibitory activity of the compound on mycelium growth (e.g., **9**, Fig. 2); (2) a decrease on the rate of brassinin detoxification due to the inhibitory activity of the compound on brassinin oxidase (e.g., **17**, Fig. 3); (3) a low to no detectable effect on the rate of brassinin detoxification (**10**, **12**, **13**, **15**, **18**, **19**, and **21**–**75**). It is also possible that the decrease on the rate of brassinin detoxification is due to strong inhibitory activity of the compound on both mycelium growth and brassinin oxidase; however, this hypothesis can be confirmed only by co-incubating compounds with brassinin oxidase or brassinin oxidase containing extracts. Therefore, further testing of compounds **9**, **11**, **17**, and **20** in brassinin oxidase containing fractions is necessary. In addition, it is necessary to understand the mechanistic details of brassinin oxidase mediated conversion of brassinin (**1**) to be able to design more effective inhibitors. Although compound **17** is a very good lead structure, compounds that are not metabolized in culture are more likely to protect oilseed crucifers against *L. maculans* infestation. It is expected that isolation and characterization of the brassinin oxidase involved in the detoxification of brassinin (**1**) will greatly facilitate the design of more effective and selective inhibitors.

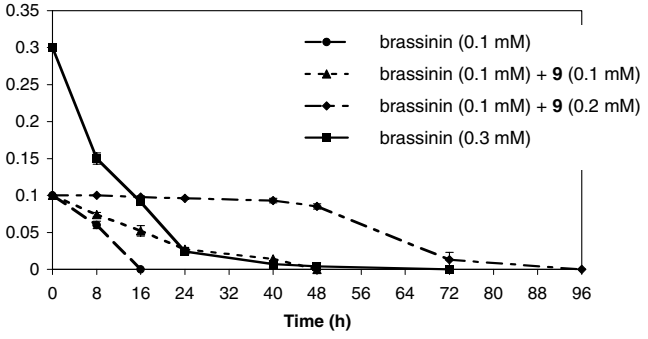


Figure 2. Progress curves for detoxification of brassinin (**1**, 0.1 mM) in the presence of different concentrations of *N*′-methylbrassiin (**9**, 0.1 and 0.2 mM) in cultures of *Leptosphaeria maculans*.

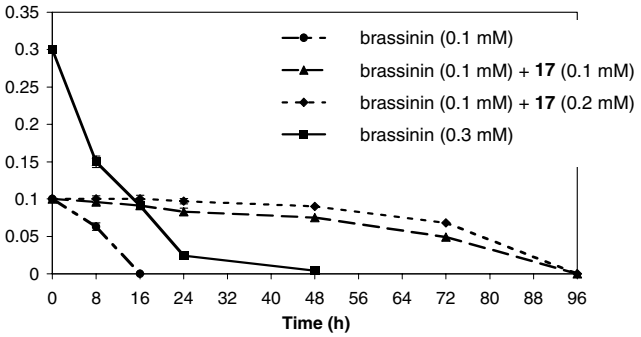


Figure 3. Progress curves for detoxification of brassinin (**1**, 0.1 mM) in the presence of tryptophol dithiocarbonae (**17**, 0.1 and 0.2 mM) in cultures of *Leptosphaeria maculans*.

4. Experimental

4.1. General experimental procedures

All chemicals were purchased from Sigma–Aldrich Canada Ltd, Oakville, ON. All solvents were of HPLC grade and used as such, except for CH_2Cl_2 and $CHCl_3$ that were redistilled. Solvents used in syntheses were dried over the following drying agents prior to use: THF and diethyl ether over sodium/benzophenone, CH_2Cl_2 and benzene over CaH_2. 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 to 100% CH_3CN, for 35 min, linear gradient, and a flow rate 1.0 mL/min. UV spectra were recorded on a Varian-Cary spectrophotometer in MeOH. NMR spectra were recorded on Bruker Avance 500 spectrometers; δ values were referenced as follows: for ^1H (500 MHz), $CDCl_3$ ($CHCl_3$ at 7.27 ppm), CD_3CN (CD_2HCN at 1.94 ppm), and $(CD_3)_2SO$ (CHD_2SOCD_3 at 2.50 ppm); for ^{13}C (125.8 MHz), $CDCl_3$ (77.2 ppm), CD_3CN (118.7 ppm), and $(CD_3)_2SO$ (39.5 ppm). Fourier transform infrared (FTIR) spectra

were recorded on Bio-Rad FTS-40 spectrometers. Mass spectra (MS) were obtained on a VG 70 SE mass spectrometer using a solid probe or on a Q Star XL, Applied Biosystems.

4.2. Fungal cultures

Fungal cultures of *L. maculans* 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.[36]

4.3. Antifungal bioassays

Virulent isolates of *L. maculans* were grown on potato dextrose agar (PDA) plates at 24 ± 1 °C under constant light for 7 days. The antifungal activity of compounds was determined following a mycelial radial growth bioassay, as described previously.[37] All bioassays were carried out in triplicate, at least two times.

4.4. Brine shrimp larvae bioassays

The cytotoxic activity of compounds was determined using brine shrimp larvae (*A. salina*). Shrimp eggs (1 scoop) were incubated in Petri dishes containing saline water (3.8% NaCl solution) under constant light and eggs were allowed to hatch for 24 h. Solutions of the compounds to be tested were prepared by serial dilutions (final concentrations 0.5, 0.2, and 0.1 mM in saline water with 1% MeOH, prepared from 50 mM stock solutions in MeOH) and dispensed (4 mL) into vials; 10 living shrimps were then added to each vial and final volume was made up to 5 mL using saline water. Control solutions (1% MeOH in saline water) containing 10 living larvae were prepared similarly. The vials were incubated for 24 h and the number of surviving larvae in each vial was counted and compared with that in the control vial. The experiments were performed in triplicate at least twice.

4.5. Metabolism and screening of potential detoxification inhibitors

Erlenmeyer flasks (125 mL, containing 50 mL of defined media) were inoculated with spores (10^8 spores/100 mL) of *L. maculans* and incubated at 24 ± 1 °C on shaker at 120 rpm under constant light for 48 h. The compound (stock solution in DMSO) to be screened was added to cultures (0.1 and 0.2 mM) and to uninoculated medium (control), and flasks were then incubated for 10 min on shaker at 120 rpm. Brassinin (final concentration 0.1 mM) was then added to the culture and cultures further incubated. Control cultures containing brassinin (**1**) or the compound only were incubated separately. Samples (2 mL) were withdrawn at different times and either frozen or immediately extracted with EtOAc (2× 4 mL). The organic phases were concentrated and analyzed by HPLC.

4.6. Syntheses

4.6.1. Brassinin (1). Carbon disulfide (45 μL, 0.75 mmol) was added to a solution of indol-3-ylmethylamine (**76**, 100 mg, 0.68 mmol) and triethylamine (191 μL, 1.44 mmol) in pyridine (1 mL) at 0 °C. After stirring for 20 min, MeI (49 μL, 0.75 mmol) was added and the reaction mixture was stirred for an additional 30 min. The reaction mixture was acidified with H_2SO_4 (5 mL, 1.5 M), was extracted with Et_2O, the organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. Fractionation by FCC (silica gel, EtOAc/hexane, 50:50) afforded brassinin (**1**, 153 mg, 95% yield) as a white solid. Mp: 132–133 °C, CH_2Cl_2 (lit.[38] 132–133 °C). HPLC t_R = 18.7 min. Spectroscopic data are identical to published data.

4.6.2. *N*′-Methylbrassinin (9). Preparation and separation as reported above for brassinin (**1**) substituting **77** (100 mg, 0.63 mmol) for indol-3-ylmethylamine (**76**). Red solid, 134 mg, 86% yield based on amine **77**. Mp: 68–69 °C, CH_2Cl_2. HPLC t_R = 21.7 min. ^{1}H NMR (500 MHz, CD_3CN) mixture of rotamers (1:2): δ 9.32 (br s, 1H, D_2O exchangeable), 7.73 (d, J = 7.5 Hz, 0.7H), 7.45 (d, J = 7.5 Hz, 1H), 7.37 (br s, 0.7H), 7.18 (dd, J = 7, 7 Hz, 1H), 7.08 (m, 1H), 5.53 (s, 1.3H), 3.22 (s, 2H), 2.66 (s, 3H); additional signals for minor rotamer 7.63 (br s, 0.3H), 7.31 (br s, 0.3H), 5.16 (s, 0.7H), 3.42 (s, 1H). ^{13}C NMR (500 MHz, CD_3CN) mixture of rotamers: δ 197.9, 136.5, 126.6, 125.3, 122.0, 119.4, 119.2, 111.5, 110.1, 51.1, 37.6, 19.7; additional signals for minor rotamer 197.1, 124.7, 119.6, 118.6, 111.7, 109.3, 49.4, 42.2. FTIR ν_{max} (KBr): 3409, 480, 1388, 745 cm^{-1}. HREIMS: m/z measured 250.0604 (250.0598 calculated for $C_{12}H_{14}N_2S_2$). EIMS m/z (% relative abundance) 250 (M^+, 35), 130 (100), 121 (15).

4.6.3. Methyl *N*-(naphthalen-2-ylmethyl)dithiocarbamate (10). Preparation as reported above for brassinin (**1**), substituting **78** (200 mg, 1.27 mmol) for indol-3-ylmethylamine (**76**). The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/hexane, 80:20) to afford methyl *N*-(naphthalen-2-ylmethyl)dithiocarbamate (**10**, 283 mg, 90% yield based on amine **78**) as a white solid. Mp: 72–73 °C, CH_2Cl_2/hexane. HPLC t_R = 24.2 min. Spectroscopic data are identical to previously reported data.[5]

4.6.4. Methyl *N*-methyl-*N*-(naphthalen-2-ylmethyl)dithiocarbamate (11). Preparation as reported above for brassinin (**1**), substituting **79** (100 mg, 0.58 mmol) for indol-3-ylmethylamine (**76**). The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/hexane, 80:20) to afford methyl *N*-methyl-*N*-(naphthalen-2-ylmethyl)dithiocarbamate (**11**, 134 mg, 88% yield based on amine **79**) as a white solid. Mp: 83–84 °C, CH_2Cl_2/hexane. HPLC t_R = 28.6 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) mixture of rotamers (1:2): δ 7.90 (m, 3H), 7.62 (br s, 0.7), 7.51 (br s, 2H), 7.43 (d, J = 8 Hz, 0.7H), 5.51 (s, 1.4H), 3.33 (s, 2H), 2.62 (s, 2H); additional signals for minor rotamer 7.71 (br s, 0.3H), 7.39 (d, J = 8 Hz, 0.3H), 5.22 (s, 0.7H), 3.50 (s, 1H), 2.58 (s, 1H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 199.3, 134.5, 133.7, 133.2, 129.2, 128.5, 128.4, 122.2, 126.9, 126.8, 126.4, 59.6, 21.0; additional signals for minor rotamer 192.2, 133.9, 129.4, 127.3, 127.0, 125.8, 57.8, 20.8. FTIR ν_{max} (KBr): 3054, 2918, 1481, 1382, 959, 752 cm^{-1}. HRE-

IMS: *m/z* measured 261.0644 (261.0646 calculated for $C_{14}H_{15}NS_2$). EIMS *m/z* (% relative abundance) 225 (M^+, 41), 141 (100), 115 (25).

