Structure−Activity Study of Brassinin Derivatives as Indoleamine 2,3-Dioxygenase Inhibitors

Paul Gaspari,[†] Tinku Banerjee,[‡,§] William P. Malachowski,*[,†] Alexander J. Muller,*[,‡] George C. Prendergast,[‡] James DuHadaway,[‡] Shauna Bennett,[†] and Ashley M. Donovan[†]

Department of Chemistry, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010-2899, and Lankenau Institute for Medical Research, Wynnewood, Pennsylvania 19096

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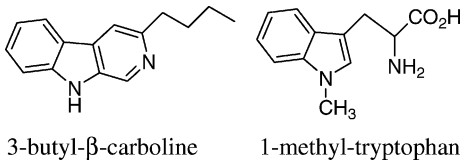
A screen of indole-based structures revealed the natural product brassinin to be a moderate inhibitor of indoleamine 2,3-dioxygenase (IDO), a new cancer immunosuppression target. A structure−activity study was undertaken to determine which elements of the brassinin structure could be modified to enhance potency. Three important discoveries have been made, which will impact future IDO inhibitor development: (i) The dithiocarbamate portion of the brassinin lead is a crucial moiety, which may be binding to the heme iron of IDO; (ii) an indole ring is not necessary for IDO inhibition; and (iii) substitution of the S-methyl group of brassinin with large aromatic groups provides inhibitors that are three times more potent in vitro than the most commonly used IDO inhibitor, 1-methyl-tryptophan.

Introduction

Understanding how tumors escape the host immune system is a rapidly developing area in the field of cancer research. Recently, the enzyme indoleamine 2,3-dioxygenase (IDO; EC 1.13.11.42) has been implicated in tumor immunosuppression.[1] Several reports identify IDO as playing a role in undermining a more vigorous immune response to tumor growth. Consequently, we have been focused on identifying novel, potent IDO inhibitors with the goal of developing a new therapeutic approach to cancer treatment. IDO inhibitors alone or in combination with other chemotherapeutics might provide another tool in the oncology armamentarium.

IDO is an extrahepatic enzyme that catalyzes the initial and rate-limiting step in the degradation of tryptophan (Trp) along the kynurenine pathway that leads to the biosynthesis of nicotinamide adenine dinucleotide.[2] IDO does not, however, handle dietary catabolism of Trp, which is instead the role of the structurally unrelated liver enzyme Trp dioxygenase (EC 1.13.11.11). IDO is a monomeric 45 kDa heme-containing oxidase that is active with the heme iron in the ferrous (Fe^{2+}) form. The ferric (Fe^{3+}) form of IDO is inactive, and substrate inhibition is believed to result from Trp binding to ferric IDO.[3] The primary catalytic cycle of IDO does not involve redox changes; nevertheless, IDO is prone to autoxidation; therefore, a reductant is necessary to reactivate the enzyme. In vivo, IDO purportedly relies on a flavin or tetrahydrobiopterin cofactor. In vitro, methylene blue and ascorbic acid are believed to substitute for the natural flavin or tetrahydrobiopterin cofactor.

Inhibition of IDO has previously been targeted for other therapies, most notably neurological disorders.[2b] Several metabolites of the kynurenine pathway are neurotoxic or are implicated in neurodegeneration, e.g., quinolinic acid; therefore, attention has focused on IDO. A recent review[4] summarizes the range of compounds that have been tested as IDO inhibitors. Strikingly, almost all IDO inhibitors, whether competitive or noncompetitive, retain the indole ring of the natural substrate. Currently, the most potent IDO inhibitor reported is 3-butyl-β-carboline (Figure 1), a noncompetitive inhibitor with a K_i = 3.3 μM.[5] However, the most commonly used IDO inhibitor is 1-methyl-Trp (Figure 1),[6] a commercially available compound that is a competitive inhibitor with a K_i = 34 μM.



3-butyl-β-carboline 1-methyl-tryptophan

Figure 1. IDO inhibitors.

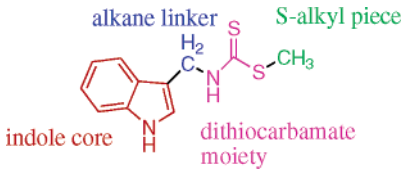


Figure 2. Brassinin (**1**) structure.

We undertook a screen of commercially available indole-based molecules to find novel IDO inhibitors. Interestingly, the natural product brassinin (**1**; Figure 2) was found to be a moderately active competitive inhibitor, K_i = 97.7 μM. Brassinin is a phytoalexin in cruciferous plants[7] and has demonstrated some antifungal[8] and anticancer activity.[9] We undertook a structure−activity relationship study of brassinin with the goal of obtaining a more potent IDO inhibitor. We divided the brassinin structure into four components: the indole core, the alkane linker, the dithiocarbamate moiety, and the S-alkyl piece. Analogues of brassinin that varied each of the four components were synthesized. The study determined the significance and flexibility of each of the four portions of the brassinin structure. The experimentation led to more potent inhibitors and several important developments in the field of IDO inhibition. Details of this study and our findings are reported herein.

Results and Discussion

Chemistry. Brassinin dithiocarbamate analogues were synthesized by adding an amine to carbon disulfide at 0 °C, stirring for 1 h, and then adding an alkyl halide. Modification of the indole core or alkane linker occurred by adding different amines. Many amines were commercially available, although several

* To whom correspondence should be addressed. (W.P.M.) Tel: 610-526-5016. Fax: 610-526-5086. E-mail: wmalacho@brynmawr.edu. (A.J.M.) Tel: 610-645-8034. Fax: 610-645-2095. E-mail: mullera@mlhs.org.
[†] Bryn Mawr College.
[‡] Lankenau Institute for Medical Research.
[§] Current address: NewLink Genetics, Wynnewood, PA 19096.

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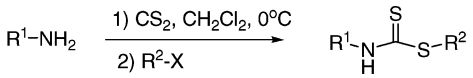
Scheme 1. Dithiocarbamate Synthesis



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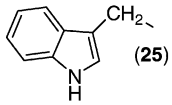
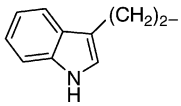
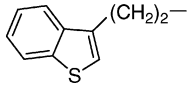
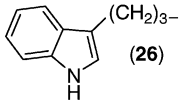
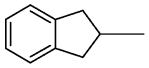
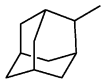
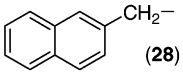
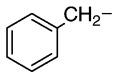
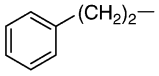
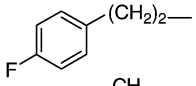
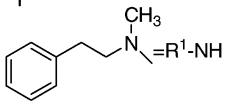
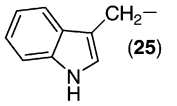
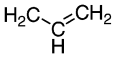
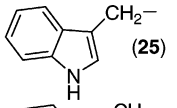
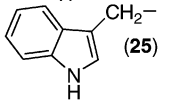
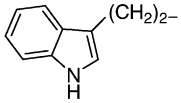
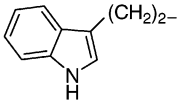
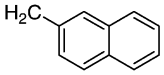
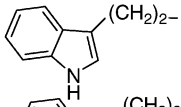
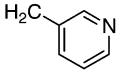
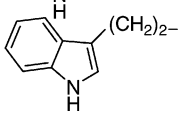
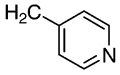
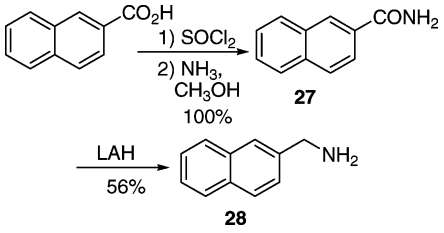
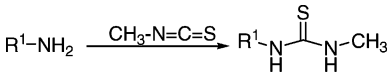
1		CH_3	43
2		CH_3	87
3		CH_3	22
4		CH_3	61
5		CH_3	74
6		CH_3	98
7		CH_3	54
8		CH_3	74
9		CH_3	85
10		CH_3	89
11		CH_3	85
12			52
13		H_2C-Ph	50
14		$-(CH_2)_5CH_3$	57
15		H_2C-Ph	86
16			59
17			40
18			50

