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Identification of indole scaffold-based dual inhibitors of NOD1 and NOD2

Kaja Keček Plešec, Dunja Urbančič, Martina Gobec, Aleksandra Pekošak, Tihomir Tomašič, Marko Anderluh, Irena Mlinarič-Raščan, Žiga Jakopin *

Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, SI-1000 Ljubljana, Slovenia

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ABSTRACT

NOD1 and NOD2 are important members of the pattern recognition receptor family and play a crucial role within the context of innate immunity. However, overactivation of NODs, especially of NOD1, has also been implicated in a number of diseases. Surprisingly, NOD1 remains a virtually unexploited target in this respect. To gain additional insight into the structure–activity relationships of NOD1 inhibitors, a series of novel analogs has been designed and synthesized and then screened for their NOD1-inhibitory activity. Selected compounds were also investigated for their NOD2-inhibitory activity. Two compounds **4** and **15**, were identified as potent mixed inhibitors of NOD1 and NOD2, displaying a balanced inhibitory activity on both targets in the low micromolar range. The results obtained have enabled a deeper understanding of the structural requirements for NOD1 and NOD2 inhibition.

1. Introduction

The initial response of the innate immune system to invading infectious agents is based on recognition of their distinct molecular patterns through specific pattern recognition receptors. The response to bacterial peptidoglycan components is mediated mainly by the cytoplasmic nucleotide-binding oligomerization domain (NOD)-like receptors, NOD1 and NOD2.[1,2] These receptors are structurally very similar as they belong to the AAA+ (ATPases associated with various cellular activities) ATPase family of proteins.[3,4] Both possess a tripartite structure composed of a C-terminal sensor domain comprising leucine-rich repeats and a centrally-located nucleotide-binding domain, and differ solely in the arrangement of their N-terminal effector domains that consist of either one (NOD1) or two (NOD2) caspase activation and recruitment domains.[5]

NOD1 and NOD2 exist mainly in an inactive state, however, recognition of their respective ligands enables them to bind ATP[6,7] and, in turn, to trigger signaling cascades that ultimately bring about an inflammatory response, typified by proinflammatory cytokine and chemokine secretion as well as secretion of antimicrobial peptides.[1,2,8] The dipeptide D-Glu-*meso*-DAP (iE-DAP) is the key sequence necessary and sufficient for ligand recognition and consequent triggering of NOD1 activation[9–11] while NOD2 is activated by muramyl dipeptide (MDP).[12,13] The downstream signaling pathways of NODs are tightly controlled by numerous accessory proteins.[8] Both pathways lead to activation of nuclear factor κB (NF-κB) and mitogen-associated protein kinases (MAPKs) while also playing important roles in apoptosis and autophagy.[8,14–16]

Although the role of NODs in the induction of inflammatory processes is still largely unknown, dysregulation of their function caused either by single nucleotide polymorphisms or by other mutations in their encoding genes, has been linked to numerous inflammatory disorders that affect NF-κB activity.[8,14,15] Similarly, overexpression of both NOD1 and NOD2 has been reported to elicit NF-κB activation.[8,14,15] Furthermore, ligand-elicited overactivation of NOD1 has been associated with the progression of multiple sclerosis,[17] coronary arteritis,[18] ocular inflammation,[19] fetal inflammation[20] and adipose tissue inflammation.[21,22] In addition, activation of NOD1 has recently been reported to augment cancer cell

Abbreviations: C12-iE-DAP, lauroyl-γ-D-glutamyl-*meso*-diaminopimelic acid; iE-DAP, γ-D-glutamyl-*meso*-diaminopimelic acid; IL, interleukin; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; NOD, nucleotide-binding oligomerization domain protein; PAMP, pathogen-associated molecular pattern; RA, residual activity; RIP2, receptor-interacting serine–threonine protein kinase; SAR, structure–activity relationship; SEAP, secreted embryonic alkaline phosphatase.

