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Synthesis of 2-arylindole derivatives and evaluation as nitric oxide synthase and NFκB inhibitors†

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Abstract

Development of small molecule drug-like inhibitors blocking both nitric oxide synthase and NFκB could offer a synergistic therapeutic approach in the prevention and treatment of inflammation and cancer. During the course of evaluating the biologic potential of a commercial compound library, 2-phenylindole (**1**) displayed inhibitory activity against nitrite production and NF κB with IC_{50} values of 38.1±1.8 and 25.4±2.1 μM, respectively. Based on this lead, synthesis and systematic optimization have been undertaken in an effort to find novel and more potent nitric oxide synthase and NF κB inhibitors with antiinflammatory and cancer preventive potential. First, chemical derivatizations of **1** and 2-phenylindole-3-carboxaldehyde (**4**) were performed to generate a panel of *N*-alkylated indoles and 3-oxime derivatives **2**–**7**. Second, a series of diversified 2-arylindole derivatives (**10**) were synthesized from an array of substituted 2-iodoanilines (**8**) and terminal alkynes (**9**) by applying one-pot palladium catalyzed Sonogashira-type alkynylation and base-assisted cycloaddition. Subsequent biological evaluations revealed 3-carboxaldehyde oxime and cyano substituted 2-phenylindoles **5** and **7** exhibited the strongest nitrite inhibitory activities (IC_{50} = 4.4±0.5 and 4.8±0.4 μM, respectively); as well as NFκB inhibition (IC_{50} = 6.9±0.8 and 8.5±2.0 μM, respectively). In addition, the 6′-MeO-naphthalen-2′-yl indole derivative **10at** displayed excellent inhibitory activity against NFκB with an IC_{50} value of 0.6±0.2 μM.

Introduction

The indole heterocyclic ring constitutes an important structural scaffold in natural products and pharmaceutical molecules (Fig. 1).[1–3] Among them, indolyl alkaloids represent a distinct class of bioactive molecules in chemical and medicinal fields. As examples, alkaloid reserpine was developed as one of the first drugs for the treatment of central nervous system (CNS) and cardiovascular system (CVS)-related diseases,[4, 5] and vincristine, a member of Vinca alkaloids and a mitotic inhibitor, was discovered in the 1960s and clinically used for cancer chemotherapy.[4] Since then, indole chemistry has received increasing interest in diverse drug discovery programs. Derivatives have been developed as antiinflammatory,[6–8] antidepression,[2] antihypertensive,[9, 10] antiosteoporosis[11] agents, among others. In addition, indole-based molecules have been used as molecular probes and pharmacological tools.[12–14] In this regard, a great deal of effort has been expended for the development of novel and advanced synthetic methods for the preparation of indole and related chemical derivatives, such as using transition metal-catalyzed cross coupling reactions,[15] multi-component

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reactions,[16] microwave-assisted organic synthesis (MAOS),[17] and solid-phase organic synthesis (SPOS).[18, 19]

It is generally agreed that chronic inflammation is an important contributing factor in the process of carcinogenesis.[20] Various transcriptional factors, including nuclear factor-κB (NFκB), interferon regulatory factor-1 (IRF-1), signal transducer and activator of transcription-1α (STAT-1α), activating protein-1 (AP-1), T-cell factor 4 (TCF-4), forkhead (Drosophila) homolog rhabdomyosarcoma-like 1 (FKHRL1), nuclear receptors, glucocorticoid receptor-α, and -β, and estrogen receptor- α and -β, have been reported to regulate inducible nitric oxide synthase (iNOS) expression.[21] Nitric oxide (NO) is produced from L-arginine by NOS.[22] This substance serves as a cellular signaling molecule and may be generated as an inflammatory response due to cellular stress and bacterial infection.[23] However, overproduction can lead to tumor promotion and progression,[24] so NOS inhibitors have therapeutic potential for the prevention and treatment of inflammation and cancer.

In addition, in part due to facilitating iNOS expression, NFκB has been recognized for a dominant role in inflammation and cancer.[25–27] Inactivation of the NFκB pathway can decrease the probability of tumor initiation and progression, and activate apoptosis and decrease cell proliferation.[28–34] Accordingly, investigations have been conducted to discover inhibitors of NFκB with potential as promising anticancer and cancer chemoprevention agents.[35]

With LPS-stimulated RAW 264.7 cells, NFκB binds to the promoter region of iNOS gene and plays an important role in iNOS expression and NO production.[36] Thus, this cell line can be used as a model for studying inhibitory activity. In our ongoing effort of developing antiinflammatory and cancer preventive agents, we have employed natural product-inspired and high throughput screening approaches.[37] Recently, during the course of evaluating the biologic potential of a commercial compound library, 2-phenylindole **1** was identified as an inhibitor of nitrite production and NFκB with modest IC_{50} values of 38.1±1.8 and 25.4±2.1 μM, respectively.

Thus far, no phenylindole derivatives have been described in the literature as inhibitors of NO production and NFκB regulation. Accordingly, on the basis of this 2-phenylindole lead, we sought to synthesize a diverse array of indole derivatives by systematically modifying the functional groups on the 2-phenylindole motif using one-pot palladium-catalyzed Sonogashira-type alkynylation and base-assisted cycloaddition. Here we report the synthesis and biological evaluation of 2-phenylindole derivatives.[38]

Results and discussion

Chemistry

Our investigation started with chemical derivatizations of 2-phenylindole **1**. *N*-Alkylation of **1** took place in moderate to high yields on the treatment with NaH and DMF or THF at room temperature (Scheme 1).[39] Deprotonation of **1** with NaH as the base, followed by reaction with MeI[40] or MOMCl, afforded the *N*-alkylation derivatives **2a** or **2c** in 94% yields, respectively. In contrast, when benzyl, propargyl, and prenyl bromides were used as alkylating agents, lower yields were obtained due to the formation of their corresponding 1,3-disubstituted indole derivatives (**2b′**, **2d′**, and **2e′**). The reaction of **1** with $(Boc)_2O$ proceeded smoothly and afforded *N*-Boc-2-phenylindole (**2f**) in 81% yield.[41] For comparison, the reduction of **1** was studied to examine if the aromatic indole system plays an important role in its biological activity. The 2-phenylindoline (**3**) was afforded in 72% yield under $NaBH_3CN$ in acetic acid (Scheme 1).[42]

Based on our initial screening data, compared with **1**, the 2-phenylindole-3-carboxaldehyde **4** showed much less inhibitory activity in the nitrite and NFκB assays. Then we chose to synthesize 3-substituted 2-phenylindole derivatives to evaluate the effects of different substituents in the 3-position of the 2-phenylindole scaffold. Chemical transformations based on **4** were undertaken to generate a series of 3-functionalized derivatives, such as oxime and cyano derivatives (Scheme 2). Several substituted 2-phenyl-1*H*-indole-3-carboxaldehyde oximes **5** and **6a**–**b** were then prepared by reacting **4** and the hydrochloride salt of hydroxylamine, methoxylamine, and *O*-benzylhydroxylamine, respectively. All reactions proceeded smoothly to produce the corresponding oxime-derivatives in excellent yields.[43, 44] Under ultrasound irradiation for 2 h with $Cu(OAc)_2$ as catalyst, the 3-cyano-2-phenylindole **7** could be obtained from **5** in 84% yield.[45]

To further expand the chemical diversity of 2-phenylindole derivatives, a focused 2-phenylindole library **10** was next synthesized employing one-pot, two-step, Sonogashira-type alkynylation and base-assisted cycloaddition from an array of commercially available 2-iodoanilines **8** and terminal alkynes **9** (Table 1).[46] The reaction was monitored by HPLC to make sure that the diaryl alkyne intermediate was transformed into the final cyclized indole product. Based on the optimized procedure, the scope and generality of Sonogashira-type alkynylation and base-assisted cycloaddition were examined.

As shown in Table 1, in general, good functional group compatibility was observed among the terminal alkynes tested. Both electron-donating and electron-withdrawing substituents at the *para*, *meta*, or *ortho* positions of the 2-aryl ring were tolerated, producing the corresponding indole products **10aa**-**10at** in moderate to good yields from 2-iodoaniline **8a** (R^1 = H) (entries 1–21, Table 1). Importantly, all the Sonogashira-type alkynylation reactions occurred smoothly within 1 h. With regarding to base-assisted cycloaddition, phenylacetylene with electron-withdrawing groups reacted relatively faster than those with electron-donating groups. Unfortunately, 3-ethynylbenzoic acid was found to be a poor substrate under these conditions (entry 14). Overall, terminal alkynes with heteroaromatic ring systems, such as pyridine and thiophene moieties, also reacted well, providing the corresponding indole derivatives in moderate yields (entries 18 and 20). However, the reaction of 2-ethynylpyridine was totally repressed compared with 3-ethynylpyridine, possibly due to the chelation of the 2′-nitrogen atom and the alkyne group with the palladium catalyst (entry 19).[47] In contrast to the aromatic alkynes, when aliphatic terminal alkynes such as ethynylcyclohexane and ethynylcyclopentane were introduced in the reaction, no pure products could be isolated (data not shown). Meanwhile, the scope of substrate **8** was also briefly surveyed; different substituted 2-iodoanilines proved to be reactive under the same conditions (entries 22–28). It is important to note that electron-withdrawing groups on the substrates significantly facilitate the reaction, which is consistent with a previous report.[46] For example, with the nitro or nitrile groups, the reaction proceeded to completion even at room temperature and the final products were obtained in high yields (entries 22, 23, and 26). The 5-amino derivative **10ha** was also synthesized by reduction of 5-nitro-2-phenyl-1*H*-indole **10ba** in 67% yield, employing Pd/C (10%) as catalyst under a hydrogen atmosphere (entry 29).[48]

Biological activities

All target compounds were tested as inhibitors of NO production and as the regulators of NFκB activity. Initial determinations were carried out at a compound concentration of 50 μM. If a compound displayed over 60% inhibition, further evaluations were then performed for the determination of IC_{50} values.