Scheme 2



Scheme 3. Thiourea Synthesis



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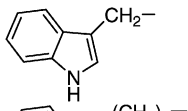
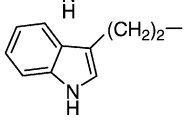
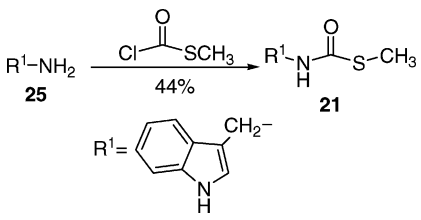
19		54
20		92

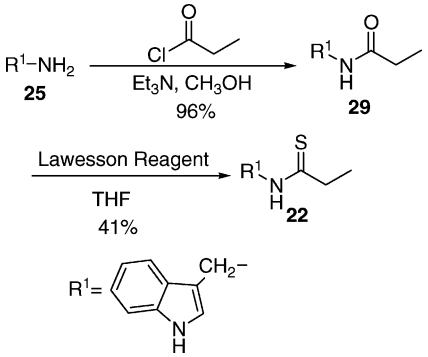
Scheme 4. Brassitin Synthesis



required synthesis. The indole-3-methanamine **25** of brassinin **1** was prepared through the reductive amination of indole-3-carboxaldehyde. Although there are several different reductive amination procedures reported in the literature,[9,10] we found Mehta's procedure[9a] to be the most effective. Homotryptamine **26**, the amine reagent for **4**, was synthesized in three steps from indole-3-propanoic acid following literature precedent.[11] 2-Aminomethyl-naphthalene **28** was also synthesized in three steps from 2-naphthoic acid (Scheme 2). Modifications of the S-alkyl piece occurred by substituting various alkyl halides for iodomethane, e.g., **12**−**18** (Scheme 1).

Modifications to the dithiocarbamate moiety included thioureas (**19** and **20**; Scheme 3), S-alkyl thiocarbamates (**21**; Scheme 4), thioamides (**22**; Scheme 5), and thiazoles (**23** and **24**; Scheme 6). The thioureas were synthesized by reacting amines with methyl isothiocyanate (Scheme 3).[35] The S-alkyl thiocarbamate **21**, a phytoalexin called brassitin, came from the reaction of S-alkyl thiochloroformate with **25** (Scheme 4). The thioamide **22** was synthesized by reaction of **25** with an acid chloride and then treatment with Lawesson's reagent (Scheme 5). Thiazoles were synthesized by reaction of thioformamide or thioacetamide with α-bromoketones **31** (Scheme 6). The

Scheme 5. Thioamide Synthesis



Scheme 6. Thiazole Synthesis

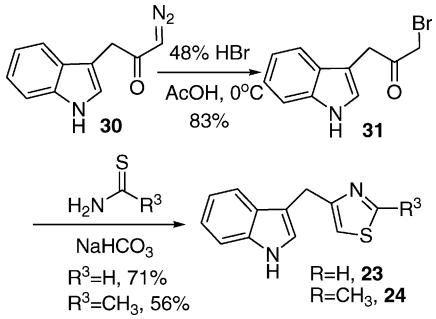


Table 1. IDO Inhibition Data

compound	K_i (μM)	compound	K_i (μM)	compound	K_i (μM)
1	97.7	**9**	62.36	**17**	28.38
2	82.54	**10**	149.35	**18**	20.48
3	40.95	**11**	1267	**19**	NI[a]
4	33.97	**12**	36.95	**20**	342.3
5	42.06	**13**	13.22	**21**	NI
6	179.6	**14**	363.6	**22**	202
7	47.57	**15**	17.15	**23**	1292
8	72.41	**16**	11.55	**24**	328.7

[a] NI, no inhibition detected.

α-bromoketones **31** were generated from the corresponding α-diazoketone derivative **30**,[12] which was synthesized in three steps from indole-3-acetic acid following a literature procedure.[13]

Enzyme Studies. Brassinin analogues were analyzed for inhibition of extracted and purified recombinant human IDO produced in bacteria. The assay was conducted according to a literature protocol,[14] with ascorbic acid and methylene blue serving the role of reductant.[15] Catalase was added to prevent IDO decomposition from peroxide side products.[16] The enzyme assay was monitored for formation of N-formylkynurenine by hydrolyzing the formyl group and spectrophotometrically analyzing for the conjugated imine generated from kynurenine and 4-(dimethylamino)benzaldehyde. In all cases where inhibition was seen, the brassinin analogues demonstrated competitive inhibition. The inhibitory constants shown are an average of two or three trials. The array of analogues tested allowed for an evaluation of the four components of the brassinin structure and resulted in some important discoveries (Table 1).

Variation of the Indole Core. One of the most suprising discoveries was the range of groups that could be substituted for indole and still retain some IDO inhibitory activity. Indeed, not only were flat aromatic structures (e.g., **5** and **7**−**10**) effective substitutes, but the adamantyl structure **6** could also bind in the substrate pocket, based on the competitive inhibition witnessed for all of these analogues. Although IDO is relatively promiscuous, there are still very few reports of substrates or inhibitors that lack the indole core.[4] The current demonstration of inhibition with benzene and cycloalkyl-based structures expands the range of structures that behave as IDO inhibitors. Furthermore, benzene aromatic structures are more easily derivatized with available synthetic methods than indole compounds. Indole derivatives can also be a liability given the neuroactivity of some indole-containing compounds, e.g., serotonin and related indolealkylamines.

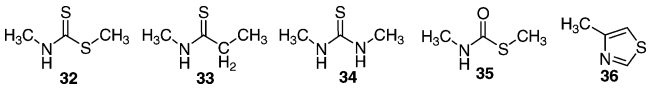


Figure 3. Dithiocarbamate analogues.

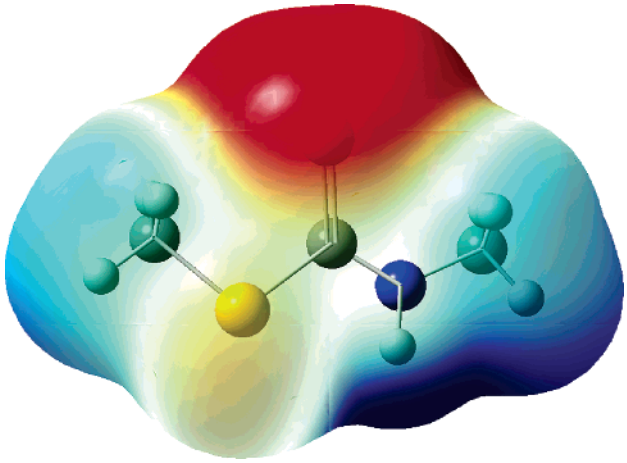


Figure 4. Molecular ESP mapped onto electron distribution for **32**.

Variation of the Alkane Linker. Linker variation was possible, and in the brassinin series, it was found that the longer linker led to more potent compounds, cf. **1** vs **2** vs **4**. However, analogues that modified two brassinin components did not replicate this trend (cf. **13** vs **15**). Taken together, the results with the alkane linker modifications and the indole core changes indicate that the IDO active site is rather accommodating.

Variation of the Dithiocarbamate. The most interesting results came from isosteric modifications of the dithiocarbamate. The transformation of brassinin's dithiocarbamate moiety to a thiourea (**19** and **20**), thiocarbamate (**21**), thioamide (**22**), or thiazole (**23** and **24**) led to weaker or no inhibition. Notably, the S-methyl-thiocarbamate analogue **21** (brassitin),[29] suffered a complete loss in inhibitory activity with the substitution of a carbonyl for the thiocarbonyl group. Given the recognized metal-coordinating properties of dithiocarbamates,[17,18] it is likely that the dithiocarbamate moiety is chelating to the heme iron at the active site of IDO.[19] In fact, pyrrolidine dithiocarbamate reportedly inhibits IDO,[17a] besides being a well-known antioxidant and NF-κB inhibitor.[20]

Computational Experiments to Explore the Electronic Nature of Dithiocarbamate. If the dithiocarbamate moiety is binding to the heme iron, then the electronic nature or charge of the group should be important to achieve optimum binding. We performed several computational experiments to understand the electronic nature of the dithiocarbamate sulfur vs the sulfur/oxygen in the isosteric analogues. To reduce the computational time, most of the experiments involved simplified analogues **32**−**36** (Figure 3), which lacked the indole ring. Figure 4 shows the molecular electrostatic potential (ESP) mapped onto the electron distribution of **32**, the dithiocarbamate analogue. Clearly, the sulfur of **32** projects the greatest electron density and therefore is the richest and most available Lewis basic site for iron binding.