4.6.5. Methyl *N*-(naphthalen-1-ylmethyl)dithiocarbamate (12). Preparation as reported above for brassinin (**1**), substituting **80** (150 mg, 0.95 mmol) for indol-3-ylmethylamine (**76**). The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/hexane, 80:20) to afford methyl *N*-(naphthalen-1-ylmethyl)dithiocarbamate (**12**, 212 mg, 90% yield from amine **80**) as a yellowish solid Mp: 85–86 °C, CH_2Cl_2/hexane. HPLC t_R = 23.9 min. Spectroscopic data are identical to previously reported data.[5]

4.6.6. Methyl *N*-methyl-*N*-(naphthalen-1-ylmethyl)dithiocarbamate (13). Preparation as reported above for brassinin (**1**), substituting **81** (100 mg, 0.58 mmol) for indol-3-ylmethylamine (**76**). The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/hexane, 80:20) to afford methyl *N*-methyl-*N*-(naphthalen-1-ylmethyl)dithiocarbamate (**13**, 130 mg, 85% yield from amine **81**) as a white solid. Mp: 132–135 °C, CH_2Cl_2/hexane. HPLC t_R = 27.8 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) mixture of rotamers (1:2): ^{1}H NMR (500 MHz, $(CD_3)_2SO$) mixture of rotamers (1:2): δ 7.99 (m, 2H), 7.89 (d, J = 8 Hz, 1H), 7.57 (m, 3H), 7.49 (br s, 1H, D_2O exchangeable), 7.21 (d, J = 6.5 Hz, 0.7H), 5.58 (s, 1.2H), 3.28 (s, 2H), 2.64 (s, 2H); additional signals for minor rotamer 7.07 (d, J = 6.5 Hz, 0.3H), 5.56 (s, 0.7H), 3.53 (s, 1H), 2.55 (s, 1H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 199.1, 134.2, 132.0, 131.6, 129.5, 128.8, 127.4, 126.9, 126.4, 125.6, 124.0, 58.0, 44.8, 21.0; additional signals for minor rotamer 198.8, 131.4, 131.1, 127.0, 126.5, 123.7, 123.6, 55.9, 20.8. FTIR ν_{max} (KBr): 2924, 1633, 1340, 1484, 1383, 800 cm^{-1}. HREIMS: *m/z* measured 261.0652 (261.0646 calculated for $C_{14}H_{15}NS_2$). EIMS *m/z* (% relative abundance) 261 (M^+, 44), 141 (100), 115 (21).

4.6.7. Tryptophol dithiocarbonate (17). Carbon disulfide (82 μL, 1.36 mmol) was added to a mixture of tryptophol (**88**, 200 mg, 1.24 mmol) and NaH (95 mg, a 60% suspension in oil, 2.48 mmol) in THF (5.0 mL) at 0 °C. After stirring the reaction mixture for 5 min, MeI (150 μL, 1.36 mmol) was added and the reaction mixture was allowed to stir for an additional 10 min at 0 °C. After dilution with H_2O (20 mL) and extraction with EtOAc, the extract was dried (Na_2SO_4) and concentrated to afford **17** (148 mg, 93% yield) as an off-white solid. Mp: 54–55 °C, EtOAc. HPLC t_R = 28.0 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.15 (br s, 1H, D_2O exchangeable), 7.64 (d, J = 8 Hz, 1H), 7.42 (d, J = 8 Hz, 1H), 7.16 (m, 2H), 7.08 (dd, J = 7.5, 7.5 Hz, 1H), 4.87 (t, J = 7 Hz, 2H), 3.26 (t, J = 7, 2 H), 2.53 (s, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 216.6, 136.8, 127.8, 123.5, 122.0, 119.3, 118.8, 111.8, 111.0, 74.5, 24.3, 18.6. FTIR ν_{max} (KBr): 3424, 1218, 1064, 744 cm^{-1}. HREIMS: *m/z* measured 251.0431 (251.0438 calculated for $C_{12}H_{13}NOS_2$). EIMS *m/z* (% relative abundance) 251 (M^+, 5), 143 (100), 115 (10).

4.6.8. Methyl *N*-(indol-3-ylmethyl)carbamate (18). A solution of *N*,*O*-bis(trimethylsilyl)acetamide (367 μL, 1.5 mmol) in CH_2Cl_2 (1 mL) was added to a solution of indol-3-ylmethylamine (**76**, 146 mg, 1.0 mmol) in solvent CH_2Cl_2 (6 mL) and the mixture was stirred at rt for 30 min. The reaction was then cooled to 0 °C and a solution of methyl chloroformate (115 μL, 1.5 mmol) in CH_2Cl_2 (1.5 mL) was added. The reaction mixture was allowed to stir at 0 °C for an additional 60 min, was quenched with H_2O (10 mL), and was extracted with EtOAc, and the organic extract was dried (Na_2SO_4) and concentrated. The residue obtained was subjected to FCC (silica gel, CH_2Cl_2) to give methyl *N*-(indol-3-ylmethyl)carbamate (**18**) (163 mg, 80% yield from amine **76**) as a white solid. Mp: 82–83 °C, CH_2Cl_2. HPLC t_R = 9.2 min. Spectroscopic data are identical to previously reported data.[10]

4.6.9. Methyl *N*-(indol-3-ylmethyl)-*N*-methylcarbamate (19). Preparation as reported above for methyl *N*-(indol-3-ylmethyl)carbamate (**18**) substituting **77** for indol-3-ylmethylamine (**76**). The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford methyl *N*-(indol-3-ylmethyl)-*N*′-methylcarbamate (**19**) as a light brown oil (185 mg, 85% yield). HPLC t_R = 12.4 min. ^{1}H NMR (500 MHz, CD_3CN) Mixture of rotamers: δ 9.22 (br s, 1H, D_2O exchangeable), 7.63 (br s, 1H), 7.43 (d, J = 8 Hz, 1H), 7.23 (s, 1H), 7.16 (dd, J = 7, 8 Hz, 1H), 7.07 (dd, J = 7.5, 7 Hz, 1H), 4.61 (s, 2H), 3.74 (br s, 1H), 3.69 (br s, 2H), 3.77 (s, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 156.7, 137.0, 127.2, 124.8, 122.1, 119.6, 119.4, 111.9, 111.8, 52.4, 43.7, 32.4. FTIR ν_{max} (KBr): 3308, 1684, 1454, 741 cm^{-1}. HREIMS: *m/z* measured 218.1059 (218.1055 calculated for $C_{12}H_{14}N_2O_2$). EIMS *m/z* (% relative abundance) 218 (M^+, 67), 130 (100) 118 (11).

4.6.10. Methyl 3-phenyldithiocarbazate (20). Carbon disulfide (65 μL, 1.0 mmol) was added to a mixture of phenylhydrazine (**84**, 100 μL, 0.93 mmol) and KOH (156 mg, 2.80 mmol) in ethanol at 0 °C. After stirring the reaction mixture for 30 min, MeI (66 μL, 1.02 mmol) was added and the reaction mixture was allowed to stir for an additional 60 min at 0 °C. The excess of solvent was removed and the residue was diluted with H_2O (20 mL), was extracted with EtOAc, the extract was dried (Na_2SO_4) and concentrated. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/hexane, 80:20) to afford **20** (174 mg, 95% yield) as an off-white solid. Mp: 133–134 °C (lit.[11] 133 °C), CH_2Cl_2/hexane. HPLC t_R = 17.5 min. Spectroscopic data are identical to previously reported data.[39]

4.6.11. Methyl 3-benzyldithiocarbazate (21). Preparation as reported above for methyl 3-phenyldithiocarbazate (**20**) substituting benzylhydrazine dihydrochloride (**85**, 150 mg, 0.77 mmol) for phenylhydrazine (**84**). The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/hexane, 80:20) to afford methyl 3-benzyldithiocarbazate (**21**) (94 mg, 87% yield) as a white solid. Mp: 76–77 °C. HPLC t_R = 20.3 min. Spectroscopic data are identical to previously reported data.[12]

4.6.12. Indol-3-ylmethylurea (22). A mixture of urea (2.0 g, 33.3 mmol), gramine (**89**, 500 mg, 2.87 mmol),

and crushed sodium hydroxide (100 mg, 2.5 mmol) was heated at 134 °C. After heating the reaction mixture for 120 min, ice-cold water (50 mL) was added with vigorous stirring. The white precipitate formed was filtered and crystallized to afford **22** (510 mg, 94% yield) as white crystals. Mp: 149–151 °C, water. HPLC t_R = 4.8 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.27 (br s, 1H, D_2O exchangeable), 7.63 (d, J = 8 Hz, 1H), 7.42 (d, J = 8 Hz, 1H), 7.16 (m, 2H), 7.07 (dd, J = 8, 8 Hz, 1H), 5.42 (br s, 1H, D_2O exchangeable), 4.72 (br s, 2H, D_2O exchangeable), 4.42 (d, J = 5 Hz, 2H). ^{13}C NMR (500 MHz, CD_3CN): δ 158.8, 136.6, 126.6, 123.2, 121.6, 119.0, 118.7, 113.7, 111.3, 35.2. FTIR ν_{max} (KBr): 3396, 1630, 1591, 742 cm^{-1}. HREIMS: *m*/*z* measured 189.0899 (189.0902 calculated for $C_{10}H_{11}N_3O$). EIMS *m*/*z* (% relative abundance) 189 (M^+, 98), 145 (50), 130 (100), 118 (40).

4.6.13. *N*-(Indol-3-ylmethyl)-*N*$_b$-methylurea (23). A solution of indol-3-ylmethylamine (**76**, 100 mg, 0.63 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a vigorously stirred mixture of $COCl_2$ (49 μL, 0.69 mmol) and $CaCO_3$ (76 mg, 0.76 mmol) in CH_2Cl_2 (3 mL) and H_2O (5 mL). After stirring for 5 min at rt, the CH_2Cl_2 layer was separated and the aqueous layer was extracted with additional CH_2Cl_2 (5 mL). The organic layer was concentrated, and the residue was re-suspended into CH_2Cl_2 (5 mL). A solution of methylamine (8.0 μL, 3.2 mmol) in THF was added to the reaction mixture and the mixture was allowed to stir for 30 min, the solvent was evaporated and the residue was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **23** (103 mg, 74% yield) as a white solid. Mp: 119–120 °C, CH_2Cl_2/MeOH. HPLC t_R = 5.3 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.26 (br s, 1H, D_2O exchangeable), 7.63 (d, J = 8 Hz, 1H), 7.41 (d, J = 8 Hz, 1H), 7.16 (m, 2H), 7.06 (dd, J = 7, 7.5 Hz, 1H), 5.26 (br s, 1H, D_2O exchangeable), 4.91 (br s, 1H, D_2O exchangeable), 4.44 (d, J = 5 Hz, 2H), 2.65 (d, J = 5 Hz, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 159.0, 136.6, 126.7, 123.1, 121.6, 118.9, 118.8, 114.0, 111.3, 35.2, 26.1. FTIR ν_{max} (KBr): 3405, 3308, 1629, 1572, 1256, 743 cm^{-1}. HREIMS: *m*/*z* measured 203.1059 (203.1058 calculated for $C_{11}H_{13}N_3O$). EIMS *m*/*z* (% relative abundance) 203 (M^+, 100), 145 (57), 130 (93), 118 (35).

4.6.14. *N*-Ethyl-*N*′-(indol-3-ylmethyl)urea (24). Ethylisocyanate (95 μL, 1.2 mmol) was added to a solution of indol-3-ylmethylamine (**76**, 146 mg, 1.0 mmol) in CH_2Cl_2 (5 mL). After allowing the reaction mixture to stir for 15 min at rt, the solvent was evaporated and the crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **24** (182 mg, 84% yield) as a white solid. Mp: 170–172 °C, CH_2Cl_2/MeOH. HPLC t_R = 7.4 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.74 (br s, 1H, D_2O exchangeable), 8.20 (d, J = 7.5 Hz, 1H), 7.98 (d, J = 8 Hz, 1H), 7.72 (m, 2H), 7.63 (dd, J = 7, 8 Hz, 1H), 5.67 (br s, 1H, D_2O exchangeable), 5.45 (br s, 1H, D_20 exchangeable), 5.0 (d, J = 5, Hz, 2H), 3.68 (m, 2H), 01.61 (t, J = 7 Hz, 3H). ^{13}C NMR (500 MHz, CD_3CN): 163.9, 142.3, 132.4, 128.9, 128.8, 127.3, 124.6, 124.5, 119.8, 117.0, 40.8, 40.3, 20.7. FTIR ν_{max} (KBr): 3440, 3234, 1580, 1352, 734 cm^{-1}. HREIMS: *m*/*z* measured 217.1214 (217.1215 calculated for $C_{12}H_{15}N_3O$). EIMS *m*/*z* (% relative abundance) 217 (M^+, 90), 172 (8), 145 (80), 130 (100), 118 (30), 77 (12).