Nitrite inhibitory activity—As summarized in Table 2, the synthetic 2-phenylindole **10aa** showed comparable inhibitory activity (IC_{50} = 24.7 μM) to commercial sample **1** (IC_{50} = 38.1 μM). To further evaluate the influence of the *N*-substituents, the alkylation derivatives of 2-phenylindole (**2a–2e**) and the *N*-Boc derivative **2f** were synthesized and tested. Compound **2d** (IC_{50} = 44.3 μM) with a propargyl substituent and compound **2f** (IC_{50} = 34.2 μM) with a *N*-Boc group showed similar moderate inhibitory effects against nitrite production. However, the inhibitory effects of **2d** and **2f** were accompanied by cytotoxicity with IC_{50} values of 46.8 and 38.2 μM, respectively. Other alkylated indole products (**2a–2c** and **2e**) were mainly inactive and exhibited less than 50% inhibition at a concentration of 50 μM. These data indicate an alkylation substituent at the N^1 position may have a detrimental effect on NO inhibitory activity.

Compound **3**, the reduced variant of **1**, was essentially inactive, indicating the importance of retaining the intact aromatic indole scaffold. From the 3-oxime series of 2-phenylindole, it was observed that 2-phenyl-1*H*-indole-3-carboxaldehyde oxime **5** showed the best inhibitory activity (IC_{50} = 4.4 μM). Compound **6a**, with the *N*-methoxyl oxime functionality, exhibited a weaker response (IC_{50} = 22.5 μM) in comparison with **5**. The bulky benzyl substituted product **6b** was less effective. On the other hand, compound **7**, with a 3-cyano group, exhibited inhibitory activity comparable with compound **5** (IC_{50} = 4.8 μM). These data suggest that both the size and electronic character of the substituent at the 3-position of 2-phenylindole have effect on the inhibitory activity.

When different functional substituents were introduced to investigate the influence of the 2-phenyl group on activity, variable results were obtained. Compared with 2-phenylindole **10aa**, compound **10ab** (2′-Me, IC_{50} = 2.8 μM) exhibited much more potent activity, whereas compound **10ac** was essentially inactive (4′-Me, 39.0% at 50 μM). However, **10ab** was also cytotoxic, yielding a cytotoxic IC_{50} value of 32.9 μM. This effect is likely due to the steric hindrance of the 2′-Me group and subsequent conformational change of the 2-phenylindole moiety.

We also investigated the effect of halogen atoms attached to the phenyl group. Derivative **10ad**, bearing two chloro atoms, showed moderate inhibitory activity (IC_{50} = 45.0 μM). A reduction in activity was obtained by introducing two fluoro atoms (**10ae**, 23.5% inhibition at 50 μM). Interestingly, the intermediate **10ae′** (IC_{50} = 4.6 μM) showed much better inhibitory activity than the final cycloaddition product **10ae**. Further improved activity was observed with compound **10af** (4′-F group; IC_{50} = 17.9 μM). In contrast, the corresponding 4′-Br derivative (**10ak**) showed less activity (42.4% at 50 μM). Other groups, such as methoxyl (**10ag-10ai**), trifluoromethoxyl (**10aj**) and long alkyl chain (**10al**) were also tested; **10ag** (IC_{50} = 29.6 μM) with 3′,5′-dimethoxyl groups demonstrated the most potent activity in this series. The mono-substituted methoxyl derivative (**10ah**, 4′-OMe, 48.6% inhibition at 50 μM; **10ai**, 3′-OMe, IC_{50} = 49.7 μM) showed much weaker inhibitory activity.

By appending an amine group in the benzene ring, compound **10an** with a 3′-amino group (IC_{50} = 30.1 μM) produced better inhibition of NO production than compound **10ao** with a 4′-amino group. No improved activity could be achieved when the amino group was substituted with two methyl groups (**10ap**, 28.8% inhibition at 50 μM). Changing the phenyl into a heteroaromatic group, the thiophen-3-yl indole derivative showed greater activity (**10as**, IC_{50} = 20.4 μM) than the pyridine moiety (**10aq**, 46.6% at 50 μM).

Finally, the effect of substituents on the indole moiety was studied. It was observed that only 5-NO_2-2-phenylindole (**10ba**) and its corresponding reduced variant, 5-NH_2-2-phenylindole (**10ha**), showed good inhibitory activities with IC_{50} values of 11.8 and 21.1 μM,

respectively. Compounds **10bb-10ga** with Cl, CN, Br and Me substituents on the indole system were only marginally active.

For direct comparison, 2-phenylbenzimidazole **11** was assessed in this assay. Only marginal activity was observed (13.8% at 50 μM), which indicates that the replacement of the pyrrole ring with the imidazole ring is detrimental for inhibitory activity.

NFκB inhibitory activity—In the NFκB assay, 2-phenylindole **10aa** displayed moderate inhibitory activity (IC_{50} = 15.4 μM), whereas the commercial sample **1** exhibited an IC_{50} value of 25.4 μM. From the *N*-alkylation and *N*-Boc series, all compounds (**2a**–**f**) were inactive at a concentration of 50 μM, except the diprenylsubstituted 2-phenylindole derivative (**2e′**, IC_{50} = 42.3 μM). In the oxime series (**5** and **6a–b**), only compound **5** with a *N*-hydroxyl group exhibited good NFκB inhibitory activity (IC_{50} = 6.9 μM). These data demonstrated the importance of the free *N*-hydroxyl group. Similar to the pattern observed in the nitrite assay, compound **7** with a cyano group in the 3-position also showed stronger inhibitory activity (IC_{50} = 8.5 μM). Taken together, it appears that small and polar substituents on the 3-position of 2-phenylindole scaffold may enhance the inhibitory effect.

In terms of different substitutents on the 2-phenyl ring, most compounds tested showed significant NFκB inhibitory activity. The substitution pattern also influenced activity. For instance, compound **10ac** bearing a 4′-Me group produced potent activity (IC_{50} = 1.5 μM), while compound **10ab** with a 2′-Me group was inactive. Introducing a mono-halogen atom into the benzene ring proved to be beneficial for activity compared with compound **10aa**. For example, compound **10af** with a 4′-F and **10ak** with a 4′-Br on the benzene ring showed slightly improved activities (**10af**, IC_{50} = 16.5 μM; **10ak**, IC_{50} = 12.5 μM). In contrast, both compounds **10ad** with two chloro atoms and **10ae** with two fluoro atoms showed a significant decrease in NFκB inhibition, with IC_{50} values of 40.2 and 48.3 μM, respectively. For comparison, the intermediate **10ae′** was also included in the assay and it displayed lower activity than the final cycloaddition product **10ae**.

As presented in Table 2, it appears the methoxy group affected the NFκB inhibition in a manner similar to halogen atoms. Compound **10ag**, bearing 3′,5′-dimethoxy on the phenyl ring, was inactive and only exhibited 56.4% inhibition at 50 μM. In contrast, when one methoxy group is attached, both compounds **10ah** (4′-OMe) and **10ai** (3′-OMe) showed much more potent inhibitory activities, with IC_{50} values of 1.4 and 5.8 μM, respectively. The most potent inhibitor obtained was compound **10at** (IC_{50} = 0.6 μM), bearing one methoxy group on the naphthalene moiety. This was probably due to the enhanced and favorable lipophilicity profile.

Compared with the free amino-substituted compounds **10an** (3′-NH_2) and **10ao** (4′-NH_2), compound **10ap** exhibited higher inhibition of NFκB with an IC_{50} of 1.5 μM. Less than 40% inhibition at 50 μM was produced when the phenyl group was replaced with heteroaromatic rings including the 3-pyridyl and 3-thiophenyl moieties. For substituents on the indole scaffold, compounds **10ba** with a 5-NO_2 group and **10ga** with a 6-Me group demonstrated significant inhibitory activities with IC_{50} values of 1.1 and 0.94 μM, respectively. For the halogen substituted compounds, **10da** and **10fa** showed intermediate activities with IC_{50} values of 9.8 and 2.4 μM, respectively. In addition, 2-phenylbenzimidazole **11** mediated an inhibitory response with an IC_{50} value of 11.2 μM.

Correlation between NOS and NFκB and preliminary SAR considerations—Several factors can affect the production of NO including the cellular level of L-arginine as an iNOS substrate and the enzymatic activity or expression level of iNOS.[49] In the assay employed for this investigation, increased production of NO results mainly from iNOS

expression following stimulation with LPS. One potential mechanism of the inhibitory effect mediated by a test compound is inhibition of NFκB activity, one of transcription factors related to iNOS expression.[21] In support of this suggestion, among the compounds tested with IC_{50} values under 50 μM in both the nitrite and NFκB assays (**1**, **2e′**, **5**, **7**, **10aa**, **10ad**, **10af**, **10an**, **10ao**, **10ba**, and **10ha**), linear regression analysis revealed a correlation coefficient of $R^2 = 0.76$ (Fig. 2).

However, a lack of correlation with other compounds is not surprising. First of all, the NFκB and nitrite assays were established using different experimental conditions, e.g., different stimuli (TNFα vs. LPS) with different signaling pathways (TNF receptor vs. Toll-like receptor 4) in different cell lines (HEK293 vs. RAW 264.7). Compounds exerting inhibitory effects on nitrite production without NFκB inhibition might suppress other signaling pathways such as JAK2/STAT1 and MAPK. On the other hand, compounds showing only NFκB inhibition might inhibit TNF receptor directly. In addition, despite structural similarities through sharing the phenylindole moiety, distinctly different mechanisms of actions may apply. For instance, it has been reported that resveratrol and an analog 4,4′-dihydroxy-*trans*-stilbene mediate an antiproliferative response in human fibroblasts via different mechanisms.[50] In the final analysis, further studies are required to clarify underlying mechanisms.

In considering compounds mediating superior activity in one of our assay systems, cleavage of the pyrrole ring in **10ae** led to loss of NFκB inhibitory activity but improved inhibition of nitrite production. The location and number of fluoro atoms at the 2-phenyl ring might be crucial for the dual activities. Compound **10ae** with the 3′,5′-difluoro substituents showed NFκB inhibition while **10af** with the 4′-F substituent exerted dual inhibition. Similarly, the location and number of methoxy (-OMe) groups affected the inhibitory activities (**10ah**, **10ai** vs. **10ag**).