* Corresponding author. Tel.: +386 1 4769 574; fax: + 386 1 4258 031.
E-mail address: ziga.jakopin@ffa.uni-lj.si (Ž. Jakopin).

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metastatic potential.[23] Compared to NOD2, which is expressed mostly in professional immune cells,[24] NOD1 has a much wider tissue distribution suggesting a broader role in inflammation.[25] Controlled suppression of NOD1 activity could therefore constitute a novel approach to treating inflammatory and autoimmune diseases which involve NOD1 in their pathogenesis. Targeting the up-stream pathways responsible for NF-κB signaling would be expected to leave most innate immunity defense mechanisms intact. In addition, novel NOD1 inhibitors would provide chemical probes that could enable further elucidation of the role of this protein in a variety of inflammatory processes.

Relatively little has been reported on the structural requirements for achieving NOD1 agonistic activity, apart from the reports of Agnihotri and Jakopin.[26,27] On the other hand, three distinct chemical classes of selective small molecule NOD1 inhibitors, 2-aminobenzimidazoles, purine-2,6-diones, and quinazolininones have thus far been identified via a high-throughput screening campaign.[28–31] Noditinib-1 (ML-130), that belongs to the 2-aminobenzimidazole class, has been found to selectively inhibit NOD1-dependent NF-κB activation.[31] The exact mechanism of action is not known, although it has been shown to interact directly with NOD1 protein without affecting the binding of ATP.[32]

Our group has been heavily engaged in the design, synthesis and evaluation of NOD1 and NOD2 agonists.[27,33–37] Here, however, we report the results of focusing on the discovery of novel inhibitors targeting NOD1 protein. Making use of the existing knowledge of the SAR of known NOD1 inhibitors, we have designed and synthesized new compounds based on an indole scaffold employing the »scaffold hopping« approach. The NOD1-overexpressing HEK-Blue cell line served as a model for studying the ability of these compounds to suppress the C12-iE-DAP (lauroyl-γ-D-glutamyl-*meso*-diaminopimelic acid)-induced activation of NF-κB by inhibition of NOD1. An analogous assay, utilizing NOD2-overexpressing HEK-Blue cells and measuring inhibition of NOD2-dependent activation upon stimulation with MDP, was used as a counterscreen to determine NOD1 versus NOD2 selectivity profiles of selected compounds.

2. Results

2.1. Design

The structure of the actual binding site of the reference NOD1 inhibitor Noditinib-1 (**1**) in the molecular target is still unknown, so novel NOD1 inhibitors cannot be designed using structure-based design, the structure–activity relationship (SAR) of known NOD1 inhibitors thus provides the only possible basis for rational drug design. Given the potent NOD1 inhibitory activity of **1** and the balanced NOD1- and NOD2-inhibitory activities of compound **2**, these compounds were identified as interesting starting points for the design of novel inhibitors. This enters an unexplored region of chemical space and involves an in-depth exploration of their SAR. Since no information regarding their binding modes is available, the ligand-based design was employed to investigate the effect of the following structural changes (shown in Fig. 1): (*i*) replacement of the central benzimidazole scaffold with an indole core (Fig. 1, in blue); (*ii*) substitution, at position 2, of the indole heterocycle (Fig. 1, Y); (*iii*) substitution at position 4 of the phenyl moiety (Fig. 1, R) and (*iv*) variation of the linker connecting the phenyl and indole moieties (Fig. 1, X).