4.6.15. *N*-(Indol-3-ylmethyl)-*N*′-propylurea (25). Preparation as reported above for urea (**24**), starting from **76** (100 mg, 0.68 mmol) and substituting propylisocyanate for ethylisocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **25** (138 mg, 87% yield) as a white solid Mp: 145–147 °C, CH_2Cl_2/MeOH. HPLC t_R = 9.8 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.18 (br s, 1H, D_2O exchangeable), 7.63 (d, J = 8 Hz, 1H), 7.41 (d, J = 8 Hz, 1H), 7.15 (m, 2H), 7.06 (dd, J = 8, 7 Hz, 1H), 5.12 (br s, 1H D_2O exchangeable), 4.94 (br s, 1H D_20 exchangeable), 4.44 (d, J = 5 Hz, 2H), 3.06 (m, 2H), 1.45 (m, 2H), 0.87 (t, J = 7 Hz, 3H). ^{13}C NMR (500 MHz, CD_3CN): 158.7, 137.1, 127.1, 123.5, 122.0, 119.3, 119.2, 114.5, 111.7, 41.9, 35.6, 23.7, 11.0. FTIR ν_{max} (KBr): 3316, 1626, 1571, 742 cm^{-1}. HREIMS: *m*/*z* measured 231.1369 (231.1371 calculated for $C_{13}H_{17}N_3O$). EIMS *m*/*z* (% relative abundance) 231 (M^+, 72), 145 (50), 130 (100), 102 (35), 73 (27).

4.6.16. *N*-(Indol-3-ylmethyl)-*N*′-phenylurea (26). Preparation as reported above for *N*-ethyl-*N*′-(indol-3-ylmethyl)urea (**24**), starting from **76** (200 mg, 1.37 mmol) and substituting phenylisocyanate for ethylisocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **26** (344 mg, 95% yield) as a white solid. Mp: 188–190 °C, CH_2Cl_2/MeOH (lit.[14] 195–196 °C). HPCL t_R = 15.3 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.18 (br s, 1 H, D_2O exchangeable), 7.68 (d, J = 8 Hz, 1H), 7.42 (m, 3H), 7.24 (m, 3H), 7.17 (m, 2H), 7.08 (dd, J = 8, 8 Hz, 1H), 6.97 (dd, J = 7.5, 7.5, 1H), 5.43 (br s, 2H, D_2O exchangeable), 4.54 (d, J = 5 Hz, 2H). ^{13}C NMR (500 MHz, CD_3CN): 155.3, 140.3, 136.7, 128.7, 126.6, 123.4, 121.8, 121.7, 119.1, 118.8, 118.5, 113.5, 111.4, 35.0. FTIR ν_{max} (KBr): 3316, 1595, 1561, 738 cm^{-1}. HREIMS: *m*/*z* measured 265.1214 (265.1215 calculated for $C_{16}H_{15}N_3O$). EIMS *m*/*z* (% relative abundance) 265 (M^+, 52), 172 (12), 130 (100), 93 (64).

4.6.17. *N*-(Indol-3-ylmethyl)-*N*-methylurea (27). A mixture of *N*-(indol-3-ylmethyl)-*N*-methylamine (**77**, 130 mg, 0.81 mmol) and KNCO (98 mg, 1.2 mmol) in MeOH (5 mL) was allowed to reflux for 60 min. The reaction mixture was cooled to rt and the solvent was evaporated. The residue was suspended into H_2O (20 mL), was extracted with EtOAc, and the organic extracts were combined, dried over Na_2SO_4, and concentrated to dryness. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **27** (145 mg, 88% yield) as a white solid Mp: 141–142 °C, CH_2Cl_2/MeOH. HPLC t_R = 4.1 min. ^{1}H NMR (500 MHz,CD_3CN): δ 9.32 (br s, 1H, D_2O exchangeable), 7.66 (d, J = 8 Hz, 1H), 7.42 (d, J = 8 Hz, 1H), 7.21 (s, 1H), 7.15 (dd, J = 7.5, 7.5. Hz, 1H), 7.06 (dd, J = 7.5, 7.5 Hz, 1H), 4.89 (br s, 2H, D_2O exchangeable), 4.60 (s, 2H), 2.75 (s, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 159.2, 137.2, 127.2, 124.3, 122.1, 119.5, 119.4, 112.6, 111.7, 43.3, 33.4. FTIR ν_{max} (KBr): 3234,

1642, 1593, 744 cm^{-1}. HREIMS: *m/z* measured 203.1053 (203.1059 calculated for $C_{11}H_{13}N_3O$). EIMS *m/z* (% relative abundance) 203 (M^+, 81), 145 (48), 130 (100), 74 (86).

4.6.18. *N*-Ethyl-*N*′-(indol-3-ylmethyl)-*N*′-methylurea (28). Ethylisocyanate (60 μL, 0.75 mmol) was added to a solution of *N*-(indol-3-ylmethyl)-*N*-methylamine (**77**, 100 mg, 0.63 mmol) in CH_2Cl_2 (5 mL). After allowing the reaction mixture to stir for 15 min at rt, the solvent was evaporated and the crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **28** (124 mg, 86% yield) as a white solid. Mp: 118–121 °C, CH_2Cl_2/MeOH. HPLC t_R = 9.2 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.19 (br s, 1H, D_2O exchangeable), 7.65 (d, J = 8 Hz, 1H), 7.41 (d, J = 8 Hz, 1H), 7.19 (d, J = 3.5 Hz, 1H), 7.15 (dd, J = 8, 8 Hz, 1H), 7.05 (dd, J = 8, 8 Hz, 1H), 5.13 (br s, 1H, D_2O exchangable), 4.78 (s, 2H), 3.20 (m, 2H), 2.73 (s, 3H), 1.10 (t, J = 7 Hz, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 158.6, 137.1, 127.3, 124.2, 122.0, 119.6, 119.4, 112.9, 111.7, 43.2, 35.7, 33.1, 15.4. FTIR ν_{max} (KBr): 3234, 1627, 1530, 743 cm^{-1}. HREIMS: *m/z* measured 231.1378 (231.1372 calculated for $C_{13}H_{17}N_3O$). EIMS *m/z* (% relative abundance) 231 (M^+, 76), 159 (18), 130 (100).

4.6.19. *N*-(Indol-3-ylmethyl)-*N*-methyl-*N*′-propylurea (29). Preparation as reported above for *N*-ethyl-*N*′-(indol-3-ylmethyl)-*N*′-methylurea (**28**), starting from **77** (220 mg, 1.37 mmol) and substituting propylisocyanate for ethylisocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **29** (269 mg, 80% yield) as a white solid. Mp: 116–118 °C, CH_2Cl_2/MeOH. HPLC t_R = 11.9 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.35 (br s, 1H, D_2O exchangeable), 7.65 (d, J = 8 Hz, 1H), 7.41 (d, J = 8 Hz, 1H), 7.19 (s, 1H), 7.15 (dd, J = 7, 8 Hz, 1H), 7.05 (dd, J = 7, 7.5 Hz, 1H), 5.19 (br s, 1H, D_2O exchangeable), 4.62 (s, 2H), 3.14 (m, 2H), 2.74 (s, 3H), 1.51 (m, 2 H), 0.89 (t, J = 7 Hz, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 158.7, 137.2, 127.3, 124.2, 122.0, 119.6, 119.4, 112.9, 111.7, 43.3, 42.7, 33.0, 23.8, 11.1. FTIR ν_{max} (KBr): 3245, 2963, 1628, 1531, 1351, 744 cm^{-1}. HREIMS: *m/z* measured 245.1538 (245.1541 calculated for $C_{14}H_{19}N_3S$). EIMS *m/z* (% relative abundance) 245 (M^+, 55), 159 (24), 130 (100), 77 (7).

4.6.20. *N*-(Indol-3-ylmethyl)-*N*-methyl-*N*′-phenylurea (30). Preparation as reported above for *N*-ethyl-*N*′-(indol-3-ylmethyl)-*N*′-methylurea (**28**), starting from **77** (200 mg, 1.25 mmol) and substituting phenylisocyanate for ethylisocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **30** (293 mg, 84% yield) as a white solid. Mp: 147–150 °C, CH_2Cl_2/MeOH. HPLC t_R = 16.8 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.29 (br s, 1H, D_2O exchangeable), 7.68 (d, J = 8 Hz, 1H), 7.47 (d, J = 8 Hz, 2H), 7.43 (d, J = 8 Hz, 1H), 3H), 7.27 (m, 3H), 7.14 (m, 2H), 7.07 (dd, J = 7, 7.5 Hz, 1H), 7.02 (dd, J = 7.5, 7.5 Hz, 1H), 4.73 (s, 2H), 2.93 (s, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 156.1, 140.8, 137.2, 129.1, 127.2, 124.5, 122.7, 122.1, 120.5, 119.6, 119.5, 112.4, 111.8, 49.3, 33.5. FTIR ν_{max} (KBr): 3281, 1643, 1526, 1444, 1238, 747 cm^{-1}. HREIMS: *m/z* measured 279.1374 (279.1372 calculated for $C_{17}H_{17}N_3O$). EIMS *m/z* (% relative abundance) 279 (M^+, 14), 160 (16), 130 (48), 119 (100), 93 (48).

4.6.21. Naphthalen-2-ylmethylurea (31). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*-methylurea (**27**) and substituting **78** (100 mg, 0.64 mmol) for **77**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **31** (103 mg, 81% yield) as a white solid. Mp: 193–194 °C, CH_2Cl_2/MeOH. HPLC t_R = 8.3 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 7.86 (m, 3H), 7.72 (s, 1H), 7.48 (m, 2H), 7.42 (d, J = 8.5 Hz, 1H), 6.52 (br s, 1 H, D_2O exchangeable), 5.57 (br s, 2H, D_2O exchangeable), 4.35 (d, J = 6 Hz, 2H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 159.1, 139.1, 133.4, 132.5, 128.2, 128.0, 127.9, 126.6, 126.3, 125.9, 125.3, 43.4. FTIR ν_{max} (KBr): 3355, 1651, 1597, 15568 cm^{-1}. HREIMS: *m/z* measured 200.0951 (200.0950 calculated for $C_{12}H_{12}N_2O$). EIMS *m/z* (% relative abundance) 200 (M^+, 100), 156 (98), 141 (24), 129 (35).

4.6.22. *N*-Ethyl-*N*′-(naphthalen-2-ylmethyl)urea (32). Preparation as reported for *N*-ethyl-*N*′-(indol-3-ylmethyl)urea (**24**) and substituting **78** (100 mg, 0.64 mmol) for **76**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **32** (117 mg, 81% yield) as a white solid. Mp: 156–158 °C, CH_2Cl_2/MeOH. HPLC t_R = 11.8 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 7.85 (m, 3H), 7.71 (s, 1H), 7.48 (m, 2H), 7.42 (d, J = 8.5 Hz, 1H), 6.40 (br s, 1 H, D_2O exchangeable), 5.92 (br s, 1H, D_2O exchangeable), 4.37 (d, J = 6 Hz, 2H), 3.05 (m, 2H), 1.01 (t, J = 7 Hz, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 158.5, 139.1, 133.4, 132.5, 128.2, 128.0, 127.9, 126.6, 126.4, 125.9, 125.4, 43.5, 34.6, 16.2. FTIR ν_{max} (KBr): 3340, 1622, 1572, 1264 cm^{-1}. HRESIMS: *m/z* measured 229.1341 (229.1340 calculated for $C_{14}H_{17}N_2O$), ESIMS *m/z* (% relative abundance) 229 ($M+1^+$, 100), 114 (29).

4.6.23. *N*-(Naphthalen-2-ylmethyl)-*N*′-propylurea (33). Preparation as reported above for *N*-ethyl-*N*′-(indol-3-ylmethyl)urea (**24**) and substituting **78** (150 mg, 0.96 mmol) for **76** and propylisothiocyanate for ethylisocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **33** (190 mg, 80% yield) as a white solid. Mp: 166–168 °C, CH_2Cl_2/MeOH. HPLC t_R = 14.2 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 7.85 (m, 3H), 7.71 (s, 1H), 7.47 (m, 2H), 7.41 (d, J = 8.5 Hz, 1H), 6.40 (br s, 1H, D_2O exchangeable), 5.97 (br s, 1H, D_2O exchangeable), 4.37 (d, J = 6 Hz, 2H), 2.99 (m, 2H), 1.39 (m, 3H), 0.84 (t, J = 7.5 Hz, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 158.6, 139.2, 133.4, 132.5, 128.2, 128.0, 127.9, 126.6, 126.4, 125.9, 125.3, 43.5, 41.7, 23.7, 11.8. FTIR ν_{max} (KBr): 3325, 1622, 1581, 1253 cm^{-1}. HRESIMS: *m/z* measured 243.1503 (243.1497 calculated for $C_{15}H_{19}N_2O$). ESIMS *m/z* (% relative abundance) 243 ($M+1^+$, 100).