Modifications of the oxime functionality (=N-OH) in **5** with a methyl or a benzyl group resulted in loss of NFκB activities, suggesting the crucial role of the free oxime functionality in phenylindoles for NFκB inhibition. Also, the introduction of the amino group ($-NH_2$) at the 3′, 4′, or 5-position of the 2-phenylindole moiety (**10an**, **10ao**, and **10ha**) showed dual activities, while NMe_2-substituted compound (**10ap**) lost inhibitory activity in nitrite production. Both hydroxyl (-OH) and amino ($-NH_2$) groups can be involved in hydrogen bonding, therefore, the inhibitory effects might be associated with hydrogen bonds with these compounds. A preliminary SAR is summarized in Fig. 3.

Conclusions

In conclusion, a series of 2-phenylindole derivatives has been synthesized. The reactions employing one-pot palladium-catalyzed Sonogashira-type alkynylation and base-assisted cycloaddition feature broad substrate scope. This indole-based chemical library was subsequently evaluated for inhibition of nitrite production and NFκB activity. The 2-phenylindole-3-carboxaldehyde oxime **5** showed the most potent inhibition of nitrite production ($IC_{50} = 4.4$ μM) among this series of compounds without appreciable cytotoxicity (90.8±2.4% survival at the highest concentration of 50 μM tested). Stronger inhibition of NO production was observed with the 2′-tolyl indole derivative **10ab** ($IC_{50} = 2.8$ μM). Although notable cytotoxicity was observed at 50 μM as 31.5±2.7% cell survival with an IC_{50} value of 32.9 μM, the cytotoxic effect of **10ab** did not affect the inhibitory activity (e.g., the cell survival was 94.5% at the IC_{80} concentration, 9.3 μM). With NFκB, most 2-phenylindole derivatives exhibited good to excellent inhibitory activities, especially the 6′-MeO-naphthalen-2′-yl indole derivative **10at** ($IC_{50} = 0.6$ μM). It is also important to note compounds **5** and **7** inhibit both NOS and NFκB with IC_{50} values ranging from 4.4 to

8.5 μM. Based on these promising results and existing SAR, further medicinal chemistry optimization is warranted to identify more potent and less toxic 2-arylindole dual NOS/NFκB inhibitors with antiinflammatory and anticancer therapeutic potential.

Experimental Section

Chemistry

All reagents and anhydrous solvents were purchased from commercial sources were used without further purification. All reactions were monitored either by thin-layer chromatography (TLC) or by analytical high performance liquid chromatography (HPLC) to detect the completion of reactions. Hydrogenation reactions were performed employing domnick hunter NITROX UHP-60H hydrogen generator, USA. Compounds were purified by flash column chromatography on silica gel using a Biotage Isolera One system and a Biotage SNAP cartridge. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance DRX-400 instrument with chemical shifts (δ, ppm) determined using TMS as internal standard. Coupling constants (*J*) are in hertz (Hz). ESI mass spectra in either positive or negative mode were provided by Varian 500-MS IT Mass Spectrometer. The purity of compounds was determined by analytical HPLC using a Gemini, 3 μm, C18, 110 Å column (50 mm × 4.6 mm, Phenomenex) and a flow rate of 1.0 mL/min. Gradient conditions: solvent A (0.1% trifluoroacetic acid in water) and solvent B (acetonitrile): 0–2.00 min 100% A, 2.00–7.00 min 0–100% B (linear gradient), 7.00–8.00 min 100% B, UV detection at 254 and 220 nm.

Representative procedure for the alkylation of 2-phenylindoles (2a–e)

A solution of 2-phenylindole (96.5 mg, 0.5 mmol) in DMF (1 mL) was added dropwise to a slurry of NaH (24 mg, 0.6 mmol, 60% dispersed in mineral oil) in DMF (2 mL) at 0 °C. After stirring for 1 h, neat iodomethane (71 mg, 0.5 mmol) was added dropwise to the reaction mixture. Upon disappearance of the starting material as indicated by TLC (1 h), the reaction mixture was poured into ice water and extracted with EtOAc (3 × 10 mL). The organic layer was combined and dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude material was purified by flash column chromatography on silica gel (hexane/EtOAc = 95/5) to yield 97.2 mg (94%) of 2-phenyl-*N*-methylindole (**2a**) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 6.8 Hz, 2H), 7.37 (t, *J* = 7.6 Hz, 2H), 7.32-7.28 (m, 2H), 7.17 (t, *J* = 8.0 Hz, 1H), 7.07 (t, *J* = 8.0 Hz, 1H), 6.49 (s, 1H), 3.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.6, 138.5, 132.9, 129.4, 128.5, 128.1, 127.9, 121.7, 120.6, 119.9, 109.7, 101.7, 31.2; ESI-MS: calc. for $C_{15}H_{13}N$ $[M + H]^+$: 208.1, found: 208.1. HPLC purity: 98.7% (254 nm), t_R: 7.47 min; 97.6% (220 nm), t_R: 7.46 min.

2-Phenyl-*N*-benzylindole **2b**: white solid; yield 64%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.44-7.36 (m, 5H), 7.28-7.22 (m, 3H), 7.17-7.15 (m, 3H), 7.03-7.02 (m, 2H), 6.67-6.65 (m, 1H), 5.36 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.9, 138.3, 138.0, 132.8, 129.3, 128.8, 128.6, 128.4, 128.1, 127.2, 126.1, 122.0, 120.6, 120.3, 110.6, 102.4, 47.8; ESI-MS: calc. for $C_{21}H_{17}N$ $[M + H]^+$: 284.1, found: 284.2. HPLC purity: 100% (254 nm), t_R: 7.83 min; 100% (220 nm), t_R: 7.82 min.

2-Phenyl-3-benzyl-*N*-benzylindole **2b′**: yellow solid; yield 28%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, J = 8.0 Hz, 1H), 7.35 (t, J = 3.2 Hz, 3H), 7.30-7.28 (m, 2H), 7.23-7.11 (m, 10H), 7.06 (t, J = 6.8 Hz, 1H), 6.95 (d, J = 6.8 Hz, 2H), 5.25 (s, 2H), 4.09 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 141.9, 138.9, 138.4, 137.0, 131.7, 130.5, 128.6, 128.4, 128.2, 127.0, 126.1, 125.6, 121.9, 119.6, 112.2, 110.3, 47.6, 30.7; ESI-MS: calc. for $C_{28}H_{23}N$ $[M + H]^+$: 374.2, found: 374.3. HPLC purity: 100% (254 nm), t_R: 8.16 min; 100% (220 nm), t_R: 8.15 min.

2-Phenyl-*N*-methoxymethylindole **2c**: white solid; yield 94%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (t, *J* = 7.2 Hz, 3H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.37 (d, *J* = 7.2 Hz, 1H), 7.24 (t, *J* = 7.2 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 6.59 (s, 1H), 5.37 (s, 2H), 3.25 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.7, 138.3, 132.4, 129.4, 128.5, 128.3, 128.1, 122.3, 120.7, 120.5, 110.2, 103.4, 74.7, 55.8; ESI-MS: calc. for $C_{16}H_{15}NO$ $[M + H]^+$: 238.1, found: 238.2. HPLC purity: 100% (254 nm), t_R: 7.37 min; 100% (220 nm), t_R: 7.37 min.

2-Phenyl-*N*-propargylindole **2d**: white solid; yield 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (dd, *J* = 8.0 and 7.2 Hz, 3H), 7.45 (dd, *J* = 8.8 and 8.4 Hz, 3H), 7.37 (t, *J* = 7.2 Hz, 1H), 7.27 (t, *J* = 8.0 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 6.57 (s, 1H), 4.76 (d, *J* = 2.4 Hz, 2H), 2.30 (t, *J* = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.9, 137.6, 132.2, 129.2, 128.7, 128.3, 128.1, 122.1, 120.7, 120.5, 110.0, 102.5, 78.9, 72.7, 34.0; ESI-MS: calc. for $C_{17}H_{13}N$ $[M+H]^+$: 232.1, found: 232.1. HPLC purity: 100% (254 nm), t_R: 7.40 min; 100% (220 nm), t_R: 7.40 min.

2-Phenyl-*N*-prenylindole **2e**: oil; yield 8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.0 Hz, 1H), 7.49 (d, J = 7.6 Hz, 2H), 7.45 (t, J = 7.2 Hz, 1H), 7.40-7.29 (m, 3H), 7.23 (t, J = 8.0 Hz, 1H), 7.20 (t, J = 8.0 Hz, 1H), 6.54 (s, 1H), 5.32-5.30 (m, 1H), 4.71 (d, J = 6.0 Hz, 2H), 1.69 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.3, 137.6, 134.2, 133.0, 129.5, 128.4, 128.2, 127.8, 121.5, 121.1, 120.5, 119.8, 110.2, 101.7, 42.6, 25.5, 17.9; ESI-MS: calc. for $C_{19}H_{19}N$ $[M + H]^{+:}$ 262.2, found: 262.2. HPLC purity: 76.4% (254 nm), t_R: 7.97 min; 81.1% (220 nm), t_R: 7.97 min; with the remaining of **2e′**.

2-Phenyl-3-prenyl-*N*-prenylindole **2e′**: oil; yield 6%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 8.0 Hz, 1H), 7.44 (t, J = 7.2 Hz, 2H), 7.38 (t, J = 6.8 Hz, 3H), 7.30 (d, J = 8.0 Hz, 1H), 7.21 (t, J = 7.2 Hz, 1H), 7.12 (t, J = 7.2 Hz, 1H), 5.29 (t, J = 7.2 Hz, 1H), 5.20 (t, J = 6.4 Hz, 1H), 4.56 (d, J = 6.4 Hz, 1H), 3.35 (d, J = 6.8 Hz, 1H), 1.65 (s, 6H), 1.52 (d, J = 4.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.3, 136.4, 133.9, 132.3, 130.9, 130.3, 128.2, 127.9, 127.8, 124.4, 121.5, 121.1, 119.2, 119.1, 112.8, 109.8, 42.3, 25.6, 25.5, 23.9, 17.74, 17.71; ESI-MS: calc. for $C_{24}H_{27}N$ $[M + H]^+$: 330.5, found: 330.3. HPLC purity: 85.1% (254 nm), t_R: 8.46 min; 86.4% (220 nm), t_R: 8.46 min; with the remaining of **2e**.