Table 2. Charge Data for Dithiocarbamate Analogues

compound	charge ESP	charge NBO	K_i of related analogue (μM) (compound no.)
32	−0.466	−0.277	97.7 (**1**)
33	−0.518	−0.318	202 (**22**)
34	−0.550	−0.390	NI (**19**)
35	−0.661	−0.711	NI (**21**)
36	−0.133	0.290	1292 (**23**)

In Table 2, the ESP and natural bond order (NBO) charge values were derived for **32**−**36**. Elimination of the indole group allowed for more rapid computational experiments and did not affect the trend witnessed.[21] For compounds **32**−**35**, a clear trend can be seen for both ESP and NBO charge calculations: the smaller the charge on sulfur/oxygen, the better the inhibition. Compound **36** breaks the trend; however, the conformationally rigid thiazole ring may prevent an effective interaction between the sulfur of the thiazole and the heme iron. The trend witnessed with compounds **32**−**35** may seem counterintuitive, i.e., a more electron rich sulfur/oxygen should be a better electron donor to the Lewis acid heme iron. Nevertheless, optimum inhibition may arise from a softer Lewis base coordinating with the softer active ferrous form of the enzyme.[22] Future inhibitor design will be guided by the insights from these computational experiments.

Variation of the S-Alkyl Piece. The greatest increases in potency were realized in modifications of the S-alkyl group. Although alkyl groups that were longer than the methyl in brassinin were less active, S-allyl brassinin **12** was two times more potent than brassinin and the benzyl analogue **13** was almost one order of magnitude more potent. Moreover, the tryptamine/naphthyl analogue **16** was as potent as **13**; pyridyl analogues **17** and **18** also demonstrated modest inhibition. Because all these compounds behaved as competitive inhibitors, these analogues reveal a large additional pocket in the IDO active site capable of accommodating flat, aromatic groups.

Sono has reported that β-carboline, a noncompetitive inhibitor, binds to the heme iron at the active site but not in the same space as the substrate.[23] Moreover, the β-carboline reportedly acts as a nitrogen donor ligand and competes with O_2 for binding to the heme iron. It is possible that the large aromatic S-alkyl pieces are binding in the same pocket that accommodates the tricyclic aromatic β-carboline structure. Nevertheless, the pyridyl analogues **17** and **18** failed to demonstrate stronger inhibition despite their similarity to the pyridyl ring of β-carboline.

Conclusion

A systematic study of the IDO inhibitory activity of brassinin has been undertaken, and three important discoveries have been made. Contrary to most previously reported IDO inhibitors, an indole ring was not necessary for inhibitory activity with the dithiocarbamate analogues of brassinin. Although indole-containing derivatives were still the most active inhibitors (i.e., **13** and **16**), the inhibitory activity retained by analogues, such as **5** and **7**−**9**, create new opportunities to further inhibitor development. Importantly, new analogues might be possible that avoid the pharmacological liabilities of the indole ring and leverage the wealth of chemical methods for benzene substitution. The dithiocarbamate moiety is an optimum group for IDO inhibition and probably chelates to the active site iron. Large unsaturated groups on the dithiocarbamate sulfur can be accommodated in the active site and lead to more potent inhibitors of IDO. Although only small increases in potency were achieved through the structure−activity study, the new inhibitors (i.e., **13** and **16**) are three times more potent than the most commonly used IDO inhibitor. In addition, the structure−activity relationship discoveries should greatly advance the search for more potent IDO inhibitors, an exciting new cancer target.

Experimental Section

Chemistry. All reactants and reagents were commercially available and were used without further purification unless otherwise indicated. Anhydrous THF was obtained by distillation from benzophenone-sodium under argon immediately before use. Anhydrous CH_2Cl_2 and Et_3N were obtained by distillation from calcium hydride under argon. Methanol was dried over Mg and distilled under argon. A saturated solution of HCl in CH_3OH was made by bubbling HCl through a drying tube, filled with $CaCl_2$, into a cooled flask of anhydrous CH_3OH under a stream of argon. A saturated solution of NH_3 in CH_3OH was made by bubbling anhydrous NH_3 into an Erlenmyer flask with a predetermined volume of CH_3OH. Concentrated refers to the removal of solvent with a rotary evaporator at normal water aspirator pressure followed by further evacuation with a two-stage mechanical pump unless otherwise indicated. Yields refer to chromatographically and spectroscopically pure (>95%) compounds, except as otherwise indicated. All new compounds were determined to be >95% pure by nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC), and/or gas chromatography (GC) as indicated (see Supporting Information). Melting points were determined using an open capillary and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively. Chemical shifts are reported in δ values (ppm) relative to an internal reference (0.05% v/v) of tetramethylsilane (TMS) for ^{1}H NMR and the solvent peak in ^{13}C NMR, except where noted. Peak splitting patterns in the NMR are reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. Rotamer peaks (about 1/4 intensity) were seen for all dithiocarbamate structures.[24] Normal phase HPLC (NP-HPLC) analysis was performed with UV detection at 254 nm and a 5 μm silica gel column (250 mm × 4.6 mm), eluted with 90:10 *n*-hexane:IPA (or gradient) at 1 mL/min. Reverse phase HPLC (RP-HPLC) analysis was performed with UV detection at 254 nm and a C_{18} column (300 mm × 3.9 mm), eluted with a gradient of H_2O + 0.1% TFA and CH_3CN + 0.1% TFA at 1 mL/min., unless otherwise indicated. GC analyses were performed with an EI-MS detector fitted with a 30 m × 0.25 mm column filled with cross-linked 5% PH ME siloxane (0.25 μm film thickness); gas pressure 7.63 psi He. IR data were obtained with an FT-IR spectrometer. Thin-layer chromatography (TLC) was performed using silica gel 60 A precoated glass-backed plates (0.25 mm thickness) with fluorescent indicators, which were scored and cut. Developed TLC plates were visualized with UV light (254 nm), iodine, or $KMnO_4$. Flash column chromatography was conducted with the indicated solvent system using normal phase silica gel 60 A, 230−400 mesh. All reactions were carried out under an inert atmosphere of argon or nitrogen unless otherwise indicated.

Indole-3-methanamine (25). Indole-3-carboxaldehyde (189 mg, 1.3 mmol) and NH_4OH·HCl (113 mg, 1.63 mmol) were dissolved in a Parr flask with 15 mL of MeOH, which was previously saturated with anhydrous ammonia. The flask was stoppered and placed on a Parr shaker for 5 h. To the resulting solution was added 200 mg of Raney nickel (50% slurry in H_2O), and the flask was pressurized to 60 psi with H_2 and allowed to shake overnight. The next day, the resulting mixture was filtered through Celite and volatiles were removed to yield 190 mg (100% yield) of a yellow solid. The product was unstable, so it was used immediately in subsequent reactions without further purification. ^{1}H NMR ($CDCl_3$/CD_3OD): δ 8.04 (br s, 1H, N*H*), 7.67 (d, 1H, Ar*H*, J = 7.8), 7.39 (d, 1H, Ar*H*, J = 7.1), 7.16 (3H, Ar*H*), 4.07 (d, 2H, ArCH_2, J = 7.4 Hz).

General Method for the Synthesis of Dithiocarbamates. The amine (1.0 equiv) was dissolved in pyridine (2−3 mL), and the solution was cooled to 0 °C. Triethylamine (1.0−1.1 equiv) and carbon disulfide (1.1 equiv) were added, and the solution was stirred

at 0 °C. After 30 min, iodomethane (1.0−1.2 equiv) was added and the reaction was allowed to slowly warm to room temperature overnight. The reaction was poured into 1 M H_2SO_4 and extracted with EtOAc (3×). The organic layer was washed with brine, dried with Na_2SO_4, and filtered. Concentration afforded a crude product that was chromatographed as described.