4.6.24. *N*-(Naphthalen-2-ylmethyl)-*N*′-phenylurea (34). Preparation as reported for *N*-ethyl-*N*′-(indol-3-ylmethyl)urea (**24**) and substituting **78** (180 mg, 1.15 mmol)

for **76** and phenylisothiocyanate for ethylisocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **34** (250 mg, 80% yield) as a white solid. Mp: 188–190 °C, CH_2Cl_2/MeOH. HPLC t_R = 19.5 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 8.59 (s, 1H, D_2O exchangeable), 7.89 (m, 3H), 7.78 (s, 1H), 7.49 (m, 3H), 7.42 (d, J = 7.5 Hz, 2H), 7.22 (t, J = 7.5, 2H), 6.89 (t, J = 7.5, 1H), 6.71 (t, J = 6 Hz, 1H, D_2O exchangeable), 4.48 (d, J = 6 Hz, 2H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 155.7, 140.9, 138.5, 133.4, 132.6, 129.1, 128.4, 128.0, 127.9, 126.6, 126.3, 126.0, 125.5, 121.6, 118.2, 43.4. FTIR ν_{max} (KBr): 3317, 1633, 1597, 1246 cm^{-1}. HRESIMS: *m*/*z* measured 277.1331 (276.1335 calculated for $C_{18}H_{17}N_2O$). ESIMS *m*/*z* (% relative abundance) 277 ($M+1^+$, 100), 149 (4).

4.6.25. *N*-Methyl-*N*-(naphthalen-2-ylmethyl)urea (35). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*-methylurea (**27**) and substituting **79** (100 mg, 0.58 mmol) for **77**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **35** (95 mg, 76% yield) as a white solid. Mp: 182–183 °C, CH_2Cl_2/MeOH. HPLC t_R = 7.8 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 7.87 (m, 3H), 7.68 (s, 1H), 7.48 (m, 2H), 7.37 (d, J = 8.5 Hz, 1H), 5.99 (s, 1H, D_2O exchangeable), 4.56 (s, 2H), 2.77 (s, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 159.4, 137.1, 133.4, 132.6, 128.5, 128.0, 127.9, 126.6, 126.3, 126.0, 125.9, 51.6, 34.5. FTIR ν_{max} (KBr): 3371, 1665, 1532, 746 cm^{-1}. HREIMS: *m*/*z* measured 214.1105 (214.1106 calculated for $C_{13}H_{14}N_2O$). EIMS *m*/*z* (% relative abundance) 214 (M^+, 100), 197 (9), 170 (63), 141 (24), 115 (23).

4.6.26. *N*-Ethyl-*N*′-methyl-*N*′-(naphthalen-2-ylmethyl)urea (36). Preparation as reported for *N*-ethyl-*N*′-(indol-3-ylmethyl)-*N*′-methylurea (**28**) and substituting **79** (100 mg, 0.58 mmol) for **77**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **36** (112 mg, 79% yield) as a white solid. Mp: 150–151 °C, CH_2Cl_2/MeOH. HPLC t_R = 14.5 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 7.87 (m, 3H), 7.67 (s, 1H), 7.48 (m, 2H), 7.35 (d, J = 8.5 Hz, 1H), 6.43 br s, 1 H, D_2O exchangeable), 4.57 (s, 2H), 3.11 (m, 2H), 2,77 (s, 3H), 1.05 (t, J = 7 Hz, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 158.8, 137.5, 133.8, 133.0, 128.9, 128.4, 128.3, 127.0, 126.5, 126.3, 52.0, 35.9, 34.5, 16.6. FTIR ν_{max} (KBr): 3341, 2963, 1629, 1534 cm^{-1}. HREIMS: *m*/*z* measured 242.1411 (242.1419 calculated for $C_{15}H_{18}N_2O$). EIMS *m*/*z* (% relative abundance) 242 (M^+, 74), 170 (94), 141 (100), 115 (29).

4.6.27. *N*-Methyl-*N*-(naphthalen-2-ylmethyl)-*N*′-propylurea (37). Preparation as reported for *N*-ethyl-*N*′-(indol-3-ylmethyl)-*N*′-methylurea (**28**), substituting **79** (100 mg, 0.58 mmol) for **77** and propylisothiocyanate for ethylisocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **37** (122 mg, 82% yield) as a white solid. Mp: 158–159 °C, CH_2Cl_2/MeOH. HPLC t_R = 14.2 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 7.86 (m, 3H), 7.67 (s, 1H), 7.48 (m, 2H), 7.35 (d, J = 7.5 Hz, 1H), 6.44 (br s, 1H, D_2O exchangeable), 4.58 (s, 2H), 3.04 (m, 2H), 2,77 (s, 3H), 1.44 (m, 2H), 0.83 (t, J = 7.5 Hz, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 158.9, 137.5, 133.8, 133.0, 128.9, 128.4, 128.3, 127.0, 126.6, 126.5, 126.3, 52.0, 42.9, 34.6, 24.0, 12.2. FTIR ν_{max} (KBr): 3344, 2962, 1630, 1534 cm^{-1}. HREIMS: *m*/*z* measured 256.1574 (256.1577 calculated for $C_{16}H_{20}N_2O$). EIMS *m*/*z* (% relative abundance) 256 (M^+, 43), 170 (64), 141 (100), 115 (33).

4.6.28. *N*-Methyl-*N*-(naphthalen-2-ylmethyl)-*N*′-phenylurea (38). Preparation as reported for *N*-ethyl-*N*′-(indol-3-ylmethyl)-*N*′-methylurea (**28**), substituting **79** (120 mg, 0.70 mmol) for **77** and phenylisothiocyanate for ethylisocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **38** (152 mg, 91% yield) as a white solid. Mp: 133–135 °C, CH_2Cl_2/MeOH. HPLC t_R = 18.7 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 8.46 (s, 1H, D_2O exchangeable), 7.89 (m, 3H), 7.76 (s, 1H), 7.49 (m, 4H), 7.42 (d, J = 8.5 Hz, 1H), 7.24 (t, J = 8, 2 H), 6.89 (t, J = 7.5, 1H), 4.72 (s, 2H), 2.96 (s 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 156.6, 141.4, 137.0, 133.8, 133.1, 129.1, 129.0, 128.4, 128.4, 127.1, 126.7, 126.6, 126.5, 122.7, 120.1, 52.2, 35.2. FTIR ν_{max} (KBr): 3326, 3055, 1642, 1532, 750 cm^{-1}. HREIMS: *m*/*z* measured 290.1421 (290.1419 calculated for $C_{19}H_{18}N_2O$). EIMS *m*/*z* (% relative abundance) 290 (M^+, 30), 170 (19), 141 (100), 115 (15).

4.6.29. Naphthalen-1-ylmethylurea (39). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*-methylurea (**27**) and substituting **80** (100 mg, 0.63 mmol) for **77**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **39** (97 mg, 74% yield). Mp: 214–216 °C, CH_2Cl_2/MeOH. HPLC t_R = 8.1 min ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 8.10, (d, J = 8 Hz, 1H), 7.94 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 8 Hz, 1H), 7.53 (m, 2H), 7.45 (m, 2H), 6.42 (br s, 1H, D_2O exchangeable), 5.52 (s, 2H, D_2O exchangeable), 4.64 (d, J = 6 Hz, 2H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 158.9, 136.5, 133.8, 131.4, 128.9, 127.8, 126.6, 126.2, 125.9, 125.5, 124.05, 41.3. FTIR ν_{max} (KBr): 3430, 3339, 1649, 1600, 773 cm^{-1}. HRESIMS: *m*/*z* measured 200.0950 (200.0949 calculated for $C_{12}H_{12}N_2O$), ESIMS *m*/*z* (% relative abundance) 200 (M^+, 95), 141 (35), 129 (42).

4.6.30. *N*-Ethyl-*N*′-(naphthalen-1-ylmethyl)urea (40). Preparation as reported for *N*-ethyl-*N*′-(indol-3-ylmethyl)urea (**24**) and substituting **80** (164 mg, 1.04 mmol) for **76**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **40** (200 mg, 84% yield) as a white solid. Mp: 183–184 °C, CH_2Cl_2/MeOH. HPLC t_R = 11.6 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 8.09 (d, J = 8 Hz, 1H), 7.93 (d, J = 7.5 Hz, 1H), 7.82 (d, J = 8 Hz, 1H), 7.54 (m, 2H), 7.44 (m, 2H), 6.29 (br s, 1H, D_2O exchangeable), 5.85 (br s, 1H, D_2O exchangeable), 4.66 (d, J = 5.5 Hz, 2H), 3.04 (m, 2H), 0.99 (t, J = 7 Hz, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 158.3, 136.6, 133.8, 131.4, 128.9, 127.7, 126.6, 126.2, 125.9, 125.5, 124.0, 41.4,

34.6, 16.2. FTIR ν_{max} (KBr): 3330, 1618, 1580, 776 cm^{-1}. HREIMS: *m/z* measured 228.1260 (228.1262 calculated for $C_{14}H_{16}N_2O$). EIMS *m/z* (% relative abundance) 228 (M^+, 60), 156 (100), 141 (58), 129 (21).

4.6.31. *N*-(Naphthalen-1-ylmethyl)-*N*′-propylurea (41). Preparation as reported for *N*-ethyl-*N*′-(indol-3-ylmethyl)urea (**24**) and substituting **80** (160 mg, 1.02 mmol) for **76** and propylisocyanate for ethylisocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **41** (200 mg, 81% yield) as a white solid. Mp: 136–137 °C, CH_2Cl_2/MeOH. HPLC t_R = 14.1 min. ^1H NMR (500 MHz, $(CD_3)_2SO$): δ 8.09 (d, J = 8 Hz, 1H), 7.93 (d, J = 7.5 Hz, 1H), 7.82 (d, J = 8 Hz, 1H), 7.53 (m, 2H), 7.44 (m, 2H), 6.28 (br s, 1H, D_2O exchangeable), 5.90 (br s, 1H, D_2O exchangeable), 4.66 (d, J = 5.5 Hz, 2H), 2.98 (m, 2H), 1.38 (m, 2H), 0.83 (t, J = 7.5 Hz, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 158.4, 136.6, 133.8, 131.4, 128.9, 127.7, 126.6, 126.2, 125.9, 125.5, 124.0, 41.6, 41.4, 23.7, 11.8. FTIR ν_{max} (KBr): 3313, 1626, 1591, 792 cm^{-1}. HRESIMS: *m/z* measured 243.1497 (243.1491 calculated for $C_{15}H_{19}N_2O$). ESIMS *m/z* (% relative abundance) 243 ($M+1^+$, 100), 114 (11).

4.6.32. *N*-(Naphthalen-1-ylmethyl)-*N*′-phenylurea (42). Preparation as reported for *N*-ethyl-*N*′-(indol-3-ylmethyl)urea (**24**), substituting **80** (160 mg, 1.02 mmol) for **76** and phenylisocyanate for ethylisocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **42** (247 mg, 88% yield) as a white solid. Mp: 221–223 °C. HPLC t_R = 19.5 min. ^1H NMR (500 MHz, $(CD_3)_2SO$): δ 8.51 (s, 1H, D_2O exchangeable), 8.13 (d, J = 8 Hz, 1H), 7.95 (d, J = 8 Hz, 1H), 7.85 (d, J = 7.5 Hz, 1H), 7.56 (m, 2H), 7.49 (m, 2H), 7.41 (d, J = 8 Hz, 2H), 7.22 (t, J = 8 Hz, 2H), 6.89 (t, J = 7.5 Hz, 1H), 6.63 (br s, 1H, D_2O exchangeable), 4.77 (d, J = 5.5 Hz, 2H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 155.5, 140.9, 135.9, 133.8, 131.3, 121.1, 129.0, 128.0, 126.7, 126.3, 125.9, 125.8, 123.9, 122.6, 118.1, 41.2. FTIR ν_{max} (KBr): 3323, 1628, 1571, 778 cm^{-1} HREIMS: *m/z* measured 276.1260 (276.1262 calculated for $C_{18}H_{16}N_2O$). EIMS *m/z* (% relative abundance) 276 (M^+, 49), 156 (10), 141 (100), 115 (15).