»Scaffold hopping« is an approach in drug design, in which the core framework of a molecule is replaced by another scaffold, with the aim to improve the drug-like/physico-chemical properties of the parent molecule or of finding similar potent compounds that exist in novel chemical space.[38] The indole scaffold is considered

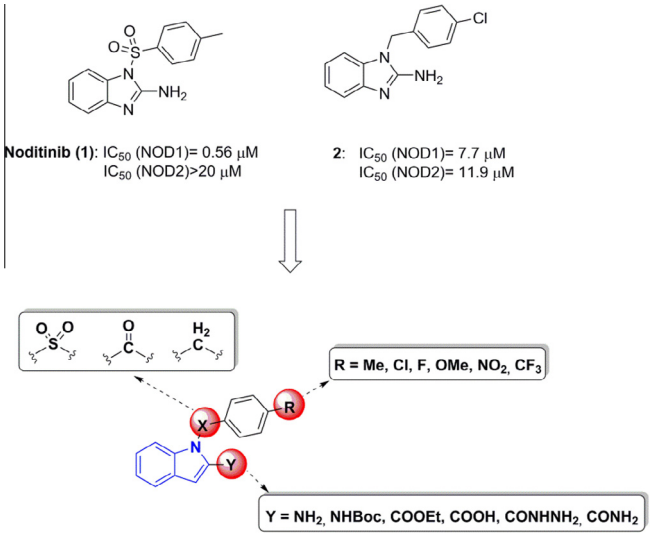
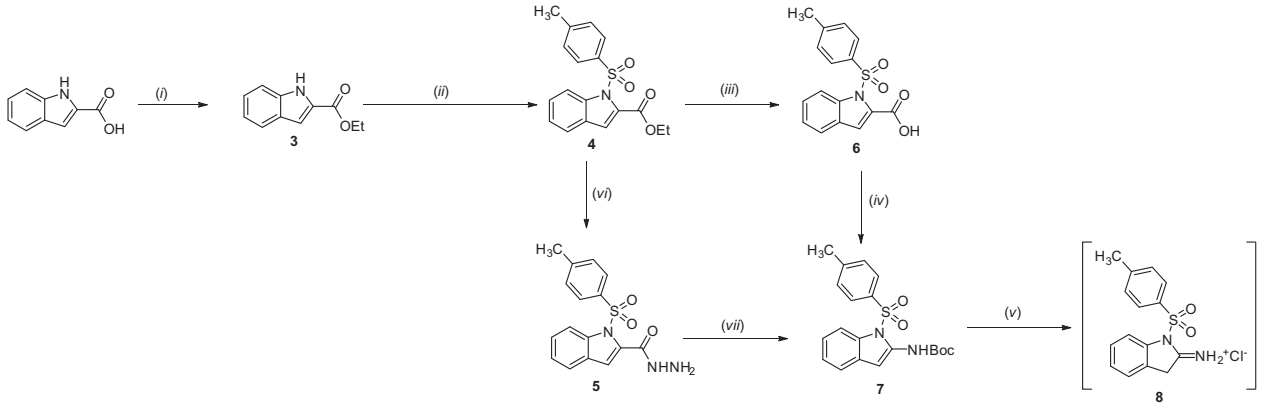


Figure 1. The design of novel NOD1 inhibitors based on structures of **1** (Noditinib-1) and **2**.

a privileged structure and is present in a number of biologically active compounds.[39] Replacement of the benzimidazole scaffold with an indole has already proved to be successful in increasing the potency of HCV NS5B polymerase inhibitors, based on greater lipophilicity of the scaffold, thus facilitating cell permeation.[40] Although SAR analysis of the benzimidazole series has shown that the presence of an NH_2 group at 2-position is essential,[28,31] we replaced it with various functionalities in order to further explore the chemical space. In addition, various substituents (R) were introduced at position 4 of the phenyl moiety, in order to shed light on the effect of the stereoelectronic nature of the substituent on bioactivity and selectivity. Finally, sulfonyl, carbonyl and methylene linkers were used in order to determine the effect of the nature of the spacer X on the bioactivity and selectivity, since it has already been demonstrated that the sulfonyl group leads to the most active compounds of the series and is also essential for their selectivity.[28,31]