Brassinin (1). Brassinin was formed from **25** according to the general method. The crude yellow solid was chromatographed on silica with EtOAc/hexanes (1:3), and the resulting yellow solid was further purified by recrystallization from CH_2Cl_2/hexanes to yield rose-colored crystals (43% yield). ^{1}H and ^{13}C NMR spectra, IR spectrum, and mp matched a previous report for **1**.[25]

N-[2-(Indol-3-yl)ethyl]-S-methyl-dithiocarbamate (2).[26] The general method was used with tryptamine and CH_2Cl_2 as the solvent. The crude product was chromatographed with CH_2Cl_2/hexanes (2:1) to afford a waxy yellow-white solid (87% yield); mp = 59−64 °C. ^{1}H NMR ($CDCl_3$): δ 8.05 (br s, 1H, N*H*), 7.61 (d, 1H, Ar*H*, J = 7.8 Hz), 7.36 (d, 1H, Ar*H*, J = 8.0 Hz), 7.22 (dt, 1H, Ar*H*, J = 8, 1 Hz), 7.14 (dt, 1H, Ar*H*, J = 8, 1 Hz), 7.01 (br s, 1H, N*H*), 4.05 (q, 2H, CH_2NH, J = 12.4, 6.6 Hz), 3.11 (t, 2H, ArCH_2, J = 6.7 Hz), 2.56 (s, 3H, CH_3) and signals due to a minor rotamer (ca. 29%) at 3.73 (m), 2.68 (s). ^{13}C NMR ($CDCl_3$): δ 198.8, 136.4, 127.1, 122.4, 122.1, 119.7, 118.7, 112.3, 111.3, 47.2, 23.9, 18.0, and signals due to a minor rotamer at 201.6, 126.8, 118.4, 46.1, 24.6, 18.9. GC: t_R = 15.00 min. EI-MS m/z (%): 202 (27, M^+-SCH_3), 143 (4), 130 (100). NP-HPLC t_R = 5.9 min.

N-[2-(Benzo[*b*]thiophen-3-yl)ethyl]-S-methyl-dithiocarbamate (3). The general method was used with 2-(benzo[*b*]thiophen-3-yl)ethanamine and CH_2Cl_2 as the solvents. The crude product was chromatographed with EtOAc/hexanes (1:9) to yield a light amber oil, which slowly crystallized (22% yield); mp = 81−84 °C. ^{1}H NMR ($CDCl_3$): δ 7.90 (m, 1H, Ar*H*), 7.79 (m, 1H, Ar*H*), 7.43 (m, 3H, Ar*H*), 7.02 (br s, 1H, N*H*), 5.18 (d, 2H, ArCH$_2$CH_2, J = 4.17 Hz), 2.63 (m, 5H, SCH_3 overlapping with ArCH_2) and a signal due to a minor rotamer (ca. 17%) at 4.91 (m). ^{13}C NMR ($CDCl_3$): δ 199.2, 140.6, 137.7, 130.7, 125.9, 124.9, 124.7, 123.1, 121.7, 45.1, 18.3, 14.2 and signals due to minor rotamer peaks at 60.4, 21.0. IR (KBr) ν_{max} cm^{-1}: 3336, 3229, 3079, 2995, 2916, 1499, 1379, 1302, 1075, 926. NP-HPLC t_R = 4.8 min. RP-HPLC t_R = 12.1 min.

N-[3-(Indol-3-yl)propyl]-S-methyl-dithiocarbamate (4). The general method was used with 3-(indol-3-yl)-propan-1-amine, 2 equiv of Et_3N, and MeOH as the solvents. After the reaction was complete, the volatiles were removed and the residue was dissolved in EtOAc (60 mL). The solution was washed with 0.5 M HCl (2 × 30 mL), H_2O (20 mL), and brine (20 mL). The organic solution was dried with Na_2SO_4, filtered, and concentrated. The crude product was chromatographed with EtOAc/hexanes (1:3) to yield an off-white oil, which crystallized overnight (61% yield); mp = 54−56 °C. ^{1}H NMR ($CDCl_3$): δ 8.04 (br s, 1H, N*H*), 7.60 (d, 1H, Ar*H*, J = 7.6), 7.35 (d, 1H, Ar*H*, J = 8.0), 7.20 (m, 1H, Ar*H*), 7.12 (m, 1H, Ar*H*), 7.04 (m, 1H, Ar*H*), 6.91 (br s, 1H, N*H*), 3.82 (q, 2H, ArCH$_2$CH$_2$CH_2, J = 7.0 Hz), 2.86 (t, 2H, ArCH_2CH$_2$ J = 7.2 Hz), 2.52 (s, 3H, SCH_3), 2.1 (m, 2H, ArCH$_2$CH_2) and signals due to a minor rotamer (ca. 30%) 3.50 (q, J = 6.3 Hz), 2.68 (s). ^{13}C NMR ($CDCl_3$): δ 198.7, 136.4, 127.1, 122.2, 121.6, 119.4, 118.7, 115.1, 111.3, 47.2, 28.3, 22.7, 18.0 and signals due to a minor rotamer at 46.0, 28.9, 18.7. IR (KBr) ν_{max} cm^{-1}: 3410, 3321, 2919, 1888, 1504, 1337, 1094. EI-MS: m/z (%) 216 (57, M^+-SCH_3), 183 (5), 156 (10), 131 (23). NP-HPLC t_R = 12.3 min. RP-HPLC t_R = 11.9 min.

N-(Indan-2-yl)-S-methyl-dithiocarbamate (5). The general method was used with 2-aminoindan HCl. The crude product in EtOAc was decolorized with charcoal and filtered through Celite, and the volatiles were removed to yield a clear oil. The oil was chromatographed with EtOAc/hexanes (1:9) to yield an off-white solid (74% yield); mp = 106−108 °C. ^{1}H NMR ($CDCl_3$): δ 7.23 (4H, Ar*H*), 7.10 (br s, 1H, N*H*), 5.31 (m, 1H, CH$_2$C*H*CH$_2$), 3.44 (m, 2H, C*H*CHC*H*), 2.98 (dd, 2H, C*H*CHC*H*, J = 16.5 Hz, 3.7 Hz), 2.62 (s, 3H, SCH_3) and signals due to a minor rotamer (ca. 38%) 4.78 (m), 2.70 (s). ^{13}C NMR ($CDCl_3$): δ 198.6, 140.5, 127.0, 124.9, 57.9, 39.4, 18.2 and signals due to a minor rotamer at 57.1, 39.8, 18.5. IR (KBr) ν_{max} cm^{-1}: 3226, 2948, 2916, 2088, 1483, 1371, 1337, 1070. NP-HPLC t_R = 4.5 min. RP-HPLC t_R = 12.2 min.

N-(Adamant-2-yl)-S-methyl-dithiocarbamate (6). The general method was used with 2-adamantylamine HCl and 2 equiv of Et_3N to afford a white solid (98% yield); mp 128−129 °C. ^{1}H NMR ($CDCl_3$): δ 7.25 (br s, 1H, N*H*), 4.65 (t, 1H, C*H*NH, J = 3.6 Hz), 2.63 (s, 3H, SCH_3), 2.13 (m, 2H, CH_2), 1.73 (m, 12H, CH_2) and signals due to a minor rotamer (ca. 36%) 4.08 (m), 2.68 (s). ^{13}C NMR ($CDCl_3$): δ 197.8, 97.5, 61.1, 37.3, 32.7, 31.4, 27.4, 18.5 and signals due to a minor rotamer at 37.8, 32.0, 27.3, 19.3. IR (KBr) ν_{max} cm^{-1}: 3351, 2918, 2852, 1497, 1384, 1117, 942. NP-HPLC t_R = 4.1 min. RP-HPLC t_R = 13.2 min.