4.6.33. *N*-Methyl-*N*-(naphthalene-1-ylmethyl)urea (43). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*-methylurea (**27**) and substituting **81** (100 mg, 0.58 mmol) for **77**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **43** (98 mg, 78% yield) as a white solid. Mp: 193–194 °C. HPLC t_R = 8.4 min. ^1H NMR (500 MHz, $(CD_3)_2SO$): δ 8.15, (br s, 1H), 7.93 (br s, 1H), 7.84 (d, J = 8 Hz, 1H), 7.52 (br s, 2H), 7.47 (t, J = 7 Hz, 1H), 7.31 (d, J = 7 Hz, 1H), 6.02 (br s, 1H, D_2O exchangeable), 4.88 (s, 2H), 2.74 (s, 3 H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 159.7, 134.9, 134.3, 132.2, 129.3, 128.9, 128.3, 126.9, 126.6, 126.5, 126.2, 125.7, 1245, 49.8, 34.6. FTIR ν_{max} (KBr): 3433, 3338, 1650, 1600, 774 cm^{-1}. HREIMS: *m/z* measured 214.1112 (214.1106 calculated for $C_{13}H_{14}N_2O$). EIMS *m/z* (% relative abundance) 214 (M^+, 21), 170 (88), 141 (100), 15 (19).

4.6.34. *N*-Ethyl-*N*′-methyl-*N*′-(naphthalen-1-ylmethyl)urea (44). Preparation as reported for *N*-ethyl-*N*′-(indol-3-ylmethyl)-*N*′-methylurea (**28**) and substituting **81** (100 mg, 0.58 mmol) for **77**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **44** (101 mg, 81% yield) as a white solid. Mp: 174–175 °C. HPLC t_R = 14.1 min. ^1H NMR (500 MHz, $(CD_3)_2SO$): δ 8.13 (br s, 1H), 9.93 (br s, 1H), 7.83 (d, J = 8 Hz, 1H), 7.52 (br s, 2H), 7.47 (t, J = 7.5 Hz, 1H), 7.27 (d, J = 7 Hz, 1H), 6.43 (br s, 1H, D_2O exchangeable), 4.89 (s, 2H), 3.11 (m, 2 H), 2.74 (s, 3H), 1.04(t, J = 7 Hz, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 158.7, 134.9, 134.3, 132.0, 129.3, 128.3, 126.9, 126.6, 126.2, 125.8, 124.5, 49.8, 35.9, 34.3, 16.6. FTIR ν_{max} (KBr): 3347, 1630, 1532, 785 cm^{-1}. HREIMS: *m/z* measured 242.1421(228.1419 calculated for $C_{15}H_{18}N_2O$). EIMS *m/z* (% relative abundance) 242 (M^+, 93), 170 (82), 141 (100), 15 (54).

4.6.35. *N*-Methyl-*N*-(naphthalen-1-ylmethyl)-*N*′-propylurea (45). Preparation as reported for *N*-ethyl-*N*′-(indol-3-ylmethyl)-*N*′-methylurea (**28**), substituting **81** (100 mg, 0.58 mmol) for **77** and propylisocyanate for ethylisocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **45** (119 mg, 80% yield) as a white solid. Mp: 147–148 °C. HPLC t_R = 14.2 min. ^1H NMR (500 MHz, $(CD_3)_2SO$): δ 8.12 (dd, J = 6, 6 Hz, 1H), 7.93 (dd, J = 6, 6 Hz, 1H), 7.83 (d, J = 8 Hz, 1H), 7.52 (dd, J = 6, 6 Hz, 2H), 7.47 (t, J = 7.5 Hz, 1H), 7.27 (t, J = 7 Hz, 1H), 6.41 (br s, 1H, D_2O exchangeable), 4.89 (s, 2H), 3.05 (m, 2H), 2.74 (s, 3H), 1.44 (m, 2H), 0.84 (t, J = 7.5 Hz, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 158.7, 135.0, 134.3, 132.0, 129.3, 128.3, 126.9, 126.6, 126.4, 125.7, 124.5, 49.9, 42.9, 34.3, 24.0, 12.2. FTIR ν_{max} (KBr): 3321, 1625, 1587 cm^{-1}. HREIMS: *m/z* measured 256.1574 (256.1575 calculated for $C_{16}H_{20}N_2O$). EIMS *m/z* (% relative abundance) 256 (M^+, 68), 170 (87), 141 (58), 15 (22).

4.6.36. *N*-Methyl-*N*-(naphthalen-1-ylmethyl)-*N*′-phenylurea (46). Preparation as reported for *N*-ethyl-*N*′-(indol-3-ylmethyl)-*N*′-methylurea (**28**), substituting **81** (100 mg, 0.58 mmol) for **77** and phenylisocyanate for ethylisocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **46** (146 mg, 86% yield) as a white solid. Mp: 153–154 °C, CH_2Cl_2/MeOH. HPLC t_R = 23.7 min. ^1H NMR (500 MHz, $(CD_3)_2SO$): δ 8.43 (s, 1H, D_2O exchangeable), 8.13 (d, J = 7.5 Hz, 1H), 7.96 (d, J = 7 Hz, 1H), 7.86 (d, J = 8 Hz, 1H), 7.54 (m, 5H), 7.37 (d, J = 7 Hz, 1H), 7.24 (t, J = 8 Hz, 2 H), 6.95 (t, J = 7.5 Hz, 1H), 5.04 (s, 2H), 2.93 (s, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 156.0, 141.0, 134.1, 133.9, 131.6, 129.0, 128.7, 128.0, 126.7, 126.3, 125.9, 125.5, 124.0, 122.3, 120.4, 49.6, 34.7. FTIR ν_{max} (KBr): 3328, 1631, 1531, 1245, 759 cm^{-1}. EIMS: *m/z* measured 290.1421 (290.1419 calculated for $C_{19}H_{18}N_2O$). EIMS *m/z* (% relative abundance) 290 (M^+, 38), 141 (100), 115 (11).

4.6.37. 3-Indolylmethylthiourea (47). A solution of 1-Boc-indol-3-ylmethylamine (220 mg, 0.85 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a vigorously

stirred mixture of $CSCl_2$ (71 μL, 0.94 mmol) and $CaCO_3$ (107 mg, 1.07 mmol) in CH_2Cl_2 (6 mL) and H_2O (10 mL). After stirring for 5 min at rt, the CH_2Cl_2 layer was separated and the aqueous layer was extracted with additional CH_2Cl_2 (5 mL). The organic layers were combined and concentrated, and the residue was subjected to FCC (silica gel, CH_2Cl_2) to afford 1-Boc-indol-3-ylmethylisothiocyanate (**91**) (196 mg, 71% yield) as a light yellow solid. Ammonia gas was bubbled into a solution of isothiocyanate **91** in CH_2Cl_2 (10 mL). After 60 min, CH_2Cl_2 was evaporated, and the residue was dissolved in MeOH, and Na (100 mg, 4.30 mmol cut in small pieces) was added to the solution. The mixture was stirred at rt for 60 min, the solvent was evaporated, and the residue was dissolved in H_2O (20 mL) and extracted with EtOAc. The organic layer was then evaporated and the residue was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **47** (59 mg, 40% yield) as an off-white solid. Mp: 132–133 °C, CH_2Cl_2/MeOH. HPLC t_R =: 6.7 min. ^{1}H NMR (500 MHz, CD_3CN) mixture of rotamers (1:3): δ 9.24 (br s, 1 H, D_2O exchangeable), 7.67 (br s, 1H), 7.44 (d, J = 8 Hz, 1H), 7.27 (s, 1H), 7.18 (dd, J = 7, 7.5 Hz, 1H), 7.09 (dd, J = 7.5, 7 Hz, 1H), 6.75 (br s, 1H, D_2O exchangeable), 5.98 (br s, 1H, D_2O exchangeable), 4.85 (br s, 2H); Additional peaks of minor rotamer 6.93 (br s, 0.5H, D_2O exchangeable), 5.98 (br s, 1H, D_2O exchangeable), 4.47 (br s, 0.5H). ^{13}C NMR (500 MHz, CD_3CN): δ 183.1, 136.5, 126.6, 123.8, 121.7, 119.1, 118.7, 111.7, 111.5, 40.2. FTIR ν_{max} (KBr): 3295, 1611, 1539, 1457, 1346, 716 cm^{-1}. HREIMS: *m/z* measured 205.0672 (205.0673 calculated for $C_{10}H_{11}N_3S$). EIMS *m/z* (% relative abundance) 205 (M^+, 45), 130 (100), 76 (25).

4.6.38. *N*-(Indol-3-ylmethyl)-*N′*-methylthiourea (48). Preparation as reported for indol-3-ylmethylthiourea (**47**) and substituting methylamine for ammonia. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **48** (76 mg, 50% yield) as an off-white solid. Mp: 110–112 °C, CH_2Cl_2. HPLC t_R = 8.5 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.22 (br s, 1H, D_2O exchangeable), 7.68 (d, J = 8.5 Hz, 1H), 7.43 (d, J = 8 Hz, 1H), 7.27 (s, 1H), 7.71 (dd, J = 7.5, 7.5 Hz, 1H), 7.08 (dd, J = 7.5, 7, Hz, 1H), 6.51 (br s, 1H, D_2O exchangeable), 6.32 (br s, 1H, D_2O exchangeable), 4.82 (br s, 2H), 2.89 (br s, 3H). ^{13}C NMR (500 MHz, CD_3CN) mixture of rotamers: ™ 190.9, 136.4, 126.7, 124.2, 121.8, 119.2, 118.7, 111.4, 111.1, 56.5, 40.6; additional peaks of minor rotamer 190.2, 126.5, 124.2, 119.3, 118.4, 111.5, 111.3, 57.5, 38.0. FTIR ν_{max} (KBr): 3347, 1520, 1454, 1337, 1201, 1144, 745 cm^{-1}. HREIMS: *m/z* measured 219.0827 (219.0830 calculated for $C_{11}H_{13}N_3S$). EIMS *m/z* (% relative abundance) 219 (M^+, 50), 130 (100), 90 (81).

4.6.39. *N*-(Indol-3-ylmethyl)-*N′*-propylthiourea (49). Indol-3-ylmethylamine (**76**, 210 mg, 1.44 mmol) was dissolved in CH_2Cl_2 (5 mL) and propylisothiocyanate (163 μL, 1.58 mmol) was added. After allowing the reaction mixture to stir for 15 min at rt, the solvent was evaporated and the crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **49** (305 mg, 86% yield) as a white solid. Mp: 114–116 °C, CH_2Cl_2. HPLC t_R = 14.8 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.21 (br s, 1H, D_2O exchangeable), 7.68 (d, J = 8 Hz, 1H), 7.43 (d, J = 8 Hz, 1H), 7.27 (d, J = 2 Hz, 1H), 7.18 (dd, J = 8, 8, Hz, 1H), 7.08 (dd, J = 8, 8 Hz, 1H), 6.45 (br s, 1H, D_2O exchangeable), 6.31 (br s, 1H, D_2O exchangeable), 4.82 (br s, 2H), 3.37 (br s, 2H), 1.53 (m, 2H), 0.87 (t, J = 7 Hz, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 183.1, 136.6, 126.6, 123.8, 121.8, 119.2, 118.3, 112.1, 111.4, 45.5, 39.6, 22.0, 10.5. FTIR ν_{max} (KBr): 3251, 1548, 1458, 1135, 744 cm^{-1}. HREIMS: *m/z* measured 247.1137 (247.1143 calculated for $C_{13}H_{17}N_3S$). EIMS *m/z* (% relative abundance) 247 (M^+, 50), 145 (12), 130 (100), 77 (11), 61 (39).