2.2. Chemistry

Initially, the synthesis was attempted of compounds **8** and **12**, that are counterparts to their benzimidazole reference compounds **1** and **2**. The synthesis of 1-arylsulfonyl-indole derivative **8** is depicted in Scheme 1. First, the carboxylic group of indole-2-carboxylic acid was protected in the form of ethyl ester **3**, followed by reaction with *p*-toluenesulfonyl chloride in THF in the presence of potassium *tert*-butoxide and a catalytic amount of 18-crown-6 to obtain compound **4**.[41] The hydrazide **5** was then obtained by reacting compound **4** with hydrazine hydrate. Treatment of hydrazide **5** with a $NaNO_2$ and HCl mixture gave the acyl azide which, on heating in toluene, underwent a Curtius rearrangement to the corresponding isocyanate that, in turn, reacted with *tert*-butanol to afford the desired *tert*-butyl carbamate **7**.[42,43] This procedure, however, produced poor yields and an alternative synthetic method was therefore adopted. Alkaline hydrolysis of the ester **4** furnished the corresponding acid **6**,[41] which was transformed directly into *tert*-butyl carbamate **7** in a convenient one-pot reaction, via a Curtius rearrangement reaction employing diphenyl phosphorazidate (DPPA) as described.[44,45] In the final step, acidolytic removal of the *N*-protecting group was attempted by reacting the carbamate **7** with 4 M HCl in dioxane. Although the reaction yielded the desired product **8**, the latter proved to be unstable.

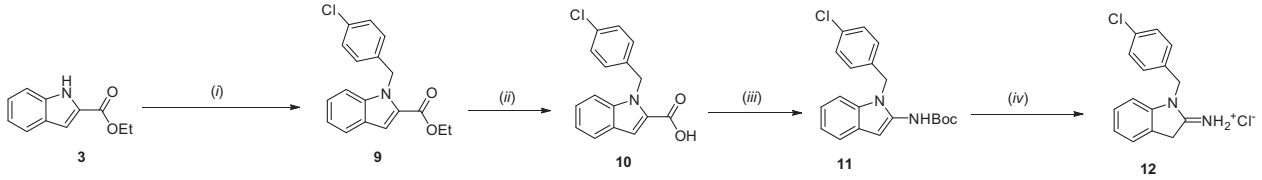


Scheme 1. Synthesis of the 1-arylsulfonyl-indole derivative **8**. Reagents and conditions: (i) $SOCl_2$, EtOH, reflux; (ii) *p*-toluenesulfonyl chloride, ^{t}BuOK, 18-crown-6, THF; (iii) 2 M KOH, EtOH; (iv) DPPA, Et_3N, 4 Å molecular sieves, ^{t}BuOH, 82 °C; (v) 4 M HCl, dioxane; (vi) $NH_2NH_2 \times H_2O$, EtOH; (vii) $NaNO_2/H^+$, then ^{t}BuOH, toluene, 110 °C.