Synthesis of 2-phenyl-*N*-Boc-indole (2f)

Under nitrogen atmosphere, $(Boc)_2O$ (*d* 0.95 g/mL, 0.125 mL, 0.55 mmol) was added to a solution of 2-phenylindole (96.5 mg, 0.5 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (1.8 mg, 0.015 mmol) in dry acetonitrile (3 mL) at room temperature. The solution was stirred at room temperature for 24 h, and then was evaporated under reduced pressure. After water was added, the resulting mixture was extracted twice with EtOAc (2 × 10 mL). The combined organic layer was washed with brine, dried with Na_2SO_4, and evaporated under reduced pressure. The residue was purified with flash column chromatography on silica gel (EtOAc/hexane = 1/50) to give the pure product **2f** (118.4 mg, 81%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 8.4 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.42-7.30 (m, 6H), 7.24 (t, *J* = 7.2 Hz, 1H), 6.54 (s, 1H), 1.29 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.2, 140.5, 137.4, 135.0, 129.2, 128.7, 127.7, 127.5, 124.2, 122.9, 120.4, 115.2, 109.9, 83.3, 27.5; ESI-MS: calc. for $C_{19}H_{19}NO_2$ $[M - H]^-$: 292.1, found: 292.3. HPLC purity: 100% (254 nm), t_R: 7.98 min; 100% (220 nm), t_R: 7.97 min.

Typical procedure for the reduction of 2-phenylindole to 2-phenylindoline 3

To a solution of 2-phenylindole (96.5 mg, 0.5 mmol) in AcOH (2 mL) was added $NaBH_3CN$ (189 mg, 3 mmol), and the resulting mixture was stirred until no starting material could be detected by TLC analysis (24 h). Then, 6 mL of H_2O was added and additional NaOH pellets were added until pH > 12, the resulting solution was extracted with Et_2O (3 × 10

mL). The organic phases were combined, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure to give the residue, which was purified by flash column chromatography on silica gel to afford the pure product 2-phenylindoline **3** (70.5 mg, 72%). Oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, J = 7.2 Hz, 2H), 7.31 (t, J = 7.6 Hz, 2H), 7.24 (t, J = 7.6 Hz, 1H), 7.07-7.03 (m, 2H), 6.71 (t, J = 7.2 Hz, 1H), 6.63 (d, J = 7.6 Hz, 1H), 4.91 (t, J = 8.8 Hz, 1H), 3.41 (dd, J = 8.8 and 15.6 Hz, 1H), 2.96 (dd, J = 8.8 and 15.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.9, 144.6, 128.5, 128.0, 127.5, 127.3, 126.2, 124.5, 118.8, 108.8, 63.4, 39.5; ESI-MS: calc. for $C_{14}H_{13}N$ [M + H] +: 196.1, found: 196.2. HPLC purity: 100% (254 nm), t_R: 5.68 min; 100% (220 nm), t_R: 5.68 min.

Synthesis of 2-phenylindole-3-carboxaldehyde oxime 5

Hydroxylamine hydrochloride (104 mg, 1.5 mmol) was added to a solution of 2-phenylindole-3-carboxaldehyde **4** (221 mg, 1 mmol) and sodium hydroxide (60 mg, 1.5 mmol) in methanol (5 mL). The reaction mixture was stirred at room temperature until no starting material was detected by TLC and HPLC analysis (3 h). The reaction mixture was evaporated *in vacuo* and EtOAc was added to the residue. The solution was washed with water, and the organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography on silica gel to give the 2-phenylindole-3-carboxaldehyde oxime **5** (197.2 mg, 83%) as a white solid. ^{1}H NMR (400 MHz, d^6-DMSO) δ 11.75 (s, 1H), 10.73 (s, 1H), 8.30 (s, 1H), 8.11 (d, J = 7.6 Hz, 1H), 7.62 (t, J = 8.8 Hz, 2H), 7.56 (t, J = 7.6 Hz, 2H), 7.49-7.44 (m, 3H), 7.22 (t, J = 7.2 Hz, 1H), 7.12 (t, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 144.4, 139.7, 136.4, 131.4, 129.0, 128.6, 125.6, 122.8, 121.9, 120.5, 111.5, 105.9; ESI-MS: calc. for $C_{15}H_{13}N_2O$ [M+ H] $^+$: 237.1, found: 237.1. HPLC purity: 100% (254 nm), t_R: 6.49 min; 100% (220 nm), t_R: 6.49 min.

Representative procedure for the preparation of oximes 6a and 6b

Methoxylamine hydrochloride (50 mg, 0.6 mmol, 1.2 equiv) was suspended in 1 mL of absolute ethanol, and anhydrous pyridine (158 mg, 2 mmol, 4.0 equiv) was added quickly dropwise. Then 2-phenylindole-3-carboxaldehyde **4** (110.5 mg, 0.5 mmol) was added and the reaction was stirred at room temperature for 4 h. The ethanol was removed and the residue was redissolved in dichloromethane and washed with water. The organic layer was dried with Na_2SO_4, filtered and evaporated to give a crude oil, which was purified by flash chromatography to afford a clear oil as product **6a** in 91% yield. 2-Phenyl-1*H*-indole-3-carboxaldehyde *O*-methyl oxime **6a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.38-8.36 (m, 2H), 7.46-7.44 (m, 5H), 7.37-7.36 (m, 1H), 7.30-7.28 (m, 2H), 4.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.2, 140.6, 136.1, 131.2, 129.1, 128.9, 128.8, 125.9, 123.7, 122.9, 121.6, 111.0, 106.6, 61.9; ESI-MS: calc. for $C_{16}H_{14}N_2O$ $[M + H]^+$: 251.3, found: 251.2. HPLC purity: 100% (254 nm), t_R: 7.19 min; 100% (220 nm), t_R: 7.19 min.

2-Phenyl-1*H*-indole-3-carboxaldehyde *O*-benzyl oxime **6b**: Oil, yield: 89%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.28 (s, 1H), 8.18 (s, 1H), 7.46 (d, J = 7.2 Hz, 2H), 7.33-7.28 (m, 7H), 7.28-7.22 (m, 4H), 5.23 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.6, 140.7, 137.9, 136.0, 131.2, 129.0, 128.9, 128.7, 128.5, 127.9, 126.0, 123.6, 123.0, 121.6, 111.0, 106.6, 76.3; ESI-MS: calc. for $C_{22}H_{18}N_2O$ $[M+ H]^+$: 327.4, found: 327.2. HPLC purity: 100% (254 nm), t_R: 7.63 min; 100% (220 nm), t_R: 7.63 min.

Synthesis of 2-phenylindole-3-carbonitrile 7

2-Phenylindole-3-carboxaldehyde oxime **5** (38.7 mg, 0.16 mmol), $Cu(OAc)_2$ (1.5 mg, 5 mol %) and acetonitrile (1.0 mL) were added to a 10 mL tube. After being capped, the reaction mixture was under continuous ultrasound irradiation for 2 h. Following sonication, the reaction mixture was concentrated *in vacuo* and the residue was purified by flash column

chromatography on silica gel (EtOAc/hexane = 1/8) to afford 2-phenylindole-3-carbonitrile **7** (29.3 mg, 84%) as a white solid. ^{1}H NMR (400 MHz, d^6-DMSO) δ 12.59 (s, 1H), 7.98 (d, J = 7.6 Hz, 2H), 7.65-7.60 (m, 3H), 7.58-7.52 (m, 2H), 7.34-7.24 (m, 2H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 144.7, 135.5, 129.9, 129.3, 128.2, 127.0, 123.9, 122.0, 118.3, 116.9, 112.6, 81.4; ESI-MS: calc. for $C_{15}H_{10}N_2$ $[M + H]^+$: 219.1, found: 219.1. HPLC purity: 95.3% (254 nm), t_R: 6.87 min; 99.9% (220 nm), t_R: 6.87 min.

Representative procedure for the synthesis of functionalized 2-phenylindole derivatives 10

A mixture of 2-iodoaniline (109.5 mg, 0.5 mmol), phenylacetylene (76.5 mg, 0.75 mmol), $PdCl_2(PPh_3)_2$ (10.5 mg, 3 mol %), CuI (2.85 mg, 3 mol %) in DMA (3 mL) was stirred in a sealed tube at room temperature until no starting material was detected by HPLC (1 h). NaOH (200 mg, 5 mmol) was added and the reaction temperature was raised to 140 °C for several hours (the completion of the cyclization was monitored by HPLC). The reaction was cooled to room temperature, and EtOAc and water was added. The separated aqueous phase was extracted with EtOAc (3 × 10 mL) and the combined organic layers were washed with water (2 × 20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane = 1/20) to afford 2-phenyl-1*H*-indole **10aa** (67.5 mg, 70%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.61 (d, J = 7.2 Hz, 3H), 7.40 (t, J = 7.2 Hz, 2H), 7.35 (dd, J = 0.8 and 8.4 Hz, 1H), 7.29 (t, J = 7.2 Hz, 1H), 7.21-7.18 (m, 1H), 7.16-7.09 (m, 1H), 6.80 (d, J = 0.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.9, 136.8, 132.4, 129.3, 128.9, 127.7, 126.1, 122.3, 120.6, 120.2, 110.9, 99.9; ESI-MS: calc. for $C_{14}H_{11}N$ $[M - H]^-$: 192.1, found: 192.3. HPLC purity: 100% (254 nm), t_R: 7.12 min; 100% (220 nm), t_R: 7.11 min.

2-*o*-Tolyl-1*H*-indole **10ab**: brown solid; yield: 67%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.64 (d, J = 7.6 Hz, 1H), 7.48-7.45 (m, 1H), 7.39 (d, J = 8.0 Hz, 1H), 7.32-7.27 (m, 3H), 7.23-7.20 (m, 1H), 7.19 (t, J = 7.8 Hz, 1H), 6.61 (d, J = 1.2 Hz, 1H), 2.51 (d, J = 1.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.5, 136.23, 136.18, 132.7, 131.1, 129.1, 128.9, 128.0, 126.2, 122.1, 120.6, 120.1, 110.6, 103.0, 21.2; ESI-MS: calc. for $C_{15}H_{13}N$ $[M–H]^–$: 206.1, found: 206.3. HPLC purity: 100% (254 nm), t_R: 7.23 min; 100% (220 nm), t_R: 7.22 min.