4.6.40. *N*-(Indol-3-ylmethyl)-*N′*-phenylthiourea (50). Preparation as reported for *N*-(indol-3-ylmethyl)-*N′*-propylthiourea (**49**), starting from **76** (200 mg, 1.37 mmol) and substituting phenylisothiocyanate for propylisothiocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **50** (345 mg, 90% yield) as a white solid. Mp: 153–155 (dec) °C, CH_2Cl_2. HPLC t_R = 18.0. ^{1}H NMR (500 MHz, CD_3CN): δ 9.21 (br s, 1H, D_2O exchangeable), 8.10(br s, 1H, D_2O exchangeable), 7.70 (d, J = 8 Hz, 1H), 7.43 (d, J = 8 Hz, 1H), 7.34 (dd, J = 8, 7.5 Hz, 2H), 7.28 (d, J = 7.5 Hz, 3H), 7.19 (m, 2H), 7.09 (dd, J = 7, 8 Hz, 1H), 6.71 (br s, 1H D_2O exchangeable), 4.94 (d, J = 5 Hz, 2H). ^{13}C NMR (500 MHz, CD_3CN): δ 181.3, 138.3, 136.5, 129.6, 127.1, 126.3, 125.2, 124.5, 122.2, 119.6, 119.2, 112.1, 111.9, 40.7. FTIR ν_{max} (KBr): 3261, 1530, 1498, 742 cm^{-1}. HREIMS: *m/z* measured 281.0981 (281.0987 calculated for $C_{16}H_{15}N_3S$). EIMS *m/z* (% relative abundance) 281 (M^+, 7), 152 (81), 135 (100), 93, (65).

4.6.41. *N*-(Indol-3-ylmethyl)-*N*-methyl-*N′*-propylthiourea (51). Propylisothiocyanate (124 μL, 1.20 mmol) was added to a solution of *N*-(indol-3-ylmethyl)-*N*-methylamine (**77**, 160 mg, 1.0 mmol) in CH_2Cl_2 (5 mL). After allowing the reaction mixture to stir for 15 min at rt, the solvent was evaporated and the crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **49** (219 mg, 84% yield) as a white solid. Mp: 129–131 °C, CH_2Cl_2. HPLC t_R = 18.1 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.26 (br s, 1H, D_2O exchangeable), 7.76 (d, J = 8 Hz, 1H), 7.42 (d, J = 8 Hz, 1H), 7.26 (s, 1H), 7.16 (dd, J = 7.5, 7.5 Hz, 1H), 7.05 (dd, J = 7.5, 7.5 Hz, 1H), 6.24 (br s, 1H, D_2O exchangeable), 5.24 (s, 2H), 3.58 (m, 2H), 3.0 (s, 3H), 1.62 (m, 2H), 0.91 (t, J = 7.5 Hz, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 182.1, 137.1, 127.1, 124.6, 122.2, 119.8, 119.5, 111.9, 111.8, 48.6, 47.7, 35.9, 22.7, 11.0. FTIR ν_{max} (KBr): 3278 (br), 2962, 1532, 1381, 1343, 745 cm^{-1}. HREIMS: *m/z* measured 261.1306 (261.1299 calculated for $C_{14}H_{19}N_3S$). EIMS *m/z* (% relative abundance) 261 (M^+, 55), 160 (16), 132 (100), 130 (95), 102 (30).

4.6.42. *N*-(Indol-3-ylmethyl)-*N*-methyl-*N′*-phenylthiourea (52). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*-methyl-*N′*-propylthiourea (**51**), starting from **77** (200 mg, 1.25 mmol) and substituting phenylisothiocyanate for propylisothiocyanate. The crude reaction mix-

ture was subjected to FCC (silica gel, CH_2Cl_2) to afford **52** (291 mg, 83% yield) as a white solid. Mp: 159–161 °C, CH_2Cl_2. HPLC t_R = 19.6 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.29 (br s, 1H, D_2O exchangeable), 7.81 (d, J = 8 Hz, 2H), 7.46 (d, J = 8 Hz, 1H), 7.36 (d, J = 6.5 Hz, 4H), 7.19 (m, 3H), 7.10 (m, 1H), 5.33 (s, 2H), 3.17 (s, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 182.2, 141.3, 137.1, 128.5, 127.1, 126.4, 125.4, 124.9, 122.3, 119.8, 119.7, 111.8, 111.4, 49.1, 37.0. FTIR ν_{max} (KBr): 3361, 3240, 1520, 1382, 1329, 753 cm^{-1}. HREIMS: m/z measured 295.1146 (279.1143 calculated for $C_{17}H_{17}N_3S$). EIMS m/z (% relative abundance) 295 (M^+, 3), 160(35), 135 (100), 130 (80), 77 (60).

4.6.43. *N*-(Naphthalen-2-ylmethyl)-*N*′-propylthiourea (53). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*-propylthiourea (**49**) and substituting **78** (150 mg, 0.95 mmol) for **76**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **53** (192 mg, 78% yield) as a white solid. Mp: 116–117 °C, CH_2Cl_2. HPLC t_R = 19.3 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 7.87 (m, 3H), 7.74 (s, 1H), 7.57 (br s, 1H, D_2O exchangeable), 7.48 (m, 3 H), 4.82 (br s, 2H), 3.34 (br s, 2H), 1.51 (m 2H), .85 (t, J = 7 Hz, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 138.0, 133.7, 133.0, 128.7, 128.4, 128.3, 127.0, 126.7, 126.5, 126.2, 47.9, 46.2, 40.5, 22.9, 12.2. FTIR ν_{max} (KBr): 3257, 3073, 1556, 1359 cm^{-1}. HREIMS: m/z measured 258.1197 (258.1191 calculated for $C_{15}H_{18}N_2S$). EIMS m/z (% relative abundance) 258 (M^+, 50), 156 (25), 141 (100), 115 (20).

4.6.44. *N*-(Naphthalen-2-ylmethyl)-*N*′-phenylthiourea (54). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*′-propylthiourea (**49**), substituting **78** (200 mg, 1.27 mmol) for **76** and phenylisothiocyanate for propylisothiocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **54** (327 mg, 88% yield) as a white solid. Mp: 172–173 °C, CH_2Cl_2. HPLC t_R = 21.8 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 9.66 (br s, 1H, D_2O exchangeable), 8.26 (br s, 1H, D_2O exchangeable), 7.89 (d, J = 8 Hz, 3H), 7.80 (s, 1H), 7.49, (m, 3H), 7.45 (d, J = 8 Hz, 2H), 7.33 (dd, J = 7.5, 8 Hz, 2 H), 7.12 (dd, J = 7, 7 Hz, 1H), 4.91 (d, J = 5 Hz, 2 H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 181.3, 139.5, 137.1, 133.3, 132.6, 129.1, 128.3, 127.9, 126.7, 126.4, 126.1, 125.9, 124.9, 123.9, 118.2, 47.8. FTIR ν_{max} (KBr): 3382, 3174, 1544, 1588, 1270 cm^{-1}. HRESIMS: m/z measured 293.1116 (293.1106 calculated for $C_{18}H_{17}N_2S$). ESIMS m/z (% relative abundance) 293 ($M+1^+$, 100), 163 (15), 114 (30).

4.6.45. *N*-Methyl-*N*-(naphthalen-2-ylmethyl)-*N*′-propylthiourea (55). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*-methyl-*N*′-propylthiourea (**51**) and substituting **79** (100 mg, 0.58 mmol) for **77**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **55** (126 mg, 79% yield) as a light yellow solid. Mp: 67–69 °C, CH_2Cl_2. HPLC t_R = 23.0 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 7.87 (m, 3H), 7.69 (s, 1H), 7.59 (br s, 1H, D_2O exchangeable), 7.50 (m, 2 H), 7.42 (d, J = 8.5, 1H), 5.23 (s, 2H), 3.49 (m, J = 7.5, 6 Hz, 2H), 3.03 (s, 3H), 1.57 (m, 2H), 0.85 (t, J = 7.5 Hz, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 182.6, 136.5, 133.7, 133.1, 128.9, 128.4, 128.3, 127.1, 126.6, 126.4, 126.3, 56.7, 48.2, 37.6, 22.9, 12.1. FTIR ν_{max} (KBr): 3325, 1581 1253 cm^{-1}. HREIMS: m/z measured 272.1341 (272.1347 calculated for $C_{16}H_{20}N_2S$). EIMS m/z (% relative abundance) 272 (M^+, 17), 202 (17), 171 (100), 155 (20), 127 (27).

4.6.46. *N*-Methyl-*N*-(naphthalen-2-ylmethyl)-*N*′-phenylthiourea (56). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*-methyl-*N*′-propylthiourea (**51**), substituting **79** (100 mg, 0.58 mmol) for **77** and phenylisothiocyanate for propylisothiocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **57** (146 mg, 82% yield) as a white solid. Mp: 183–184 °C, CH_2Cl_2. HPLC t_R = 23.9 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 9.24 (s, 1H, D_2O exchangeable), 7.91 (m, 3H), 7.79 (s, 1H), 7.52 (m, 3H), 7.37 (d, J = 8 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2 H), 7.14 (t, J = 7 Hz, 1H), 5.34 (s, 2H), 3.22 (s, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 182.4, 141.9, 136.0, 133.8, 132.2, 129.0, 128.4, 128.7, 128.5, 127.1, 127.0, 126.7, 126.6, 126.5, 125.5, 57.0, 38.6 FTIR ν_{max} (KBr): 3239, 1526, 1337 cm^{-1}. HREIMS: m/z measured 306.1184 (306.1191 calculated for $C_{19}H_{18}N_3S$). EIMS m/z (% relative abundance) 306 (M^+, 7), 170 (70), 141 (100), 115 (38), 77 (86).

4.6.47. *N*-(Naphthalen-1-ylmethyl)-*N*′-propylthiourea (57). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*′-propylthiourea (**49**), substituting **80** (100 mg, 0.64 mmol) for **76**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **57** (133 mg, 81% yield) as a white solid. Mp: 114–115 °C, CH_2Cl_2. HPLC t_R = 19.5 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 8.08 (d, J = 7.5 Hz, 1H), 7.95 (d, J = 8 Hz, 1H), 7.85 (d, J = 8 Hz, 1H), 7.76 (br s, 1H, D_2O exchangeable), 7.55 (m, 2H), 7.47 (m, 2H), 5.08 (br s, 2H), 3.33 (br s, 2H), 1.48 (m, 2H), 0.85 (t, J = 7 Hz, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 182.3, 135.4, 134.1, 131.8, 129.4, 128.4, 127.1, 126.7, 126.3, 124.4, 46.3, 40.5, 22.9, 12.1. FTIR ν_{max} (KBr): 3257, 1550, 793 cm^{-1}. HREIMS: m/z measured 258.1200 (258.1191 calculated for $C_{15}H_{18}N_2S$). EIMS m/z (% relative abundance) 258 (M^+, 35), 156 (18), 141 (100), 115 (21).

4.6.48. *N*-(Naphthalen-1-ylmethyl)-*N*′-phenylthiourea (58). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*′-propylthiourea (**49**), substituting 80 (100 mg, 0.64 mmol) for 76 and phenylisothiocyanate for propylisothiocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **58** (156 mg, 84% yield) as a white solid. Mp: 195–196 °C, CH_2Cl_2. HPLC t_R = 21.9 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 9.58 (br s, 1H, D_2O exchangeable), 8.15 (br s, 1H, D_2O exchangeable), 7.88 (dd, J = 6.5, 6.5 Hz, 1H), 7.58 (m, 2H), 7.48 (m, 4H), 7.30 (dd, J = 7.5, 8 Hz, 2H), 7.10 (t, J = 7.5 Hz, 1H), 5.18 (d, J = 5 Hz, 2H). ^{13}C NMR (500 MHz, CD_3CN): δ 181.5, 140.2, 134.9, 134.2, 131.8, 129.4, 128.6, 127.2, 126.8, 126.6, 126.3, 125.0, 124.4, 123.8, 46.3. FTIR ν_{max} (KBr): 3275, 1525, 1339 cm^{-1}. HREIMS: m/z measured 292.1036

(292.1034 calculated for $C_{18}H_{16}N_2S$). EIMS *m/z* (% relative abundance) 292 (M^+, 50), 160 (18), 141 (100), 115 (20), 93 (35).