N-[(Naphth-2-yl)methyl]-S-methyl-dithiocarbamate (7). The general method was used with **28** and 2 equiv of Et_3N and MeOH as the solvents. The crude product was chromatographed on silica with EtOAc/hexanes (15:85) to yield a yellow solid (54% yield); mp 70−72 °C. ^{1}H NMR, ^{13}C NMR, and IR spectra matched a previous report for **7**.[27]

N-Benzyl-S-methyl-dithiocarbamate (8). The general method was used with benzylamine, and the crude product was chromatographed with EtOAc/hexanes (1:10) to yield an off-white oil (74% yield). ^{1}H NMR, ^{13}C NMR, and IR spectra matched a previous report for **8**.[28]

N-Phenethyl-S-methyl-dithiocarbamate (9). The general method was used with phenethylamine, and the crude product was chromatographed with EtOAc/hexanes (1:10) to yield an off-white solid (85% yield); mp 50−51 °C. ^{1}H NMR ($CDCl_3$): δ 7.28 (5 overlapping H, Ar*H*), 6.91 (br s, 1H, *NH*$_2$), 4.02 (t, 2H, ArCH$_2$CH_2, J = 6.9), 2.98 (t, 2H, ArCH_2CH$_2$, J = 7.0), 2.61 (s, 3H, SCH_3) and signals due to a minor rotamer (ca. 24%) at 3.71 (m), 2.69 (s). ^{13}C NMR ($CDCl_3$): δ 199.1, 138.2, 128.8, 128.7, 126.8, 48.0, 34.2, 18.1 and signals due to a minor rotamer at 47.3, 34.9, 18.5. IR (KBr) ν_{max} cm^{-1}: 3340, 3240, 3026, 2918, 1946, 1496, 1337, 1095. NP-HPLC t_R = 4.6 min. RP-HPLC t_R = 11.5 min.

N-4-Fluorophenethyl-S-methyl-dithiocarbamate (10). The general method was used with 4-fluorophenethylamine, and the solvent was CH_2Cl_2. The volatiles were removed, and the residue was dissolved in EtOAc. The organic layer was washed with 1 M H_2SO_4 (40 mL), H_2O (40 mL), and brine (30 mL). The resulting organic solution was dried with Na_2SO_4 and filtered. The volatiles were removed to yield a beige solid, which was chromatographed with EtOAc/hexanes (8/92) to yield a white solid (89% yield); mp 59−60 °C. ^{1}H NMR ($CDCl_3$): δ 7.18 (m, 2H, Ar*H*), 7.01 (m, 2H, Ar*H*), 3.96 (q, 2H, ArCH$_2$CH_2, J = 7.0 Hz), 2.96 (t, 2H, ArCH_2, J = 7.1 Hz), 2.62 (s, 3H, SCH_3) and signals due to a minor rotamer (ca. 24%) at 3.69 (q, J = 6.6 Hz), 2.68 (s). ^{13}C NMR ($CDCl_3$): δ 199.4, 161.8 (d, J = 243 Hz), 133.9, 130.2, 115.8, 48.0, 33.5, 18.1 and signals due to a minor rotamer at 130.1, 47.2, 30.9, 18.5. IR (KBr) ν_{max} cm^{-1}: 3250, 3002, 2921, 1886, 1506, 1385, 1222, 940.8. NP-HPLC t_R = 5.1 min. RP-HPLC t_R = 11.6 min.

N,S-Dimethyl-N-phenethyldithiocarbamate (11). The general method was used with N-methylphenthylamine, and the solvent was CH_2Cl_2. The crude product was chromatographed with EtOAc/hexanes (1/19) to yield a white oil (85% yield). ^{1}H NMR ($CDCl_3$): δ 7.29 (m, 5H, Ar*H*), 4.25 (t, 2H, ArCH$_2$CH_2, J = 6.9 Hz), 3.20 (s, 3H, NCH_3), 3.01 (q, 2H, ArCH_2, J = 6.8 Hz), 2.66 (s, 3H SCH_3) and signals due to a minor rotamer (ca. 42%) at 3.89 (m), 3.47 (s). ^{13}C NMR ($CDCl_3$): δ 198.6, 138.9, 138.1, 129.3, 129.2, 129.1, 127.0, 59.5, 40.9, 32.9, 20.7 and signals due to a minor rotamer at 56.6, 44.6, 34.0. IR (KBr) ν_{max} cm^{-1}: 3025, 2917, 1949, 1808, 1485, 1386, 1292, 1185, 1100, 992.5. NP-HPLC t_R = 4.2 min. RP-HPLC t_R = 12.7 min.

S-Allyl-brassinin (12). The general method was used with **25**, but allyl bromide was substituted for iodomethane. The crude product was purified by chromatography on silica with EtOAc/hexanes (3/7) to afford an orange oil (52% yield). ^{1}H NMR ($CDCl_3$): δ 8.17 (br s, 1H, N*H*), 7.64 (d, 1H, Ar*H*, J = 7.8 Hz), 7.43 (d, 1H, Ar*H*, J = 8.1 Hz), 7.21 (3H, Ar*H*), 7.03 (br s, 1H, N*H*), 5.93 (m, 1H, SCH$_2$C*H*═CH$_2$), 5.22 (m, 2H, SCH$_2$CH═CH_2),

5.05 (d, 2H, ArC*H*$_2$, J = 4.4 Hz), 3.92 (d, 2H, SCH_2CH=CH_2, J = 7.7 Hz) and signals due to a minor rotamer (ca. 16%) at 4.69 (m), 4.09 (d, J = 7 Hz). ^{13}C NMR ($CDCl_3$): δ 196.3, 136.2, 132.7, 126.4, 122.7, 120.2, 118.6, 118.5, 111.5, 110.3, 43.1, 38.3 and signals due to a minor rotamer at 41.0, 39.5. IR (KBr) ν_{max} cm^{-1}: 3402, 2915, 1852, 1635, 1377, 1063. NP-HPLC t_R = 6.7 min. RP-HPLC t_R = 11.6 min.

S-Benzyl-brassinin (13). The general method was used with **25**, but benzyl bromide was substituted for iodomethane and CH_2Cl_2 was used as the solvent. The crude product was chromatographed on silica EtOAc/hexanes (3/7) to yield a translucent, yellow oil, which slowly solidified. Recrystallization from CH_2Cl_2/hexanes yielded a bright yellow solid (50% yield); mp 101−102 °C. ^{1}H NMR ($CDCl_3$): δ 8.22 (br s, 1H, N*H*), 7.62 (d, 1H, Ar*H*, J = 7.9 Hz), 7.28 (9H, Ar*H* + Ph*H*), 6.98 (br s, 1H, N*H*), 5.11 (d, 2H, ArCH_2, J = 3.9 Hz), 4.55 (s, 2H, CH_2Ph) and signals due to a minor rotamer (ca. 19%) at 4.77 (d, J = 4.5 Hz), 4.67 (s). ^{13}C NMR ($CDCl_3$): δ 196.4, 136.6, 136.3, 129.0, 128.6, 127.5, 126.5, 124.0, 122.8, 120.3, 118.7, 111.4, 110.7, 43.2, 39.9. IR (KBr) ν_{max} cm^{-1}: 3417, 3334, 3058, 1890, 1494, 1455, 1067. NP-HPLC t_R = 6.8 min. RP-HPLC t_R = 12.5 min.

S-Hexyl-brassinin (14). The general method was used with **25**, but 1-iodohexane was substituted for iodomethane. The crude product was chromatographed on silica with EtOAc/hexanes (3/7) to yield a golden oil (57% yield). ^{1}H NMR ($CDCl_3$): δ 8.18 (br s, 1H, N*H*), 7.65 (d, 1H, Ar*H*, J = 7.8 Hz), 7.43 (d, 1H, Ar*H*, 8.1 Hz), 7.22 (3H, Ar*H*), 6.99 (br s, 1H, N*H*), 5.06 (d, 2H, ArCH_2, J = 4.4 Hz), 3.26 (t, 2H, SCH_2, J = 7.5 Hz), 1.70 (m, 2H, SCH$_2$CH_2-CH$_2$CH$_2$CH$_2$CH$_3$), 1.39 (6H, SCH$_2$CH$_2$CH_2CH_2CH_2CH$_3$), 0.88 (t, 3H, CH_3, J = 7.5 Hz) and signals due to a minor rotamer (ca. 19%) at 4.79 (d, J = 4.8 Hz), 3.39 (t, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$): δ 197.6, 136.2, 126.4, 124.0, 122.6, 120.1, 118.6, 111.5, 110.5, 43.0, 35.4, 29.0, 28.5, 22.5, 14.0 and signals due to a minor rotamer at 42.0, 36.5. IR (KBr) ν_{max} cm^{-1}: 3409, 3328, 2955, 2927, 2855, 1620, 1494, 1456, 1379, 1094. NP-HPLC t_R = 6.0 min. RP-HPLC t_R = 13.8 min.