2-*p*-Tolyl-1*H*-indole **10ac**: yellow solid; yield: 68%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.62-7.59 (m, 1H), 7.50 (dd, J = 2.0 and 6.8 Hz, 2H), 7.34 (dd, J = 2.0 and 6.8 Hz, 1H), 7.22 (dd, J = 1.2 and 7.2 Hz, 2H), 7.19-7.16 (m, 1H), 7.15-7.08 (m, 1H), 6.77-6.76 (m, 1H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 137.8, 136.8, 129.8, 129.7, 129.5, 125.2, 122.3, 120.7, 120.3, 111.0, 99.5, 21.4; ESI-MS: calc. for $C_{15}H_{13}N$ $[M + H]^+$: 208.1, found: 208.2. HPLC purity: 100% (254 nm), t_R: 7.29 min; 100% (220 nm), t_R: 7.28 min.

2-(3,4-Dichlorophenyl)-1*H*-indole **10ad**: white solid; yield: 59%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.56 (d, J = 2.0 Hz, 1H), 7.52 (d, J = 7.6 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.30 (d, J = 2.0 Hz, 1H), 7.27 (dd, J = 2.0 and 8.0 Hz, 1H), 7.12 (t, J = 7.2 Hz, 1H), 7.05 (t, J = 7.2 Hz, 1H), 6.69 (d, J = 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.0, 135.3, 133.1, 132.3, 131.3, 130.9, 129.0, 126.7, 124.2, 123.0, 120.9, 120.6, 111.1, 101.2; ESI-MS: calc. for $C_{14}H_9Cl_2N$ $[M + H]^+$: 262.0, found: 262.0. HPLC purity: 100% (254 nm), t_R: 7.63 min; 100% (220 nm), t_R: 7.64 min.

2-(3,5-Difluorophenyl)-1*H*-indole **10ae**: white solid; yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.25-7.21 (m, 1H), 7.16-7.12 (m, 3H), 6.83 (d, J = 1.2 Hz, 1H), 6.76-6.72 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 164.7, 162.3, 162.2, 137.0, 135.5, 128.9, 123.3, 121.1, 120.7, 111.1, 108.0, 107.9, 107.8, 107.7, 103.0, 102.8, 102.5, 101.8; ESI-MS: calc. for $C_{14}H_9F_2N$ $[M–H]^–$:

228.1, found: 228.2. HPLC purity: 100% (254 nm), t_R: 7.29 min; 100% (220 nm), t_R: 7.30 min.

2-((3,5-Difluorophenyl)ethynyl)aniline **10ae′**: brown oil; yield: 95%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (t, J = 8.0 Hz, 1H), 7.18-7.14 (m, 1H), 7.04-6.99 (m, 2H), 6.81-6.70 (m, 3H), 4.20 (s, br, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 163.9, 161.6, 161.5, 148.1, 132.4, 120.5, 126.1, 118.1, 114.6, 114.5, 114.3, 114.2, 106.9, 104.6, 104.3, 104.1, 92.53, 92.49, 92.46, 88.1; ESI-MS: calc. for $C_{14}H_9F_2N$ $[M + H]^+$: 230.1, found: 230.2. HPLC purity: 100% (254 nm), t_R: 7.19 min; 100% (220 nm), t_R: 7.19 min.

2-(4-Fluorophenyl)-1*H*-indole **10af**: white solid; yield: 66%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.65 (d, J = 7.6 Hz, 1H), 7.62-7.58 (m, 2H), 7.38 (d, J = 8.0 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 7.18-7.12 (m, 3H), 6.76 (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9, 161.4, 137.2, 137.1, 129.5, 129.0, 128.9, 127.1, 127.0, 122.6, 120.8, 120.6, 116.3, 116.1, 111.1, 100.2; ESI-MS: calc. for $C_{14}H_{10}FN$ $[M + H]^+$: 212.2, found: 212.2. HPLC purity: 100% (254 nm), t_R: 7.14 min; 100% (220 nm), t_R: 7.14 min.

2-(3,5-Dimethoxyphenyl)-1*H*-indole **10ag**: white solid; yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.61 (d, J = 7.2 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.23-7.09 (m, 2H), 6.80 (s, 3H), 6.44 (s, 1H), 3.84 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.3, 137.8, 136.7, 134.3, 129.1, 122.4, 120.7, 120.2, 110.9, 103.6, 100.3, 99.7, 55.4; ESI-MS: calc. for $C_{16}H_{15}O_2N$ $[M+ H]^+$: 254.1, found: 254.2. HPLC purity: 100% (254 nm), t_R: 7.15 min; 100% (220 nm), t_R: 7.14 min.

2-(4-Methoxyphenyl)-1*H*-indole **10ah**: brown solid; yield: 59%; ^{1}H NMR (400 MHz, d^6-DMSO) δ 11.39 (s, 1H), 7.77 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 7.6 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.05-6.97 (m, 4H), 6.73 (d, J = 2.0 Hz, 1H), 3.79 (s, 3H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 159.0, 138.0, 137.1, 129.0, 126.6, 125.1, 121.3, 119.9, 119.5, 114.6, 111.3, 97.6, 55.4; ESI-MS: calc. for $C_{15}H_{13}ON$ $[M + H]^+$: 224.1, found: 224.2. HPLC purity: 97.8% (254 nm), t_R: 7.06 min; 100% (220 nm), t_R: 7.06 min.

2-(3-Methoxyphenyl)-1*H*-indole **10ai**: white solid; yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.32-7.29 (m, 2H), 7.18-7.08 (m, 4H), 6.82 (dd, J = 2.0 and 7.6 Hz, 1H), 6.78 (d, J = 2.0 Hz, 1H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.2, 137.9, 136.9, 133.9, 130.2, 129.3, 122.5, 120.8, 120.4, 117.8, 113.2, 111.1, 111.0, 100.3, 55.4; ESI-MS: calc. for $C_{15}H_{13}ON$ $[M + H]^+$: 224.1, found: 224.2. HPLC purity: 100% (254 nm), t_R: 7.11 min; 100% (220 nm), t_R: 7.10 min.

2-(4-(Trifluoromethoxy)phenyl)-1*H*-indole **10aj**: white solid; yield: 71%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.65 (t, J = 9.2 Hz, 3H), 7.39 (d, J = 8.0 Hz, 1H), 7.28 (d, J = 8.0 Hz, 2H), 7.23 (t, J = 7.2 Hz, 1H), 7.12 (t, J = 7.6 Hz, 1H), 6.81 (d, J = 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.6, 137.0, 136.5, 131.2, 129.8, 129.1, 126.4, 122.7, 121.6, 120.8, 120.5, 111.0, 100.7; ESI-MS: calc. for $C_{15}H_{10}F_3ON$ $[M + H]^+$: 276.2, found: 276.2. HPLC purity: 100% (254 nm), t_R: 7.49 min; 100% (220 nm), t_R: 7.49 min.

2-(4-Bromophenyl)-1*H*-indole **10ak**: yellow solid; yield: 56%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.54 (dd, J = 1.6 and 6.4 Hz, 2H), 7.49 (dd, J = 1.6 and 6.4 Hz, 2H), 7.36 (d, J = 7.6 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1H), 7.12 (t, J = 7.2 Hz, 1H), 6.79 (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.1, 136.8, 132.3, 131.5, 129.3, 126.7, 122.9, 121.7, 120.9, 120.6, 111.1, 100.7; ESI-MS: calc. for $C_{14}H_{10}BrN$ $[M+ H, {}^{81}Br]^+$: 274.0, found: 274.1; calc. for $C_{14}H_{10}BrN$ $[M+ H, {}^{79}Br]^+$: 272.0, found: 272.1. HPLC purity: 98.9% (254 nm), t_R: 7.42 min; 96.6% (220 nm), t_R: 7.42 min.

2-(4-Pentylphenyl)-1*H*-indole **10al**: yellow solid; yield: 61%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.55 (d, *J* = 8.0 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 8.0 Hz, 2H), 7.17 (t, *J* = 8.0 Hz, 1H), 7.10 (t, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 1.2 Hz, 1H), 2.63 (t, *J* = 7.6 Hz, 2H), 1.66-1.60 (m, 2H), 1.36-1.32 (m, 4H), 0.90 (t, 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.8, 138.2, 136.8, 129.9, 129.4, 129.1, 125.2, 122.2, 120.6, 120.2, 110.9, 99.5, 35.7, 31.6, 31.1, 22.6, 14.1; ESI-MS: calc. for $C_{19}H_{21}N$ $[M + H]^+$: 264.2, found: 264.4. HPLC purity: 100% (254 nm), t_R: 7.99 min; 100% (220 nm), t_R: 7.97 min.

3-(1*H*-Indol-2-yl)aniline **10an**: white solid; yield: 60%; ^{1}H NMR (400 MHz, d^6-DMSO) δ 11.37 (s, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.13-7.02 (m, 2H), 7.00-6.96 (m, 3H), 6.70 (t, *J* = 1.2 Hz, 1H), 6.56-6.53 (m, 1H), 5.15 (s, 2H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 149.0, 138.7, 136.9, 132.8, 129.4, 128.6, 121.2, 119.8, 119.2, 113.5, 113.1, 111.2, 110.5, 97.9; ESI-MS: calc. for $C_{14}H_{12}N_2$ $[M + H]^+$: 209.2, found: 209.2. HPLC purity: 100% (254 nm), t_R: 5.49 min; 100% (220 nm), t_R: 5.49 min.

4-(1*H*-Indol-2-yl)aniline **10ao**: brown solid; yield: 52%; ^{1}H NMR (400 MHz, d^6-DMSO) δ 11.19 (s, 1H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 7.6 Hz, 1H), 7.00-6.93 (m, 2H), 6.63 (d, *J* = 8.4 Hz, 2H), 6.58 (s, 1H), 5.31 (s, 2H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 148.5, 139.2, 136.7, 129.1, 126.1, 120.3, 119.9, 119.2, 119.0, 114.0, 110.8, 95.5; ESI-MS: calc. for $C_{14}H_{12}N_2$ $[M + H]^+$: 209.2, found: 209.2. HPLC purity: 100% (254 nm), t_R: 5.51 min; 100% (220 nm), t_R: 5.51 min.