4.6.49. *N*-Methyl-*N*-(naphthalen-1-ylmethyl)-*N′*-propylthiourea (59). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*-methyl-*N′*-propylthiourea (**51**) and substituting **81** (100 mg, 0.58 mmol) for **77**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **59** (126 mg, 79% yield) as a white solid. Mp: 81–82 °C, CH_2Cl_2. HPLC t_R = 22.7 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 8.11 (d, J = 6 Hz, 1H), 7.94 (d J = 6 Hz, 1H), 7.84 (d, J = 8 Hz, 1H), 7.63 (br s, 1H, D_2O exchangeable), 7.54 (m, 2H), 7.47 (t, J = 7.5 Hz, 1H), 7.19 (d, J = 7 Hz, 1H), 5.52 (s, 2H), 3.52 (m, 2H), 3.05 (s, 3H), 1.59 (m, 2H), 0.86 (t, J = 7.5, 1H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 182.5, 134.3, 133.8, 131.8, 129.4, 128.4, 127.0, 126.7, 126.3, 125.1, 124.4, 54.8, 48.2, 38.0, 22.9, 12.1. FTIR ν_{max} (KBr): 3361, 1530, 1544, 742 cm^{-1}. HREIMS: *m/z* measured 272.1346 (272. 1347 calculated for $C_{16}H_{20}N_2S$). EIMS *m/z* (% relative abundance) 272 (M^+, 100), 170 (52), 141 (55), 115 (13).

4.6.50. *N*-Methyl-*N*-(naphthalen-1-ylmethyl)-*N′*-phenylthiourea (60). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*-methyl-*N′*-propylthiourea (**51**), substituting **81** (100 mg, 0.58 mmol) for **77** and phenylisothiocyanate for propylisothiocyanate. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **60** (147 mg, 82% yield) as a white solid. Mp: 153–154 °C, CH_2Cl_2. HPLC t_R = 23.7. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 9.28 (br s, 1H, D_2O exchangeable), 8.12 (d, J = 8 Hz, 1H), 7.97 (d, J = 8 Hz, 1H), 7.88 (d, J = 8 Hz, 1H), 7.55 (m, 3H), 7.33 (m, 5H), 7.14 (t, J = 7 Hz, 1H), 5.62 (s, 2H), 3.22 (s, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 182.8, 141.9, 134.3, 133.4, 131.8, 129.4, 128.8, 128.4, 127.1, 126.9, 126.8, 126.4, 125.5, 125.2, 55.1, 39.0. FTIR ν_{max} (KBr): 3257, 1538, 1341 cm^{-1}. HRMS: *m/z* measured 306.1181 (306.1190 calculated for $C_{19}H_{18}N_2S$). EIMS *m/z* (% relative abundance) 306 (M^+, 51), 170 (31), 141 (100), 77 (46).

4.6.51. Indol-3-ylmethylsulfamide (61). A mixture of indol-3-ylmethylamine (**76**, 200 mg, 1.37 mmol) and sulfamide (147 mg, 1.50 mmol) in H_2O (5 mL) was heated at 100 °C for 120 min. The reaction mixture was cooled to rt, was extracted with EtOAc, the extract was dried, and the solvent was evaporated. The residue was subjected to FCC (CH_2Cl_2/MeOH, 98:2) to give **61** (135 mg, 44% yield) as a white solid. Mp: 132–134 °C, CH_2Cl_2/MeOH. HPLC t_R = 8.6 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.24 (br s, 1H, D_2O exchangeable), 7.69 (d, J = 8 Hz, 1H), 7.44 (d, J = 8 Hz, 1H), 7.28 (d, J = 2.5 Hz, 1H), 7.18 (dd, J = 8, 8 Hz, 1H), 7.11 (dd, J = 8,8 Hz, 1H), 5.17 (br s, 3H D_2O exchangeable), 4.38 (d, J = 6 Hz, 2H). ^{13}C NMR (500 MHz, CD_3CN): δ 136.6, 126.7, 124.0, 121.8, 119.2, 118.7, 111.4, 111.1, 38.7. FTIR ν_{max} (KBr): 3400, 3277, 1340, 1150, 744 cm^{-1}. HREIMS: *m/z* measured 225.0569 (225.0572 calculated for $C_9H_{11}N_3O_2S$). EIMS *m/z* (% relative abundance) 225 (M^+, 38), 144 (48), 130 (100), 118 (15).

4.6.52. *N*-(Indol-3-ylmethyl)-*N′*-methylsulfamide (62). A mixture of *N*-(indol-3-ylmethyl)-*N*-methylamine (**77**, 220 mg, 2.0 mmol) and sulfamide (211 mg, 2.20 mmol) in H_2O (5 mL) was heated at 100 °C for 120 min. The reaction mixture was cooled to rt, was extracted with EtOAc, the extract was dried, and the solvent was evaporated. The residue obtained was subjected to FCC (CH_2Cl_2/MeOH, 98:2) to give **62** (115 mg, 35% yield) as a, off-white solid. Mp: 142–144 °C, CH_2Cl_2/MeOH. HPLC t_R = 5.8 min. ^{1}H NMR (500 MHz, CD_3CN): 7.69 (d, J = 8 Hz, 1H), 7.39 (d, J = 8 Hz, 1H, 7.24 (dd, J = 8, 8 Hz, 1H), 7.19 (s, 1H), 7.12 (dd, J = 8, 8 Hz, 1H), 5.02 (br s, 1H, D_2O exchangeable), 5.16 (br s, 2H, D_2O exchangeable), 4.37 (d, J = 6 Hz, 2H), 3.78 (s, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 137.2, 127.5, 125.5, 122.4, 119.8, 119.4, 112.0, 110.1, 46.22, 34.2. FTIR ν_{max} (KBr): 3409, 1341, 1161, 746 cm^{-1}. HREIMS: *m/z* measured 239.0729 (239.0728 calculated for $C_{10}H_{13}N_3O_2S$). EIMS *m/z* (% relative abundance) 239 (M^+, 17), 147 (8), 130 (100).

4.6.53. Naphthalen-2-ylmethylsulfamide (63). Preparation as reported for indol-3-ylmethylsulfamide (**61**) and substituting **78** (200 mg, 1.27 mmol) for **76**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **63** (228 mg, 76% yield) as a white solid. Mp: 164–166 °C, CH_2Cl_2/MeOH. HPLC t_R = 11.1 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 7.91 (m, 4H), 7.52 (m, 3H), 5.57 (br s, 1H, D_2O exchangeable), 5.24 (br s, D_2O exchangeable), 4.36 (d, J = 5.5 Hz, 2H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 134.2, 133.3, 131.8, 128.9, 128.8, 127.1, 126.7, 126.4, 125.8, 124.1, 45.5. FTIR ν_{max} (KBr): 3272, 1329, 1154 cm^{-1}. HREIMS: *m/z* measured 236.0613 (236.0619 calculated for $C_{11}H_{12}N_2O_2S$). EIMS *m/z* (% relative abundance) 236 (M^+, 54), 155 (100), 141 (38), 128 (24).

4.6.54. *N*-Methyl-*N*-(naphthalen-2-ylmethyl)sulfamide (64). Preparation as reported for *N*-(indol-3-ylmethyl-*N′*-methylsulfamide (**62**) and substituting **79** (100 mg, 0.58 mmol) for **77**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **64** (109 mg, 71% yield) as a white solid. Mp: 140–142 °C, CH_2Cl_2/MeOH. HPLC t_R = 13.3 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$):™ 7.91 (m, 4H), 7.85 (s, 1H), 7.51 (m, 3H), 6.97 (s, 2H, D_2O exchangeable), 4.25 (s, 2H), 2.56 (s, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 135.4, 133.7, 133.3, 129.0, 128.5, 128.4, 127.7, 127.2, 127.1, 126.8, 54.8, 35.6. FTIR ν_{max} (KBr): 3269, 1341, 1157 cm^{-1}. HREIMS: *m/z* measured 250.0777 (250.0776 calculated for $C_{12}H_{14}N_2O_2S$). EIMS *m/z* (% relative abundance) 250 (M^+, 43), 168 (54), 141 (100), 115 (11).

4.6.55. Naphthalen-1-ylmethylsulfamide (65). Preparation as reported for indol-3-ylmethylsulfamide (**61**) and substituting **80** (200 mg, 1.27 mmol) for **76**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **65** (231 mg, 77% yield) as a white solid. Mp: 117–118 °C, CH_2Cl_2/MeOH. HPLC t_R = 10.7 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 8.15 (d, J = 8 Hz, 1H), 7.94 (d, J = 7.5 Hz, 1H), 7.86

(d, $J = 8$ Hz, 1H), 7.55 (m, 3H), 7.47 (t, $J = 7.5$ Hz, 1H), 7.06 (br s, 1H, D_2O exchangeable), 6.74 (br s, D_2O exchangeable), 4.51 (d, $J = 6$ Hz, 2H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 134.4, 134.0, 131.9, 129.3, 128.6, 127.1, 127.0, 126.6, 126.2, 124.6, 45.2. FTIR ν_{max} (KBr): 3270, 1310, 1164, 795 cm^{-1}. HREIMS: *m/z* measured 236.0622 (236.0619 calculated for $C_{11}H_{12}N_2O_2S$). EIMS *m/z* (% relative abundance) 236 (M^+, 39), 154 (100), 141 (36), 115 (15).

4.6.56. *N*-Methyl-*N*-(naphthalen-1-ylmethyl)sulfamide (66). Preparation as reported for *N*-(indol-3-ylmethyl-*N*2-methylsulfamide (**62**) and substituting **81** (100 mg, 0.58 mmol) for **77**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2/MeOH, 98:2) to afford **66** (99 mg, 68% yield) as a white solid. Mp: 145–146 °C, CH_2Cl_2/MeOH. HPLC $t_R = 14.6$ min. ^1H NMR (500 MHz, $(CD_3)_2SO$): δ 8.28 (d, $J = 8$ Hz, 1H), 7.96 (d, $J = 8$ Hz, 1H), 7.91 (d, $J = 8$ Hz, 1H), 7.53 (m, 4H), 7.50 (m, 1H), 7.02 (s, 2H, D_2O exchangeable), 4.48 (s, 2H), 2.46 (s, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 134.4, 132.6, 132.4, 129.4, 129.3, 128.6, 127.1, 126.8, 126.2, 124.9, 53.3, 35.4. FTIR ν_{max} (KBr): 3350, 3275, 1324, 1163, 782 cm^{-1}. HRMS: *m/z* measured 250.0768 (250.0776 calculated for $C_{12}H_{14}N_2O_2S$). EIMS *m/z* (% relative abundance) 250 (M^+, 47), 168 (60), 141 (100).

4.6.57. Indol-3-ylmethylmethanesulfonamide (67). Methanesulfonyl chloride (92 μL, 1.20 mmol) was added to a solution of indol-3-ylmethylamine (**76**, 146 mg, 1.0 mmol) and triethylamine (265 μL, 2.0 mmol) in THF (4 mL) at 0 °C. The reaction mixture was allowed to stir at rt for 60 min, the precipitate formed was filtered off, the filtrate was concentrated, and the residue was subjected to FCC (silica gel, CH_2Cl_2) to give indol-3-ylmethyl methanesulfonamide (**67**) (146 mg, 65% yield) as a light brown solid. Mp: 131–133 °C, CH_2Cl_2. HPLC $t_R = 6.1$ min. ^1H NMR (500 MHz, CD_3CN): δ 9.29 (br s, 1H, D_2O exchangeable), 7.71 (d, $J = 8$ Hz, 1H), 7.46 (d, $J = 8$ Hz, 1H), 7.29 (s, 1H), 7.20 (dd, $J = 7$, 8 Hz, 1H), 7.13 (dd, $J = 8$, 8 Hz, 1H), 5.45 (br s, 1H, D_2O exchangeable), 4.44 (d, $J = 5$ Hz, 1H), 2.83 (s, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 137.0, 126.9, 124.5, 124.4 119.7, 119.1, 111.9, 111.6, 39.8, 38.9. FTIR ν_{max} (KBr): 3412, 1310, 1147, 749 cm^{-1}. HRMS: *m/z* measured 224.0611 (224.0619 calculated for $C_{10}H_{12}N_2O_2S$). EIMS *m/z* (% relative abundance) 224 (M^+, 55), 144 (87), 130 (100), 80 (20).