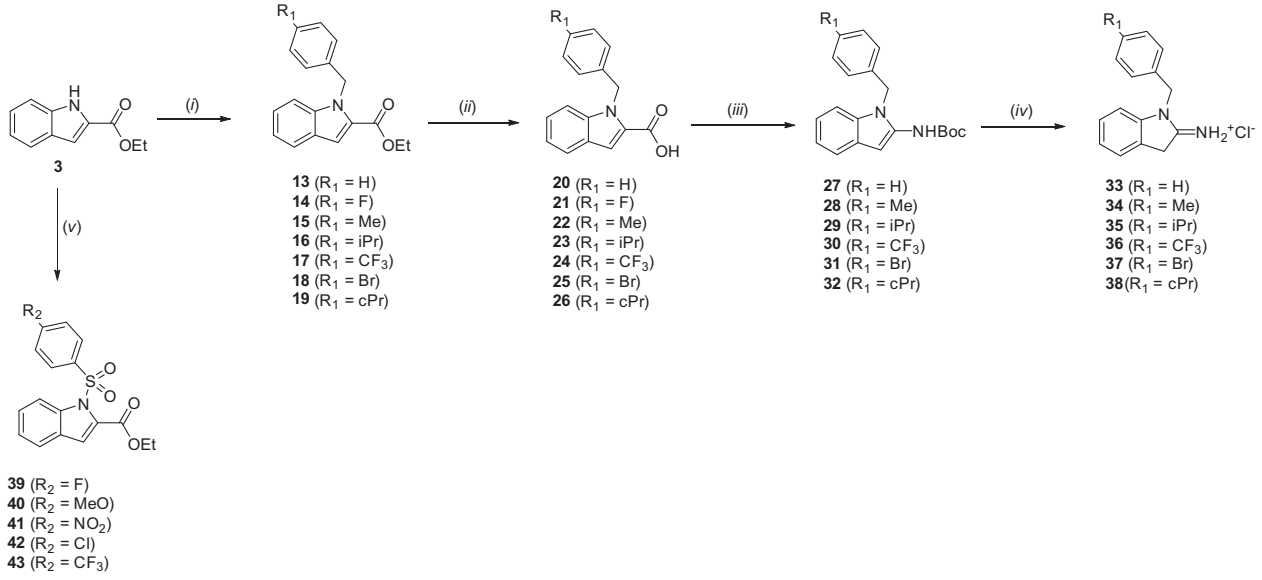
N-benzylated analog **12** was synthesized as shown in Scheme 2. N-alkylation of the starting ethyl indole-2-carboxylate (**3**), in the presence of Cs_2CO_3,[46] was followed by alkaline hydrolysis of the ester **9**, yielding *N*-benzylated indole-2-carboxylic acid **10**. Subsequent Curtius rearrangement of the latter, employing the successfully established DPPA procedure, gave the *tert*-butyl carbamate **11** in good yield.

In the final step, *N*-Boc deprotection was achieved by reacting the carbamate **11** with 4 M HCl in dioxane, affording the desired product **12** in the indoline tautomeric form.

Several other derivatives of *N*-benzylated 2-iminoindolines (compounds **33**–**38**) and of *N*-sulfonylated ethyl indole-2-carboxylates (compounds **39**–**43**) were synthesized (Scheme 3). The starting ethyl indole-2-carboxylate (**3**) was first *N*-alkylated with appropriate benzyl bromides in the presence of Cs_2CO_3,[46] affording the desired alkylated products **13**–**18** in excellent yields. Compound **19**, incorporating a cyclopropyl moiety, was obtained from the corresponding bromo-derivative **18** via a modified Suzuki cross-coupling reaction.[47] The obtained ethyl esters **13**–**19** were subjected to alkaline hydrolysis, giving the *N*-benzylated



Scheme 2. Synthesis of the *N*-benzylated compound **12**. Reagents and conditions: (i) 4-chlorobenzyl bromide, Cs_2CO_3, acetonitrile, 60 °C; (ii) 2 M KOH, EtOH; (iii) DPPA, Et_3N, 4 Å molecular sieves, ^{t}BuOH, 82 °C; (iv) 4 M HCl, dioxane.



Scheme 3. Synthesis of *N*-benzylated 2-iminoindolines (compounds **33**–**38**) and *N*-sulfonylated ethyl indole-2-carboxylates (compounds **39**–**43**). Reagents and conditions: (i) *p*-substituted benzyl bromide, Cs_2CO_3, acetonitrile, 60 °C, except for compound **19**; (ii) 2 M KOH, EtOH; (iii) DPPA, Et_3N, 4 Å molecular sieves, ^{t}BuOH, 82 °C; (iv) 4 M HCl, dioxane; (v) *p*-substituted arylsulfonyl chloride, ^{t}BuOK, 18-crown-6, THF.

indole-2-carboxylic acids **20**–**26**.[48] Subsequent Curtius rearrangement of these acids employing the established DPPA procedure, afforded the corresponding carbamates **27**–**32**.

Acidolytic removal of the *N*-Boc protecting group in the final step, using 4 M HCl in dioxane, yielded the final 2-iminoindolines **33**–**38**. The *N*-arylsulfonylindole derivatives **39**–**43** were obtained simply using the well established procedure by sulfonylating ethyl indole-2-carboxylate (**3**) with the corresponding arylsulfonyl chloride in the presence of potassium *tert*-butoxide.