N-[2-(Indol-3-yl)ethyl]-S-benzyl-dithiocarbamate (15). The general method was used with tryptamine as the amine and CH_2-Cl_2 as the solvent. Benzyl bromide was used as the alkylating agent in place of iodomethane. The crude product was chromatographed with EtOAc/hexanes (1:4) to yield white crystals (86% yield). Further purification was accomplished by recrystallization in EtOAc/hexanes to afford a 73% yield; mp = 79−81 °C. ^{1}H NMR ($CDCl_3$): δ 8.02 (br s, 1 H, N*H*), 7.58 (m, 1 H, Ar*H*), 7.37 (m, 6 H, Ar*H*), 7.32−7.18 (m, 1 H, Ar*H*), 7.13 (t, 1 H, Ar*H*, J = 9.0 Hz), 6.99 (m, 2 H, Ar*H*), 4.48 (s, 2 H, SCH_2), 4.05 (q, J = 6.0 Hz, 1 H, ArCH$_2$CH_2), 3.09 (m, 2 H, ArCH_2) and signals due to a minor rotamer (ca. 24%) at 4.59 (s), 3.74 (q, J = 6.0 Hz). ^{13}C NMR ($CDCl_3$): δ 197.2, 136.5, 136.3, 129.3, 128.9, 128.6, 127.6, 127.4, 127.1, 122.4, 122.1, 119.7, 118.7, 112.2, 111.3, 47.2, 39.8, 24.6, 23.9, and signals due to a minor rotamer at 135.7, 127.6, 118.4, 41.0, 24.6. IR (KBr) ν_{max} cm^{-1}: 3394, 3179, 1618, 1503, 1455, 1332, 1095, 936. EI-MS: *m*/*z* (%) 130 (100), 202 (37). GC t_R = 14.8 min. NP-HPLC t_R = 7.6 min. RP-HPLC t_R = 12.9 min. Anal. calcd for $C_{18}H_{18}N_2S_2$: C, 66.22; H, 5.56; N, 8.58; S, 19.64. Found: C, 66.19; H, 5.43; N, 8.42; S, 19.87.

N-[2-(Indol-3-yl)ethyl]-S-[(naphth-2-yl)methyl]dithiocarbamate (16). The general method was used with tryptamine as the amine and CH_2Cl_2 as the solvent. 2-(Bromomethyl)naphthalene was used as the alkylating agent in place of iodomethane. The crude product was chromatographed with EtOAc/hexanes (1:4) to afford the pure product (59% yield). Further purification was accomplished by recrystallization in EtOAc/hexanes to afford white crystals (29% yield); mp = 158−160 °C. ^{1}H NMR ($CDCl_3$): δ 8.05 (br s, 1 H, N*H*), 7.90 (m, 1 H, Ar*H*), 7.79 (t, J = 9.4 Hz, 4 H, Ar*H*), 7.46 (m, 5 H, Ar*H*), 7.11−7.35 (m, 4 H, Ar*H*), 6.97 (s, 1 H, Ar*H*), 4.64 (s, 2 H, SCH_2), 4.08 (q, ArCH$_2$CH_2, J = 6.0 Hz), 3.12 (t, 2 H, ArCH_2, J = 6.0 Hz), and signals due to a minor rotamer (ca. 25%) at 4.77 (s), 3.78 (q, J = 6.0 Hz). ^{13}C NMR ($CDCl_3$): δ 197.3, 136.6, 134.2, 133.5, 132.9, 128.7, 128.0, 127.9, 127.3, 127.2, 126.5, 126.2, 122.6, 122.4, 119.9, 118.9, 112.5, 111.5, 47.4, 40.3, 24.1, 1.2 and signals due to a minor rotamer (ca. 20%) at δ 46.0, 42.0. IR (KBr) ν_{max} cm^{-1}: 3436, 3191, 2914, 2837, 1592, 1515, 1451, 1387, 1358, 1326, 1300, 1204, 1089, 999, 935, 816, 736. EI-MS: *m*/*z* (%): 130 (100), 202 (24). GC t_R = 14.7 min. NP-HPLC t_R = 5.7 min. RP-HPLC t_R = 13.2 min.

N-[2-(Indol-3-yl)ethyl]-S-[(pyrid-3-yl)methyl]dithiocarbamate (17). The general method was used with tryptamine as the amine and CH_2Cl_2 as the solvent. 3-(Bromomethyl)pyridine, HBr salt, was used as the alkylating agent in place of iodomethane, and 2.0 equiv of Et_3N were used. The crude product was chromatographed with EtOAc/hexanes (3:1) to afford a powdery tan solid (21% yield); mp = °C. ^{1}H NMR ($CDCl_3$): 8.5 (m, 2 H, Ar*H*), δ 8.16 (br s, 1 H), 7.69 (m, 1 H, Ar*H*), 7.58 (t, 1 H, Ar*H*, J = 6.0 Hz), 7.37 (d, 1 H, Ar*H*, J = 6.0 Hz), 7.24−7.12 (m, 4 H, Ar*H*), 7.03 (m, 1 H, Ar*H*), 4.52 (s, 2 H, SCH_2), 4.08 (m, 2 H, ArCH$_2$CH_2), 3.12 (m, 2H, ArCH_2), and signals due to a minor rotamer (ca. 25%) at 4.58 (s), 3.75 (m). ^{13}C NMR ($CDCl_3$): δ 197.0, 150.3, 148.8, 136.8, 133, 127, 123.6, 122.7, 122.4, 120, 118.9, 112.5, 111.6, 53.5, 47.7, 36.9, 24.2 and a signal due to a minor rotamer at 54.0. IR (KBr) ν_{max} cm^{-1}: 3403, 3306, 3164, 2917, 1724, 1619, 1500, 1455, 1421, 1392, 1332, 1257, 1089, 926, 851, 739. EI-MS: *m*/*z* (%): 130 (100), 202 (35). GC t_R = 14.8 min. NP-HPLC t_R = 27.9 min. RP-HPLC t_R = 9.4 min.

N-[2-(Indol-3-yl)ethyl]-S-[(pyrid-4-yl)methyl]dithiocarbamate (18). The general method was used with tryptamine as the amine and CH_2Cl_2 as the solvent. 4-(Bromomethyl)pyridine, HBr salt, was used as the alkylating agent in place of iodomethane, and 2.0 equiv of Et_3N was used. The crude product was recrystallized with EtOAc/hexanes (3:1) to afford tan crystals (50% yield); mp = 125−127 °C. ^{1}H NMR ($CDCl_3$): 8.51 (m, 2 H, Ar*H*), 8.08 (br s, 1 H), 7.59 (m, 1 H, Ar*H*), 7.39 (d, 1 H, Ar*H*, J = 6.9 Hz), 7.28−7.00 (m, 6 H, Ar*H*), 4.51 (s, 2 H, SCH_2), 4.07 (m, ArCH$_2$CH_2, J = 6.0 Hz), 3.14 (m, 2 H, ArCH_2), and signals due to a minor rotamer (ca. 25%) at 4.60 (s), 3.75 (m). ^{13}C NMR ($CDCl_3$): δ 196.3, 150.1, 146.7, 136.7, 127.4, 124.3, 124.1, 122.7, 122.4, 120.0, 118.9, 112.5, 111.6, 47.9, 38.5, 24.2, 19.8. IR (KBr) ν_{max} cm^{-1}: 3404, 3299, 2917, 2851, 2178, 2099, 1600, 1508, 1455, 1416, 1337, 1225, 1091, 1002, 927, 743. EI-MS: *m*/*z* (%): 130 (100), 202 (29). GC t_R = 14.7 min. NP-HPLC t_R = 28.9 min. RP-HPLC t_R = 9.4 min.

General Method for the Synthesis of Thioureas. The amine was dissolved/suspended in CH_2Cl_2, cooled to 0 °C, and treated with Et_3N (2.1−2.2 equiv). Methyl isothiocyanate (1.1−1.5 equiv) was added about 5 min later, and the reaction was allowed to slowly warm to room temperature while stirring overnight.

N-[1-(Indol-3-yl)methyl]-N′-methyl-thiourea (19). The general method was used with **25**. The volatiles were removed from the reaction, and the crude residue was recrystallized from EtOAc/hexanes to yield a gold, crystalline solid (54% yield); mp 148−150 °C. ^{1}H NMR (DMSO-d_6): δ 10.9 (br s, 1H, N*H*), 7.65 (m, 1H, Ar*H*), 7.36 (m, 2H, Ar*H*), 7.10 (t, 1H, A*rH*, J = 7.2 Hz), 4.75 (br s, 2H, ArCH_2), 2.85 (br s, 3H, NHCH_3). ^{13}C NMR (DMSO-d_6): δ 183.4, 137.1, 124.9, 124.4, 122.1, 119.4, 112.7, 111.9, 40.1 (overlapped with *C*DCl$_3$), 31.5. IR (KBr) ν_{max} cm^{-1}: 3210, 1565, 1456, 1300, 1089. NP-HPLC (isocratic) t_R = 23.9 min. NP-HPLC (gradient) t_R = 22.5 min.