4-(1*H*-Indol-2-yl)-*N*,*N*-dimethylaniline **10ap**: yellow solid; yield: 40%;^{1}H NMR (400 MHz, d^6-DMSO) δ 11.26 (s, 1H), 7.66 (d, *J* = 7.2 Hz, 2H), 7.44 (d, *J* = 7.6 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.00 (t, *J* = 7.2 Hz, 1H), 6.96 (t, *J* = 7.6 Hz, 1H), 6.77 (d, *J* = 8.8 Hz, 2H), 6.63 (d, *J* = 1.6 Hz, 1H), 2.93 (s, 6H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 149.9, 138.8, 136.9, 129.2, 126.1, 120.6, 120.2, 119.4, 119.2, 112.5, 111.0, 96.1, 40.1; ESI-MS: calc. for $C_{16}H_{16}N_2$ $[M + H]^+$: 237.3, found: 237.2. HPLC purity: 100% (254 nm), t_R: 5.73 min; 100% (220 nm), t_R: 5.72 min.

2-(Pyridin-3-yl)-1*H*-indole **10aq**: white solid; yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 8.95 (d, *J* = 1.6 Hz, 1H), 8.51 (d, *J* = 4.4 Hz, 1H), 7.92-7.64 (m, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.30 (q, *J* = 4.8 Hz, 1H), 7.23-7.17 (m, 1H), 7.12 (t, *J* = 8.0 Hz, 1H), 6.85 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.4, 146.4, 137.6, 134.7, 132.7, 129.2, 128.9, 124.0, 123.1, 121.0, 120.7, 111.3, 101.5; ESI-MS: calc. for $C_{13}H_{10}N_2$ $[M + H]^+$: 195.2, found: 195.2. HPLC purity: 98.6% (254 nm), t_R: 5.28 min; 98.2% (220 nm), t_R: 5.27 min.

2-(Thiophen-3-yl)-1H-indole **10as**: yellow solid; yield: 66%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.38 (s, 3H), 7.36-7.32 (m, 1H), 7.17 (dt, *J* = 1.2 and 8.0 Hz, 1H), 7.09 (dt, *J* = 0.8 and 8.0 Hz, 1H), 6.67 (d, *J* = 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.7, 134.4, 134.2, 129.4, 126.8, 125.9, 122.5, 120.8, 120.5, 119.3, 110.8, 100.2; ESI-MS: calc. for $C_{12}H_9NS$ $[M + H]^+$: 200.3, found: 200.1. HPLC purity: 100% (254 nm), t_R: 6.99 min; 100% (220 nm), t_R: 6.99 min.

2-(6-Methoxynaphthalen-2-yl)-1*H*-indole **10at**: white solid; yield 77%; ^{1}H NMR (400 MHz, d^6-DMSO) δ 11.62 (s, 1H), 8.28 (s, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.87 (t, *J* = 8.0 Hz, 2H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 2.8 Hz, 1H), 7.20 (d, *J* = 2.8 and 8.0 Hz, 1H), 7.10 (t, *J* = 8.0 Hz, 1H), 7.00 (t, *J* = 7.2 Hz, 1H), 6.96 (d, *J* = 1.2 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 157.6, 138.0, 137.4, 133.8, 129.7, 129.0, 128.8, 127.6, 127.5, 124.4, 123.0, 121.7, 120.2, 119.6, 119.4, 111.4, 106.3, 99.0, 55.4; ESI-

MS: calc. for $C_{19}H_{15}NO$ $[M + H]^+$: 274.3, found: 274.3. HPLC purity: 100% (254 nm), t_R: 7.45 min; 100% (220 nm), t_R: 7.45 min.

5-Nitro-2-phenyl-1*H*-indole **10ba**: yellow solid; yield: 58%; ^{1}H NMR (400 MHz, d^6-DMSO) δ 12.30 (s, 1H), 8.52 (s, 1H), 8.00 (dd, *J* = 1.2 and 8.8 Hz, 1H), 7.89 (d, *J* = 7.6 Hz, 2H), 7.55 (d, *J* = 9.2 Hz, 1H), 7.49 (t, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.16 (s, 1H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 141.6, 141.1, 140.4, 131.1, 129.2, 128.6, 128.0, 125.5, 117.1, 111.8, 101.0; ESI-MS: calc. for $C_{14}H_{10}N_2O_2$ $[M + H]^+$: 239.2, found: 239.2. HPLC purity: 100% (254 nm), t_R: 7.11 min; 100% (220 nm), t_R: 7.11 min.

5-Nitro-2-*o*-toyl-1*H*-indole **10bb**: yellow solid; yield: 84%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.59 (d, *J* = 1.6 Hz, 1H), 8.11 (dd, *J* = 2.4 and 9.2 Hz, 1H), 7.45 (dd, *J* = 6.4 and 9.2 Hz, 2H), 7.34-7.29 (m, 3H), 6.76 (s, 1H), 2.50 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.1, 140.8, 139.0, 136.3, 131.3, 128.9, 128.8, 128.2, 126.3, 117.7, 117.6, 110.7, 104.7, 20.9; ESI-MS: calc. for $C_{15}H_{12}N_2O_2$ $[M + H]^+$: 253.1, found: 253.1. HPLC purity: 100% (254 nm), t_R: 7.25 min; 100% (220 nm), t_R: 7.25 min.

5-Chloro-2-phenyl-1*H*-indole **10ca**: white solid; yield: 64%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.56 (d, *J* = 1.6 Hz, 1H), 7.42 (t, *J* = 8.0 Hz, 2H), 7.32 (t, *J* = 7.6 Hz, 1H), 7.25 (t, *J* = 7.6 Hz, 1H), 7.11 (dd, *J* = 2.0 and 8.4 Hz, 1H), 6.72 (d, *J* = 1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.3, 135.1, 131.8, 130.3, 129.1, 128.1, 125.8, 125.2, 122.5, 119.9, 111.8, 99.5; ESI-MS: calc. for $C_{14}H_{10}ClN$ $[M + H]^+$: 228.1, found: 228.2. HPLC purity: 100% (254 nm), t_R: 7.40 min; 100% (220 nm), t_R: 7.39 min.

6-Chloro-2-phenyl-1*H*-indole **10da**: yellow solid; yield: 64%; ^{1}H NMR (400 MHz, d^6-DMSO) δ 11.71 (s, 1H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.48-7.42 (m, 3H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.01 (dd, *J* = 2.0 and 7.6 Hz, 1H), 6.91 (d, *J* = 1.6 Hz, 1H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 138.9, 137.6, 131.8, 129.1, 127.9, 127.5, 126.2, 125.2, 121.5, 119.9, 110.9, 98.9; ESI-MS: calc. for $C_{14}H_{10}ClN$ $[M + H]^+$: 228.1, found: 228.2. HPLC purity: 96.8% (254 nm), t_R: 7.38 min; 96.4% (220 nm), t_R: 7.38 min.

6-Carbonitrile-2-phenyl-1*H*-indole **10ea**: white solid; yield: 84%; ^{1}H NMR (400 MHz, d^6-DMSO) δ 12.14 (s, 1H), 8.05 (s, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.47 (t, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.04 (s, 1H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 140.4, 138.9, 131.2, 129.1, 128.5, 128.4, 125.6, 125.5, 124.4, 120.8, 112.6, 101.5, 99.4; ESI-MS: calc. for $C_{15}H_{10}N_2$ [M+ H] +: 219.2, found: 219.2. HPLC purity: 100% (254 nm), t_R: 6.95 min; 100% (220 nm), t_R: 6.95 min.

5-Bromo-2-phenyl-1*H*-indole **10fa**: white solid; yield: 83%; ^{1}H NMR (400 MHz, d^6-DMSO) δ 11.78 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.71 (s, 1H), 7.47 (t, *J* = 8.0 Hz, 2H), 7.36 (t, *J* = 7.6 Hz, 2H), 7.20 (d, *J* = 8.8 Hz, 1H), 6.88 (s, 1H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 139.2, 135.8, 131.7, 130.5, 129.0, 127.9, 125.2, 124.0, 122.2, 113.3, 111.9, 98.3; ESI-MS: calc. for $C_{14}H_{10}BrN$ $[M + H, {}^{81}Br]^+$: 274.2, found: 274.1; $C_{14}H_{10}BrN$ $[M + H, {}^{79}Br]^+$: 272.2, found: 272.2. HPLC purity: 100% (254 nm), t_R: 7.47 min; 100% (220 nm), t_R: 7.46 min.

6-Methyl-2-phenyl-1*H*-indole **10ga**: white solid; yield: 25%; ^{1}H NMR (400 MHz, d^6-DMSO) δ 11.36 (s, 1H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.46-7.40 (m, 3H), 7.29 (t, *J* = 7.6 Hz, 1H), 7.19 (s, 1H), 6.83 (s, 2H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 137.6, 137.0, 132.4, 130.7, 128.9, 127.2, 126.5, 124.8, 121.2, 119.8, 111.1, 98.6, 21.5; ESI-MS: calc. for $C_{15}H_{13}N$ $[M + H]^+$: 208.3, found: 208.2. HPLC purity: 98.8% (254 nm), t_R: 7.28 min; 100% (220 nm), t_R: 7.28 min.

Synthesis of 5-amino-2-phenylindole 10ha

A mixture of 5-nitro-2-phenylindole **10ba** (33 mg, 0.13 mmol) and 10% Pd/C (1.5 mg) in 2.5 mL of absolute ethanol was hydrogenated at room temperature. The reaction was detected by HPLC and was found to be completed after 4 h. The catalyst was filtered off and the solvent was evaporated. The residue was purified by flash column chromatography to afford 18 mg (67%) of 5-amino-2-phenylindole **10ha** as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.62 (d, J = 7.2 Hz, 2H), 7.40 (t, J = 7.2 Hz, 2H), 7.32-7.27 (m, 1H), 7.22 (s, 1H), 6.92 (d, J = 2.0 Hz, 1H), 6.66-6.63 (m, 2H), 3.2 (br, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.0, 138.5, 132.6, 131.9, 130.3, 129.0, 127.6, 125.1, 113.3, 111.5, 105.5, 99.2; ESI-MS: calc. for $C_{14}H_{12}N_2$ $[M+H]^+$: 209.2, found: 209.2. HPLC purity: 99.2% (254 nm), t_R: 5.32 min; 99.1% (220 nm), t_R: 5.30 min.