4.6.58. *N*-(Indol-3-ylmethyl)-*N*-methylmethanesulfonamide (68). Methanesulfonyl chloride (79 μL, 1.02 mmol) was added to a solution of indol-3-ylmethylamine (**77**, 150 mg, 0.93 mmol) and triethylamine (247 μL, 1.86 mmol) in THF (4 mL) at 0 °C. The reaction mixture was allowed to stir at rt for 60 min, the precipitate formed was filtered off, the filtrate was concentrated, and the residue was subjected to FCC (silica gel, CH_2Cl_2) to give indol-3-ylmethyl methanesulfonamide (**68**) (156 mg, 70% yield) as a white solid. Mp: 131–132 °C, CH_2Cl_2. HPLC $t_R = 8.3$ min. ^1H NMR (500 MHz, CD_3CN): δ 9.32 (br s, 1H, D_2O exchangeable), 7.73 (d, $J = 8$ Hz, 1H), 7.43 (d, $J = 8$ Hz, 1H), 7.28 (s, 1H), 7.17 (dd, $J = 7$, 8 Hz, 1H), 7.09 (dd, $J = 7$, 8 Hz, 1H), 4.45 (s, 2H), 2.78 (s, 3H), 2.68 (s, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 137.2, 127.4, 125.6, 122.4, 119.9, 119.4, 112.0, 109.9, 45.6, 34.8, 33.9. FTIR ν_{max} (KBr): 3406, 1322, 1148, 749 cm^{-1}. HREIMS: *m/z* measured 238.0774 (238.0776 calculated for $C_{11}H_{14}N_2O_2S$). EIMS *m/z* (% relative abundance) 238 (M^+, 29), 158 (23), 130 (100).

4.6.59. *N*-(Naphthalen-2-ylmethyl)methanesulfonamide (69). Preparation as reported for indol-3-ylmethyl methanesulfonamide (**67**) and substituting **78** (250 mg, 1.60 mmol) for **76**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **69** (303 mg, 81% yield) as a white solid. Mp: 132–134 °C, CH_2Cl_2. HPLC $t_R = 13.4$ min. ^1H NMR (500 MHz, $(CD_3)_2SO$): δ 7.90 (m, 3H), 7.84 (s, 1H), 7.67 (t, $J = 6$ Hz, 1H, D_2O exchangeable), 7.51 (m, 3H), 4.33 (d, $J = 6$ Hz, 2H), 2.88 (s, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 136.7, 133.7, 133.1, 128.7, 128.5, 128.4, 127.1, 126.9, 126.8, 126.7, 47.0, 40.8. FTIR ν_{max} (KBr): 3275, 1312, 1151 cm^{-1}. HRMS: *m/z* measured 235.0671 (235.0667 calculated for $C_{12}H_{13}NO_2S$). EIMS *m/z* (% relative abundance) 235 (M^+, 28), 155 (100), 141 (19), 128 (24).

4.6.60. *N*-Methyl-*N*-(naphthalen-2-ylmethyl)methanesulfonamide (70). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*-methyl methanesulfonamide (**68**) and substituting **79** (100 mg, 0.58 mmol) for **77**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **70** (113 mg, 78% yield) as a white solid. Mp: 140–142 °C, CH_2Cl_2. HPLC $t_R = 18.0$ min. ^1H NMR (500 MHz, $(CD_3)_2SO$): δ 7.93 (m, 3H), 7.84 (s, 1H), 7.52 (m, 2H), 7.48 (d, $J = 8.5$ Hz, 4.39 (s, 2H), 2.99 (s, 3H), 2.69 (s, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): 134.9, 133.7, 133.3, 129.1, 128.5, 128.4, 127.6, 127.2, 127.0, 126.9, 54.1, 35.9, 35.2. FTIR ν_{max} (KBr): 3292, 2962, 1531, 1377 cm^{-1}. HRMS: *m/z* measured 249.0815 (249.0823 calculated for $C_{13}H_{15}NO_2S$). EIMS *m/z* (% relative abundance) 249 (M^+, 48), 168 (95), 141 (100), 115 (27).

4.6.61. *N*-(Naphthalen-1-ylmethyl)methanesulfonamide (71). Preparation as reported for indol-3-ylmethyl methanesulfonamide (**67**) and substituting **80** (100 mg, 0.64 mmol) for **76**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **71** (119 mg, 80% yield) as a white solid. Mp: 130–131 °C, CH_2Cl_2. HPLC $t_R = 13.5$ min. ^1H NMR (500 MHz, $(CD_3)_2SO$): δ 8.17 (d, $J = 8$ Hz, 1H), 7.96 (d, $J = 8$ Hz, 1H), 7.88 (d, $J = 8$ Hz, 1H), 7.57 (m, 4H), 7.49 (dd, $J = 8$, 8 Hz, 1H), 4.62 (d, $J = 6$ Hz, 2H), 2.95 (s, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): δ 134.3, 134.2, 131.7, 129.4, 128.9, 127.1, 127.0, 126.7, 126.2, 124.5, 45.0, 40.8. FTIR ν_{max} (KBr): 3279, 1310, 1142, 773 cm^{-1}. HRMS: *m/z* measured 235.0666 (235.0667 calculated for $C_{12}H_{13}NO_2S$). EIMS *m/z* (% relative abundance) 235 (M^+, 36), 154 (100), 141 (19), 129 (22).

4.6.62. *N*-Methyl-*N*-(naphthalen-1-ylmethyl)methanesulfonamide (72). Preparation as reported for *N*-(indol-3-ylmethyl)-*N*-methylmethanesulfonamide (**68**) and substituting **81** (100 mg, 0.58 mmol) for **77**. The crude reaction mixture was subjected to FCC (silica gel, CH_2Cl_2) to afford **72** (107 mg, 74% yield) as a white sol-

id. Mp: 98–99 °C, CH_2Cl_2. HPLC t_R = 14.1 min. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 8.30 (d, J = 8 Hz, 1H), 7.96 (d, J = 8 Hz, 1H), 7.93 (d, J = 8.5 Hz, 1H), 7.53 (m, 4H), 4.68 (s, 2H), 3.06 (s, 3H), 2.60 (s, 3H). ^{13}C NMR (500 MHz, $(CD_3)_2SO$): 134.4, 132.2, 132.1, 129.5, 129.4, 128.3, 127.2, 126.8, 126.2, 124.5, 52.4, 35.0, 34.9. FTIR ν_{max} (KBr): 1330, 1154, 790 cm^{-1}. HRMS: *m/z* measured 249.0822 (249.0823 calculated for $C_{13}H_{15}NO_2S$). EIMS *m/z* (% relative abundance) 249 (M^+, 50), 168 (92), 141 (100), 115 (18).

4.6.63. 3-(Indolyl-3)propanamide (73). A solution of 3-(indolyl-3)propanoic acid (**92**, 200 mg, 1.05 mmol) and triethylamine (155 μL, 1.16 mmol) in THF (8 mL) was cooled to 0 °C on an ice bath. Methyl chloroformate (92 μL, 1.16 mmol) was added dropwise to the cold solution. After allowing the reaction mixture to stir for 10 min at 0 °C, dry ammonia gas was bubbled in for 60 min. The precipitate formed was filtered off, and the filtrate was concentrated to give **73** (188 mg, 95% yield) as a white solid. Mp: 134–136 °C, THF. HPLC t_R = 6.1 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.11 (br s, 1H, D_2O exchangeable), 7.57 (d, J = 8 Hz, 1 H), 7.37 (d, J = 8 Hz, 1H), 7.12 (dd, J = 8, 7 Hz, 1 H), 7.04 (m, 2H), 6.15 (br s, 1H, D_2O exchangeable), 5.68 (br s, 1H, D_2O exchangeable), 3.0 (t, J = 7 Hz, 2H,), 2.52 (t, J = 8 Hz, 2H). ^{13}C NMR (500 MHz, CD_3CN): δ 175.8, 137.6, 128.3, 123.1, 123.0, 122.5, 119.7, 119.5, 115.6, 112.4, 37.0, 21.8. FTIR ν_{max} (KBr): 3396, 3177, 1648, 740 cm^{-1}. HREIMS: *m/z* measured 188.0949 (188.0950 calculated for $C_{11}H_{12}N_2O$). EIMS *m/z* (% relative abundance) 188 (M^+, 43), 144 (21), 130 (100).

4.6.64. *N*-Methyl-3-(indolyl-3)propanamide (74). A solution of 3-(indolyl-3)propanoic acid (**92**, 50 mg, 0.26 mmol) and triethylamine (141 μL, 1.06 mmol) in THF (3 mL) was cooled to 0 °C on an ice bath. Methyl chloroformate (23 μL, 0.29 mmol) was then added dropwise to the cold solution. After stirring for 10 min at 0 °C, methylamine hydrochloride (270 mg, 4.0 mmol) was added and the reaction mixture was stirred for 60 min at 0 °C. The precipitate formed was filtered off, the filtrate was concentrated, and the resulting yellow oil was subjected to FCC (silica gel, $CHCl_3$/MeOH, 95:5) to give **74** (46 mg, 85% yield) as an off-white solid. Mp: 99–101 °C (lit.[19] 97–99 °C), $CHCl_3$/MeOH. HPLC t_R = 6.9 min. ^{1}H NMR (500 MHz, CD_3CN): δ 9.30 (br s, 1H, D_2O exchangeable), 7.58 (d, J = 8 Hz, 1H), 7.41 (d, J = 8 Hz, 1H), 7.17 (dd, J = 7, 8 Hz, 1H), 7.07 (m, 2H), 6.46 (br s, 1H, D_2O exchangeable), 3.05 (t, J = 7 Hz, 2 H,), 2.68 (d, J = 5 Hz, 3H), 2.51 (t, J = 8 Hz, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 173.6, 137.0, 127.7, 122.4, 121.8, 119.0, 118.9, 114.9, 111.7, 37.0, 26.7, 21.5. FTIR ν_{max} (KBr): 3282, 1644, 1546, 743 cm^{-1}. HREIMS: *m/z* measured 202.1106 (202.1106 calculated for $C_{12}H_{14}N_2O$). EIMS *m/z* (% relative abundance) 202 (M^+, 44), 189 (6), 144 (25), 130 (100).

4.6.65. Methyl 3-(indolyl-3)propanoate (75). H_2SO_4 (250 μL, concd) was added to a solution of 3-(indol-3-yl)propanoic acid (**92**, 110 mg, 0.58 mmol) in MeOH (2 mL) and the mixture was allowed to reflux for 60 min. The reaction mixture was neutralized using saturated solution of Na_2CO_3 and extracted with EtOAc. The organic extracts were combined, dried, and evaporated to yield **75** (115 mg, 97% yield) as a white solid. Mp: 80–82 °C (lit.[19] 79–80 °C), MeOH. ^{1}H NMR (300 MHz, CD_3CN): δ 7.99 (br s, 1H, D_2O exchangeable), 7.62 (dd, J = 8, 1 Hz, 1H), 7.36 (dd, J = 8 Hz, 1, 1H), 7.18 (m, 2H), 7.0 (s, 1H), 3.7 (s, 3H), 3.12 (t, J = 8 Hz, 2H), 2.74 (t, J = 8 Hz, 2H).

4.7. Metabolism

4.7.1. Isolation of methyl 3-phenylthiocarbazate (94, t_R = 11.4 min), the biotransformation product of methyl 3-phenyldithiocarbazate (20). Five Erlenmeyer flasks (250 mL) containing 100 mL of media inoculated with spores (10^8 spores/100 mL) of *L. maculans* were incubated at 24 ± 1 °C on shaker at 120 rpm under constant light. After 48 h, a solution of **20** (100 μL, 0.1 M) in DMSO was added to the cultures. The flasks were then incubated on shaker at 120 rpm for further 48 h. The mycelia were filtered, the filtrates were combined, were extracted with EtOAc to yield 9.3 mg of crude extract. The crude extract was separated using prep TLC (silica gel, CH_2Cl_2/MeOH, 98:2) to yield 6.2 mg of **94** as a white solid. Mp: 151–152 °C, CH_2Cl_2/MeOH. HPLC t_R = 11.4 min. ^{1}H NMR (500 MHz, CD_3CN): δ 7.77 (br s, 1H, D_2O exchangeable), 7.25 (t, J = 7 Hz, 2H), 6.89 (br s, 1H), 6.82 (d, J = 7.5 Hz, 2H), 6.56 (s, 1H, D_2O exchangeable), 2.18 (s, 3H). ^{13}C NMR (500 MHz, CD_3CN): δ 175.0, 147.9, 129.5, 121.0, 113.8, 11.2. FTIR ν_{max} (KBr): 3308, 1661, 1245, 753 cm^{-1}. HREIMS: *m/z* measured 182.0515 (182.0514 calculated for $C_8H_{10}N_2OS$). EIMS *m/z* (% relative abundance) 182 (M^+, 52), 107 (100), 77 (35).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.03.014.

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