N-[1-(Indol-3-yl)ethyl]-N′-methyl-thiourea (20). The general method was used with tryptamine HCl. The crude product was isolated by washing the reaction mixture with 1 M H_2SO_4 (2×), saturated $NaHCO_3$, and brine and drying with Na_2SO_4. After concentration, the crude product was further purified by chromatography with EtOAc/hexanes (gradient, 1/1 to 3/1) to afford an oil that crystallizes on sitting to a light brown solid (92% yield); mp = 102−106 °C. ^{1}H NMR ($CDCl_3$): δ 8.13 (s, 1H, N*H*), 7.60 (d, 1H, Ar*H*, J = 7.8 Hz), 7.37 (d, 1H, Ar*H*, J = 8.1 Hz), 7.21 (t, 1H, A*rH*, J = 7.0 Hz), 7.12 (t, 1H, Ar*H*, J = 7.0 Hz), 7.04 (s, 1H, Ar*H*), 5.75 (br s, 2H, N*H*-C=S), 3.79 (br d, 2H, ArCH$_2$CH_2, J = 5.4 Hz), 3.06 (t, 2H, ArCH_2, J = 6.6 Hz), 2.79 (br d, 3H, CH_3, J = 4.5 Hz). ^{13}C NMR ($CDCl_3$): δ 182.3, 136.3, 127.1, 122.4, 122.3, 119.6, 118.5, 112.4, 111.4, 44.8, 30.5, 24.8. IR (KBr) ν_{max} cm^{-1}: 3394, 3320, 3323, 3051, 1561, 1342. NP-HPLC t_R = 24.2 min. RP-HPLC (1/1 MeOH/H_2O) t_R = 6.1 min.

Brassitin (21).[29] Freshly made **25** (190 mg, 1.3 mmol) and Et_3N (271 μL, 1.95 mmol) were dissolved in anhydrous MeOH (10 mL). The flask was cooled to 0 °C, and methyl chlorothiolformate (116 μL, 1.36 mmol) was added dropwise followed by stirring at room temperature for 6 h. A few drops of H_2O were added to quench excess reagent, and the volatiles were evaporated. The residue was dissolved in EtOAc (35 mL) and washed with 0.5 M HCl (2 × 20 mL), saturated $NaHCO_3$ (20 mL), and brine (15 mL). The organic solution was dried with Na_2SO_4, filtered, and concentrated to afford a crude brownish-orange solid (270 mg). After recrystallization from CH_2Cl_2/hexanes, beige crystals: 125 mg, 44% yield; mp 110−111 °C. ^{1}H NMR ($CDCl_3$): δ 8.15 (br s, 1H, N*H*), 7.64 (d, 1H, Ar*H* J = 7.9), 7.39 (d, 1H, Ar*H* J = 7.1), 7.23 (m, 1H, Ar*H*), 7.18 (m, 1H, Ar*H*), 7.13 (m, 1H, Ar*H*), 5.52 (br s, 1H, CH_2N*H*C), 4.67 (d, 2H, ArC*H*$_2$, J = 5.1), 2.38 (s, 3H, SC*H*$_3$). ^{13}C NMR ($CDCl_3$): δ 167.6, 136.3, 126.3, 123.3, 122.5, 119.9, 118.7, 112.1, 111.3, 36.9, 12.4. EI-MS *m*/*z* (%): 220 (37, M^+), 205 (9), 172 (12, M^+-SCH_3), 130 (100). NP-HPLC t_R = 9.8 min. RP-HPLC (1/1 CH_3CN/H_2O + 0.1% TFA) t_R = 9.5 min.

N-[(Indol-3-yl)methyl]propanamide (29). Compound **25** (1.00 g, 6.84 mmol) and Et_3N (1.4 mL, 10.26 mmol) were dissolved in MeOH and cooled to 0 °C. Propionyl chloride (633 mg, 6.84 mmol) was added dropwise, and the reaction was stirred at room temperature for 4 h. The volatiles were removed, and the residue was taken up in CH_2Cl_2 (40 mL), washed with 10% citric acid (20 mL), saturated $NaHCO_3$ (20 mL), and brine (20 mL). The organic layer was dried with Na_2SO_4 and filtered, and the volatiles were removed to yield 1.33 g of a white, crystalline solid (1.33 g, 96% yield). An analytical sample was recrystallized form EtOAc/hexanes; mp 91−92 °C. ^{1}H NMR ($CDCl_3$): δ 8.80 (br s, 1H, N*H*), 7.62 (d, 1H, Ar*H*, J = 7.85 Hz), 7.38 (d, 1H, Ar*H*, J = 7.2 Hz), 7.20 (3H, Ar*H*), 5.80 (br s, 1H, N*H*), 4.60 (d, 2H, ArC*H*$_2$, J = 5.1 Hz), 2.20 (q, 2H, COC*H*$_2$CH$_3$, J = 7.6 Hz), 1.14 (t, 3H, COCH$_2$C*H*$_3$, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$): δ 173.6, 136.5, 126.6, 123.3, 122.5, 119.9, 118.8, 112.8, 111.4, 35.2, 29.7, 9.9. IR (KBr) ν_{max} cm^{-1}: 3405, 1891, 1634, 1532, 1097.

N-[(Indol-3-yl)methyl]propanethioamide (22). Amide **29** (190 mg, 0.94 mmol) was dissolved in THF (20 mL). Lawesson reagent (304 mg, 0.75 mmol) was added to the resulting solution, and the reaction was stirred for 2 h at room temperature. The volatiles were removed, and the residue was dissolved in CH_2Cl_2 (20 mL) and washed with H_2O (12 mL). The organic layer was dried with Na_2SO_4 and filtered. After standing, a white precipitate formed, which was filtered, and the filtrate was concentrated. The resulting residue (380 mg) was chromatographed with EtOAc/hexanes (1:1) to yield a clear oil, which slowly crystallized (85 mg, 41% yield); mp 132−134 °C. ^{1}H NMR ($CDCl_3$): δ 8.23 (br s, 1H, N*H*), 7.63 (1H, Ar*H*), 7.41 (1H, Ar*H*), 7.23 (3H, Ar*H*), 4.98 (d, 2H, ArC*H*$_2$, J = 4.5 Hz), 2.68 (q, 2H, CSC*H*$_2$CH$_3$, J = 7.5 Hz), 1.30 (t, 3H, CSCH$_2$C*H*$_3$, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$): δ 205.9, 136.3, 126.5, 124.0, 122.8, 120.3, 118.7, 111.5, 110.8, 42.2, 40.0, 13.5. IR (KBr) ν_{max} cm^{-1}: 3331, 2975, 2931, 1523, 1413, 1090. EI-MS *m*/*z* (%): 218 (49, M^+), 163 (8), 131 (12), 130 (100). NP-HPLC t_R = 10.9 min. RP-HPLC t_R = 10.4 min.

2-Naphthoyl Chloride. A 100 mL round-bottom flask was charged with 2-naphthoic acid (2 g, 11.6 mmol) and $SOCl_2$ (15 mL). The solution was refluxed for 4 h and then concentrated to yield a yellow solid, which was used without further purification (2.21 g, 100% yield). ^{1}H NMR ($CDCl_3$): δ 8.76 (s, 1H, Ar*H*), 8.04 (2H, Ar*H*), 7.93 (d, 2H, Ar*H*, J = 8.9 Hz), 7.66 (m, 2H, Ar*H*).