Further analogs of **8** were synthesized (Scheme 4). As described in Scheme 1, ethyl indole-2-carboxylate (**3**) was sulfonylated with *p*-toluenesulfonylchloride, followed by alkaline hydrolysis of the resulting ester to afford **6**. The latter was then converted to its carboxamide counterpart **45** using the $[(Boc_2)O]$-$(NH_4)_2CO_3$ system.[49] The carbonyl linker-incorporating congener of **8**, compound **44**, was synthesized by acylation with *p*-toluoyl chloride in the presence of potassium *tert*-butoxide and 18-crown-6 in THF.[41]

The structures of all synthesized compounds were fully characterized by spectroscopy. The presence of individual signals of the amino group protons in the ^{1}H NMR spectra of *N*-benzylated products attests to the considerable double-bond character of the bond of the exocyclic nitrogen atom to the α-carbon atom, as a consequence of which these protons become non-equivalent.[50] This observation confirmed that 2-aminoindole salts existed in the imine form. Finally, since compound **8** proved unstable, we addressed the stability of other compounds belonging to the 2-iminoindoline structural class. It should be noted, that the 2-aminoindole bases are very sensitive to atmospheric oxygen and are known to undergo autoxidation,[51] whereas their salts are stable under these conditions.[50,52–54] Our compounds, although initially in the form of hydrochlorides, would be expected to be deprotonated under the conditions of the biological assays employed (pH ~7.2), rendering them prone to autoxidation. Thus, to understand the stability of the free base, it was prepared from **12** by liberating it from the salt by treatment with sodium hydrogencarbonate and subsequent extraction to ether. Its presence was confirmed by ^{1}H NMR spectroscopy immediately after the basification. The solution of free base **12b** (see Supporting information) was then left to stand in air for 3 weeks, its ^{1}H NMR spectrum being recorded at the final time-point. The spectra showed that the synthesized 2-iminoindolines are stable towards autoxidation (see Supporting information).

2.3. Biological characterization

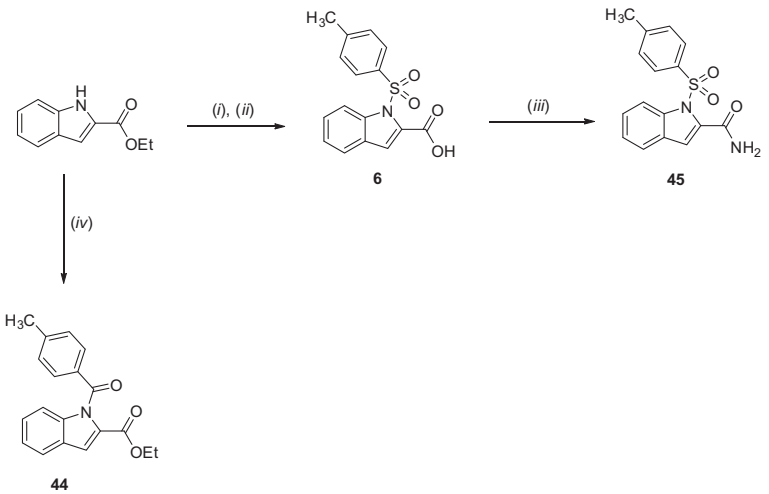
2.3.1. Evaluation of cytotoxicity of synthesized compounds

An MTS assay in which the proliferation rates of HEK-Blue NOD1 cells were measured in the presence of Noditinib-1 and of the synthesized potential NOD1 inhibitors **4**–**19** and **33**–**45** was employed to screen these compounds for potential cytotoxicity. Cells were treated for 24 h with the compound of interest at concentrations of up to 25 μM. Comparison of the resulting metabolic activities with that of the untreated control showed that all compounds were well tolerated by HEK-Blue NOD1 cells, since their residual metabolic activities did not fall below 80% at the maximum concentration tested (Fig. 2).