Biological assays

Measurement of the production of NO in LPS-stimulated RAW 264.7 murine macrophage cells (nitrite assay)—The level of NO in the cultured media was estimated by measuring the level of nitrite due to the instability of NO and its subsequent conversion to nitrite. The nitrite assay was performed as previously described.[51] Briefly, RAW 264.7 cells were incubated in 96-well culture plates at 37 °C, 5% CO_2 in a humidified air incubator for 24 h. Then cells were treated with serially diluted compounds for 15 min, followed by treatment with or without LPS (1 μg/mL) for an additional 20 h. After the incubation, nitrite released in the cultured media was measured using Griess regent [1:1 mixture (v/v) of 1% sulfanilamide in 5% H_3PO_4 and 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride solution], and absorbance was measured at 540 nm. The concentration of nitrite was calculated using a standard curve created with known concentration of sodium nitrite. Under the same experimental conditions, sulforhodamine B (SRB) assay was performed to evaluate the cytotoxic effect of tested compounds on RAW 264.7 cells. After transferring 100 μL of the cultured media, cells were fixed with 10% trichloroacetic acid, and stained with 0.4% SRB solution in 1% acetic acid. The protein-bound SRB was dissolved in 10 mM Tris base solution, and the absorbance was measured at 515 nm. Percentage of cell survival was calculated in comparison with LPS-treated control.[52] Initial screening was performed at a concentration of 50 μM. Compounds which exhibited over 50% inhibition of nitrite production, and under 50% cell survival, were considered as active and cytotoxic, respectively. Only active inhibitors of nitrite production lacking appreciable cytotoxicity were examined in greater detail to determine whether the inhibitory effect was derived from a false positive cytotoxic effect, and to compare the relative potency of the compounds (SAR).

NFκB luciferase assay—Human embryonic kidney cells 293 were used to monitor any changes occurring along the NFκB pathway. This cell line contains chromosomal integration of a luciferase reporter construct regulated by the NFκB response element. Transcription factors can bind to the response element when stimulated by certain agents, allowing transcription of the luciferase gene. Following an incubation period of 6 h with TNFα and test compounds, cells were analyzed for luciferase activity using the Luc assay system from Promega Corporation (Madison, WI). Results were expressed as a percentage, relative to control (TNFα-treated with DMSO) samples, and dose-response curves were constructed for the determination of IC_{50} values, which were generated from the results of five serial dilutions of test compounds and were the mean of two different experiments.[53] *N*-Tosyl-L-phenylalanyl chloromethyl ketone (TPCK) was used as a positive control (IC_{50} = 3.8±0.6 μM).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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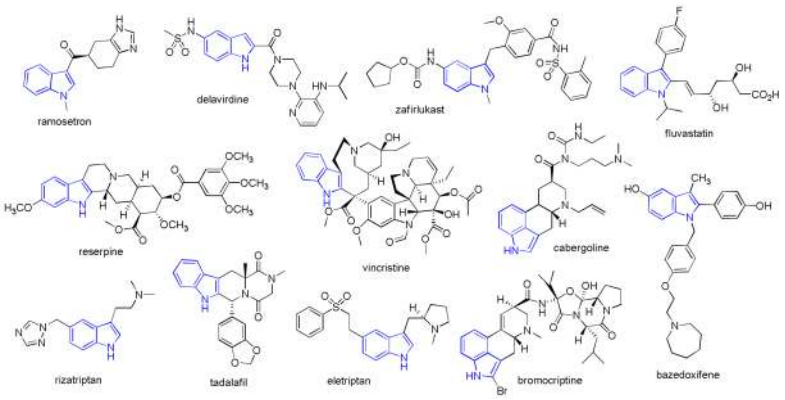


Fig. 1.
Selected examples of drug molecules with an indole scaffold.

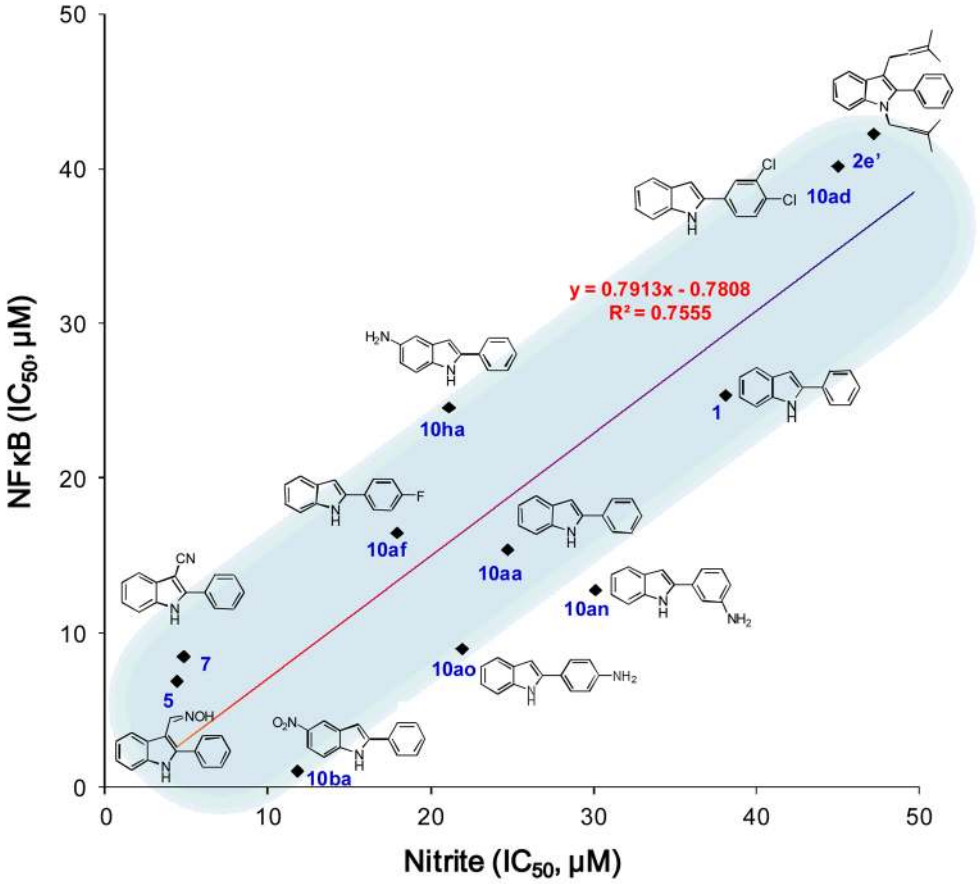


Fig. 2.

The correlation between inhibitory activities of eleven compounds (closed diamond) in nitrite and NFκB assays. Trend analysis using Microsoft Office Excel 2007: R^2= 0.76. **10ai** with an IC_{50} value of 49.7 μM (round number as 50 μM), is excluded.

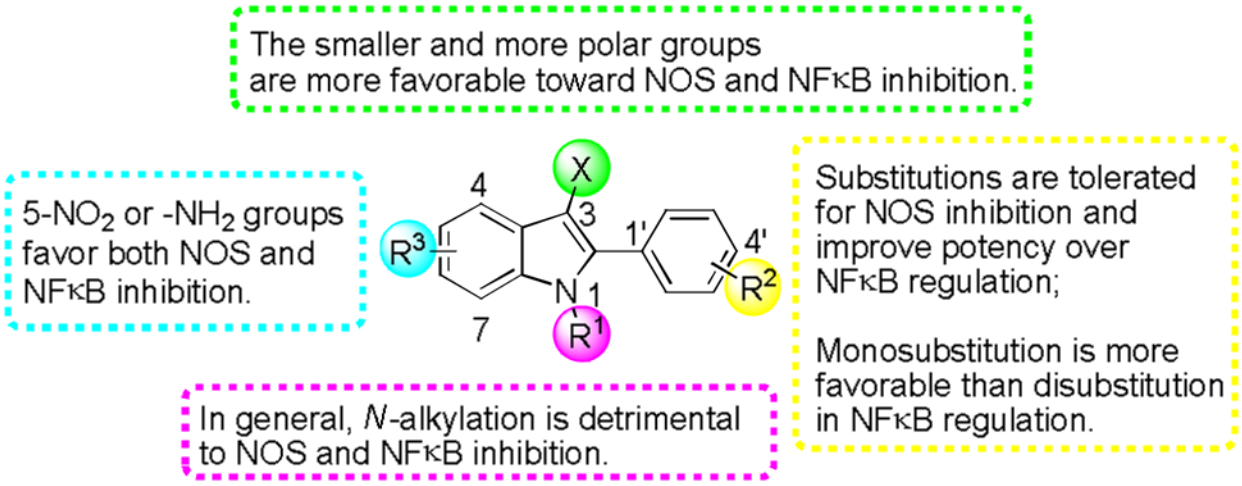
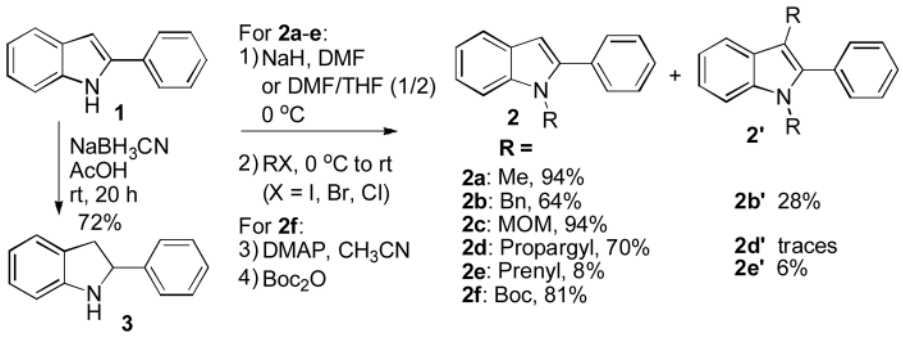
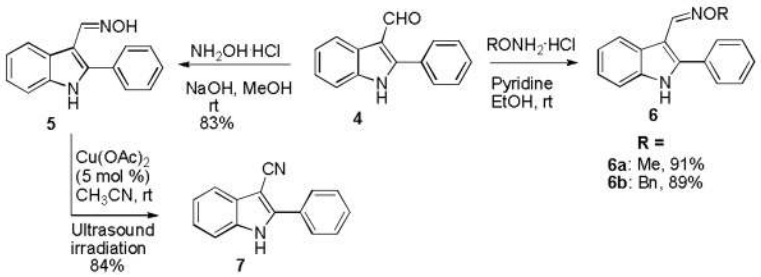


Fig. 3.
Preliminary SAR of 2-phenylindole derivatives.