2-Naphthamide (27). 2-Naphthoyl chloride (2.21 g, 11.6 mmol) was dissolved in a MeOH/NH_3 solution (2 M, 20 mL) and was allowed to stir overnight. Volatiles were removed, and the resulting white solid was triturated with EtOAc. The solid was filtered and washed with cold EtOAc to yield a white solid, which was used without further purification (1.98 g, 100% yield); mp 191−192 °C. ^{1}H NMR ($CDCl_3$): δ 8.39 (s, 1H, Ar*H*), 7.90 (4H, Ar*H*), 7.57 (m, 2H, Ar*H*). ^{13}C NMR ($CDCl_3$): δ 169.3, 135.0, 132.6, 130.5, 129.0, 128.6, 128.1, 127.9, 127.8, 126.9, 123.7. IR (Nujol) ν_{max} cm^{-1}: 3400, 3210, 1650, 1628, 1512, 1510.

2-Aminomethylnaphthalene (28). Compound **27** (1.00 g, 5.8 mmol) in THF (20 mL) was added slowly to a solution of LAH (1.76 g, 46.4 mmol) in THF (45 mL) at 0 °C. The solution was allowed to warm to room temperature, and the reaction was stirred overnight. The reaction was cooled to 0 °C and quenched with H_2O. The solids were filtered from the solution through Celite and washed with hot THF. The filtrate was concentrated, and the residue was dissolved in EtOAc (80 mL) and washed with 1 M HCl (3 × 30 mL). The aqueous layer was basified with 6 M NaOH to a pH of 12, and the precipitate was extracted with EtOAc (3 × 30 mL). The resulting organic solution was washed with brine (40 mL), dried with Na_2SO_4, and filtered. Concentration afforded a slightly yellow solid (510 mg, 56% yield); mp 55−56 °C. ^{1}H NMR ($CDCl_3$): δ 7.80 (3H, Ar*H*), 7.72 (s, 1H, Ar*H*), 7.43 (m, 3H, Ar*H*), 4.00 (s, 2H, ArC*H*$_2$). ^{13}C NMR ($CDCl_3$): δ 140.6, 133.5, 132.5, 128.2, 127.7, 126.1, 125.8, 125.5, 125.1, 46.6. IR (KBr) ν_{max} cm^{-1}: 3362, 3291, 3050, 2915, 1950, 1596, 1507, 1358, 1273. GC t_R = 9.0 min. EI-MS *m*/*z* (%): 157 (83, M^+), 156 (100), 141 (15), 129 (49), 128 (40), 127 (24), 115 (10).

1-Bromo-3-(indol-3-yl)propanone (31). The diazoketone **30** (379 mg, 1.90 mmol) was dissolved in acetic acid (4 mL) and cooled to 0 °C. HBr (48%, 0.51 mL) was added dropwise. Forty minutes later, the reaction was diluted with H_2O and then quenched at 5 °C with saturated $NaHCO_3$. The reaction mixture was extracted with CH_2Cl_2 (2×), washed with saturated $NaHCO_3$, H_2O, and brine, dried with Na_2SO_4, filtered, and concentrated to a brown oil (398 mg, 83% yield). The crude product was used immediately in the next step. ^{1}H NMR ($CDCl_3$): δ 8.26 (br s, 1H, N*H*), 7.56 (d, 1H, Ar*H*, J = 7.8 Hz), 7.39 (d, 1H, Ar*H*, J = 7.8 Hz), 7.27−7.13 (m, 3H, Ar*H*), 4.07 (s, 2H, C*H*$_2$Br), 3.95 (s, 2H, ArC*H*$_2$).

General Method for the Synthesis of Thiazoles. α-Bromoketone **31** was dissolved in EtOH and treated with thioamide (1.5 equiv) and $NaHCO_3$ (1.5 equiv). The resulting mixture was heated at reflux overnight. Upon cooling, the reaction material was partitioned between EtOAc and half saturated $NaHCO_3$. The aqueous layer was extracted with EtOAc, and the combined organic layers were washed with H_2O and brine, dried with $MgSO_4$, filtered, and concentrated to a brown oily solid. The crude thiazole product was purified by chromatography with EtOAc/hexanes (1:2).

4-[(Indol-3-yl)methyl]thiazole (23). The general method was used with thioformamide[30] to afford a 71% yield. ^{1}H NMR ($CDCl_3$): δ 8.76 (d, 1H, SC*H*N, J = 2.0 Hz), 8.11 (br s, 1H, N*H*), 7.52 (d, 1H, Ar*H*, J = 7.6 Hz), 7.36 (d, 1H, Ar*H*, J = 8.0 Hz), 7.18 (t, 1H, Ar*H*, J = 7.1 Hz), 7.08 (t, 2H, Ar*H*, J = 7.4 Hz), 6.90 (s, 1H, Ar*H*), 4.34 (s, 2H, ArC*H*$_2$). ^{13}C NMR ($CDCl_3$): δ 157.5, 152.5, 122.5, 122.1, 119.4, 119.1, 113.8, 113.5, 111.2, 27.6. IR (CH_2Cl_2) ν_{max} cm^{-1}: 3626, 3470, 3051, 2987, 1420, 1264. GC t_R = 15.1 min. EI-MS *m*/*z* (%): 214 (100, M^+), 213 (86), 186 (15), 154 (14), 130 (51). RP-HPLC (1/1 CH_3CN/H_2O + 0.1% TFA) t_R = 4.4 min.

4-[(Indol-3-yl)methyl]-2-methyl-thiazole (24). The general method was used with thioacetamide to afford a 56% yield. ^{1}H NMR ($CDCl_3$): δ 8.17 (br s, 1H, N*H*), 7.53 (d, 1H, Ar*H*, J = 7.8 Hz), 7.34 (d, 1H, Ar*H*, J = 8.1 Hz), 7.17 (t, 1H, Ar*H*, J = 7.0 Hz), 7.10−7.04 (m, 2H, Ar*H*), 6.63 (s, 1H, Ar*H*), 4.23 (s, 2H, C*H*$_2$), 2.69 (s, 3H, C*H*$_3$). ^{13}C NMR ($CDCl_3$): δ 165.6, 162.3, 156.1, 136.4, 127.3, 122.6, 122.0, 119.3, 119.1, 113.4, 111.1, 27.7, 19.1. IR (KBr) ν_{max} cm^{-1}: 3247, 3090, 2919, 1527, 1454, 1429, 1188. GC t_R = 15.4 min. EI-MS *m*/*z* (%): 228 (100, M^+), 227 (71), 186 (22), 154 (23), 130 (39). RP-HPLC (1/1 MeOH/H_2O) t_R = 18.6 min.

Computational Procedure. All electronic structure calculations were carried out using the Gaussian 03 suite of programs.[31] Natural bond orbital (NBO) population analysis was done with NBO 3.1 as implemented in Gaussian 03.[32] All compounds with terminal methyl groups were optimized at the HF/6-31G*//HF/6-31G[33] level. ESP[34] and NBO atomic charges were computed. The HF/6-31G* molecular ESP surface was mapped onto the total density surface.

Inhibition Assays with IDO. The inhibition assays were performed in a 96 well microtiter plate as described by Littlejohn et al.[14] with a small modification. Briefly, the reaction mixture contained 50 mM potassium phosphate buffer (pH 6.5), 40 mM

ascorbic acid, 400 μg/mL catalase, 20 μM methylene blue, and purified recombinant IDO(1) optimized based on its activity. The reaction mixture was added to the substrate, L-Trp, and the inhibitor. The L-Trp was serially diluted from 200 to 25 μM, and the inhibitors were tested at two concentrations, 200 and 400 μM. The reaction was carried out at 37 °C for 60 min and stopped by adding 30% (w/v) trichloroacetic acid. The plate was heated at 65 °C for 15 min to convert formylkynurenine to kynurenine and then was spun at 6000*g* for 5 min. Finally, 100 μL of supernatant from each well was transferred to a new 96 well plate and mixed with 2% (w/v) *p*-(dimethylamino)benzaldehyde in acetic acid. The yellow color generated from the reaction with kynurenine was measured at 490 nm using a Synergy HT microtiter plate reader (Bio-Tek, Winooski, VT). The data were analyzed using Graph Pad Prism 4 software (Graph Pad Software Inc., San Diego, CA).

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Supporting Information Available: Copies of ^{1}H NMR spectra for compounds **1**−**24**, **27**−**29**, and **31**. Copies of ^{13}C NMR spectra for compounds **1**−**24** and **27**−**29**. Copies of HPLC data for compounds **1**−**24**. Copies of GC data for compounds **15**, **17**, **18**, **23**, **24**, and **28**. Copies of MS data for compounds **15**, **17**, **18**, **23**, **24**, and **28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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