2.3.2. Evaluation of NOD1-inhibitory activity of synthesized compounds

These potential NOD1 inhibitors, together with Noditinib-1 (**1**), were examined for their effectiveness using the HEK-Blue assay. The cell line HEK-Blue NOD1 stably overexpresses the human NOD1 gene and an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Recognition of an NOD1 agonist triggers a signaling pathway leading to the activation of NF-κB and the production of SEAP. During these experiments, the cells were preincubated for 1 h with the newly synthesized potential NOD1 inhibitors and with Noditinib-1 at concentrations of 25 μM. They were then stimulated for 18 h with 100 nM C12-iE-DAP, followed by spectrophotometric determination of SEAP activity in the supernatant (Fig. 3).

The positive control, a known NOD1 agonist C12-iE-DAP, increased NF-κB transcriptional activity significantly relative to that in untreated cells, whereas Noditinib-1 (**1**) completely abolished this activity to its basal level (residual activity (RA) = 9%). The derivatives **4**–**7** belonging to the *N*-sulfonylated set of compounds are characterized by the presence of different groups at position 2 of the *N*-sulfonylated indole heterocycle. From this set only the ethyl ester **4** (RA = 24%) and hydrazide **5** (RA = 68%) inhibited the C12-iE-DAP-induced increase in NF-κB transcriptional activity, by 76% and 32%, respectively. The free acid analog **6** did not exhibit any NOD1 inhibitory activity, most probably due to its unfavorable cell permeation ability—the pH conditions under which the assay is executed render the compound completely ionized. Similarly, the *tert*-butyl carbamate **7** was completely devoid



Scheme 4. Synthesis of compounds **44** and **45**. Reagents and conditions: (i) *p*-toluenesulfonyl chloride, K^{t}BuO, 18-crown-6, THF; (ii) 2 M KOH, EtOH; (iii) Py, Boc_2O, $(NH_4)_2CO_3$, THF, rt; (iv) *p*-toluoyl chloride, ^{t}BuOK, 18-crown-6, THF.

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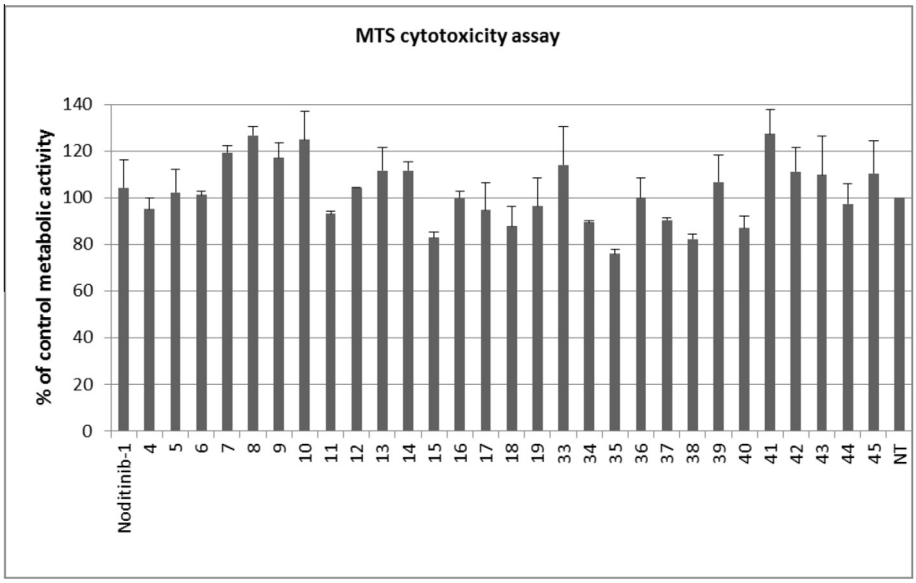


Figure 2. Proliferation rates, expressed as metabolic activities, of HEK-Blue cells after 24 h treatment with the synthesized compounds and Noditinib-1 (25 μM). The data are means of three independent experiments ± SD.