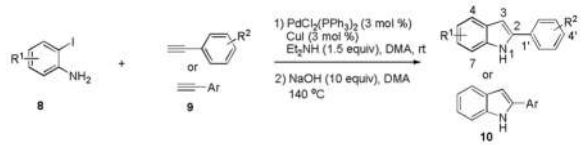
Scheme 1.
Chemical derivatizations of **1**.

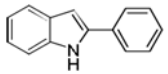
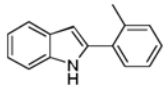
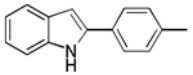
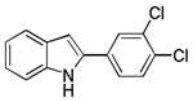
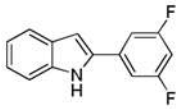


Scheme 2.
Chemical transformations of **4**.

Table 1

Synthesis of indole derivatives **10** from 2-iodoanilines **8** and terminal alkynes **9**[a]

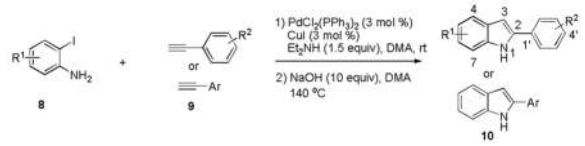


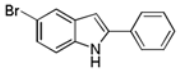
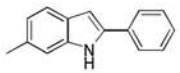
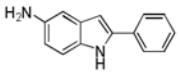
Entry	R^1	R^2	Product	Yield (%)[b]
1	H	H	 **10aa**	70
2	H	2′-Me	 **10ab**	67
3	H	4′-Me	 **10ac**	68
4	H	3′,4′-Dichloro	 **10ad**	59
5	H	3′,5′-Difluoro	 **10ae**	70

Entry	R^1	R^2	Product	Yield (%)[b]
6[c]	H	3′,5′-Difluoro	**10ae′**	95
7	H	4′-F	**10af**	66
8	H	3′,5′-Dimethoxy	**10ag**	67
9	H	4′-OMe	**10ah**	59
10	H	3′-OMe	**10ai**	70
11	H	4′-OCF_3	**10aj**	71
12	H	4′-Br	**10ak**	56

Entry	R^1	R^2	Product	Yield (%)[b]
13	H	4′-Pentyl	**10al**	61
14	H	3′-COOH	**10am**	0
15	H	3′-NH_2	**10an**	60
16	H	4′-NH_2	**10ao**	52
17	H	4′-NMe_2	**10ap**	40
18	H	3′-Pyridyl	**10aq**	60
19	H	2′-Pyridyl	**10ar**	0

Entry	R^1	R^2	Product	Yield (%)[b]
20	H	3′-Thiophenyl		66
			10as	
21	H	6′-Methoxy-naphthalen-2′-yl		77
			10at	
22	5-NO2	H		79
			10ba	
23	5-NO2	2′-Me		84
			10 bb	
24	5-Cl	H		64
			10ca	
25	6-Cl	H		59
			10da	
26	5-CN	H		84
			10ea	



Entry	R^1	R^2	Product	Yield (%)[b]
27	5-Br	H	 **10fa**	83
28	6-Me	H	 **10ga**	25
29[d]	5-NH2	H	 **10ha**	67

[a] Conditions: 0.5 mmol of substrate **8**, 0.75 mmol of substrate **9**, 3 mol % of $PdCl_2(PPh_3)_2$, 3 mol % of CuI, 1.5 equiv of $NHEt_2$, 10 equiv of NaOH, and 3 mL of DMA.

[b] Isolated yield after column chromatography on silica gel.

[c] Isolated alkynylation intermediate.

[d] 5-amino-2-phenyl-1*H*-indole **10ha** was synthesized via reduction of 5-nitro-2-phenyl-1*H*-indole **10ba**.

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Table 2

Evaluation of 2-arylindole derivatives for potential to inhibit iNOS activity and regulate NFκB[a]

	Nitrite assay				NFκB assay		
Compound	**% inhibition**	**% survival[b]**	**IC_{50} (μM)[c]**	**Cytotoxic IC_{50} (μM)[c]**	**% inhibition**	**% survival[b]**	**IC50 (μM)[c]**
1	60.4±2.2	87.3±3.9	38.1±1.8		72.3±5	113.1±2.8	25.4±2.1
2a	33.6±4.9	74.3±6.2			0.0	105.5±12.3	
2b	0.0	84.6±2.0			0.0	96.3±8.9	
2b′	0.0	101.0±6.1			0.0	124.1±6.2	
2c	33.8±4.9	67.3±6.2			21.5±3.3	106.7±2.8	
2d	96.9±1.4	35.8±3.2	44.3±2.1	46.8±1.9	46.2±2.1	105.2±5.3	
2e	33.1±3.1	75.6±4.7			47.0±2.7	113.3±5.6	
2e′	59.2±2.7	89.0±3.4	47.2±0.0		60.9±1.1	104.6±11.2	42.3±2.8
2f	92.4±4.0	27.6±2.4	34.2±1.5	38.2±5.0	0.0	107.1±16.3	
3	35.0±7.0	102.7±7.4			65.0±4.1	87.9±8.8	ND[d]
4	30.6±2.1	109.8±6.7			36.2±11.4	139.2±14.3	
5	97.7±0.7	90.8±2.4	4.4±0.5		73.6±2.6	105.5±10.3	6.9±0.8
6a	97.2±2.4	48.3±7.8	22.5±1.2	48.0±5.1	48.8±8.0	51.8±23.0	
6b	85.3±1.2	59.2±7.7	34.6±2.4		50.2±3.9	66.1±18.8	
7	70.7±3.5	82.1±4.1	4.8±0.4		70.2±3.8	91.4±2.1	8.5±2.0
10aa	79.0±2.0	66.7±4.0	24.7±2.4		76.3±8.2	79.8±5.9	15.4±3.3
10ab	97.5±0.4	31.5±2.7	2.8±0.3	32.9±2.5	0.0	121.1±4.1	
10ac	39.0±2.5	57.9±9.5			96.6±0.6	86.6±3.9	1.5±0.3
10ad	55.8±2.7	67.4±2.8	45.0±2.5		68.2±6.3	115.1±2.1	40.2±3.5
10ae	23.5±4.6	86.5±9.4			62.3±8.3	107.9±10.0	48.3±1.5
10ae′[e]	97.4±1.2	30.8±0.6	4.6±0.6	42.9±5.1	31.5±4.3	121.3±1.9	
10af	58.0±5.9	69.7±3.7	17.9±3.1		77.9±1.4	84.3±8.8	16.5±3.7
10ag	62.6±3.4	109.5±2.4	29.6±3.8		56.4±2.3	66.16±4.1	
10ah	48.6±6.9	59.8±4.0			81.3±1.9	85.0±2.9	1.4±0.1
10ai	50.7±0.9	92.8±6.4	49.7±0.2		85.5±3.0	65.8±15.0	5.8±1.1
10aj	31.2±5.7	105.7±3.5			79.7±8.8	74.8±6.6	6.5±0.1

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	Nitrite assay				NFκB assay		
Compound	**% inhibition**	**% survival[b]**	**IC_{50} (μM)[c]**	**Cytotoxic IC_{50} (μM)[c]**	**% inhibition**	**% survival[b]**	**IC50 (μM)[c]**
10ak	42.4±3.5	69.3±2.7			82.3±0.8	86.7±11.2	12.5±2.0
10al	31.9±6.8	131.8±12.6			76.1±4.1	86.6±12.1	18.1±5.0
10an	70.8±4.3	112.5±6.0	30.1±2.1		72.0±1.9	70.7±10	12.8±2.0
10ao	86.1±1.2	58.7±2.9	21.9±1.5		81.7±4.2	59.7±2.6	9.0±3.3
10ap	28.8±1.5	60.9±4.0			95.9±0.2	74.6±11.0	1.5±0.7
10aq	46.6±3.4	107.0±7.0			12.3±4.4	71.4±5.5	
10as	66.3±4.6	90.6±2.6	20.4±1.8		35.6±3.1	76.0±16.6	
10at	13.9±3.6	58.0±7.7			94.4±1.6	93.5±2.8	0.6±0.2
10ba	66.1±3.8	81.7±3.4	11.8±0.9		92.8±4.0	68.2±11.1	1.1±0.1
10bb	45.9±3.0	89.2±9.5			60.6±3.2	119.2±8.6	ND[d]
10ca	49.3±2.1	77.8±2.9			2.3±0.9	112.6±12.1	
10da	36.3±2.6	74.0±11.9			91.5±1.2	89.4±15.7	9.8±2.3
10ea	45.6±2.5	78.1±3.8			50.6±3.8	72.4±6.2	
10fa	42.4±4.0	91.8±8.7			92.2±2.7	86.1±6.8	2.4±0.3
10ga	45.1±1.6	76.0±4.5			97.3±1.7	84.2±12.0	0.94±0.4
10ha	67.4±3.3	100.2±6.7	21.1±5.7		78.4±12.1	73.8±19.6	24.6±3.6
11[f]	13.8±6.4	97.7±5.6			73.6±3.1	86.2±18	11.2±0.3
Positive control[g]			22.1±0.2				3.8±0.6

[a] Concentration for screening: 50 μM.

[b] Percentage of cell survival in comparison with vehicle-treated controls.

[c] IC_{50} (μM): the half maximal inhibitory concentration (μM); if not listed, the IC_{50} values were >50 3M.

[d] ND indicates the response was not dose-dependent.

[e] **10ae′**: 2-[(3,5-difluorophenyl)ethynyl]aniline (the intermediate of **10ae**).

[f] 2-phenylbenzimidazole **11** was purchased from Sigma-Aldrich.

[g] IC_{50} values of control inhibitors in these assays; L-*N*^{G}-monomethyl arginine for the nitrite assay and *N*-tosyl-L-phenylalanine-chloromethyl ketone for the κB assay.

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