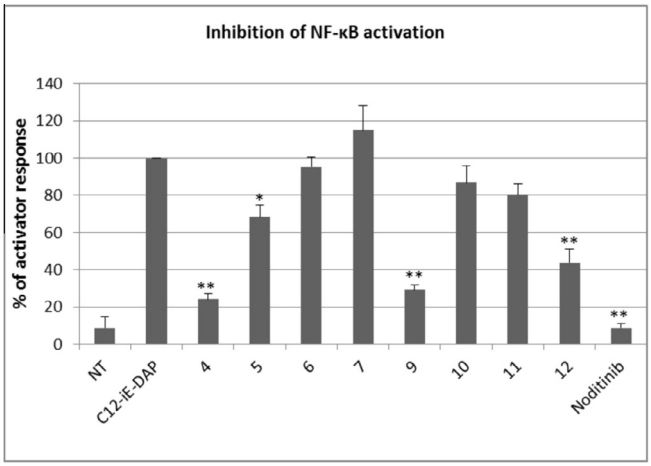


Figure 3. Effects of synthesized compounds **4**–**7**, **9**–**12** and Noditinib-1 (**1**) on the inhibition of C12-iE-DAP-induced NF-κB transcriptional activity. Secreted embryonic alkaline phosphatase (SEAP) activity in HEK-Blue cells was measured after their exposure to the NOD1 inhibitors (25 μM) for 1 h followed by stimulation with C12-iE-DAP (100 nM) for 18 h. Columns represent means of duplicates of three independent experiments. Error bars indicate ± SD; $^{*}p$ <0.05 and $^{**}p$ <0.01 vs C12-iE-DAP-treated cells.

of NOD1 inhibitory activity. Thus, the presence of the 2-carboxyethyl function is evidently pertinent for the bioactivity of this structural type of compound.

Results obtained from the *N*-benzylated series were interesting, the introduction of the 4-chlorobenzyl moiety resulting in compound **9** which inhibited the C12-iE-DAP-induced increase in NF-κB transcriptional activity by 71% (RA = 29%). In addition, within this series the 2-iminoindoline **12** was also identified as a moderate inhibitor of NOD1-dependent activity (RA = 44%). Similarly to what was observed in the *N*-sulfonyl series, the free acid **10** and *tert*-butyl carbamate **11** displayed no activity whatsoever. These results thus indicate that the presence of the 2-carboxyethyl moiety and, to a minor extent, of the 2-imino substituent, is essential for the desired bioactivity of compounds of this structural type.

Given the promising inhibitory activity in the preliminary assay of hit compound **4** (RA = 24%), further compounds incorporating differentially *para*-substituted benzenesulfonyl moieties were prepared and screened for NOD1-inhibitory activity in order to further explore the chemical space of the *N*-sulfonyl series. In this second set of compounds, we aimed to assess the effect of the size of different substituents positioned at the 4-site by comparing the obtained results to that for the parent compound **4** (*p*-methyl) (see Fig. 4). The 4-fluoro analog of the latter, compound **39**, inhibited NOD1-dependent activity (RA = 24%) to a degree similar to that of the parent compound **4** (RA = 24%), whereas the 4-chloro analog **42** (RA = 30%) decreased the NOD1 activity by 70%. Replacement of the methyl group by a trifluoromethyl bioisosteric moiety, however, giving compound **43**, led to a slightly smaller decrease in activity (RA = 37%). The 4-methoxy analog **40** (RA = 36%) acted similarly. Surprisingly, compound **41**, which bears a bulkier 4-nitro substituent, inhibited NOD1 activation effectively (RA = 27%), its results being on par with those of **4** and **39**. These results demonstrate a remarkable tolerance of diverse 4-substituents in the *N*-sulfonylated series of compounds for NOD1 inhibitory activity.

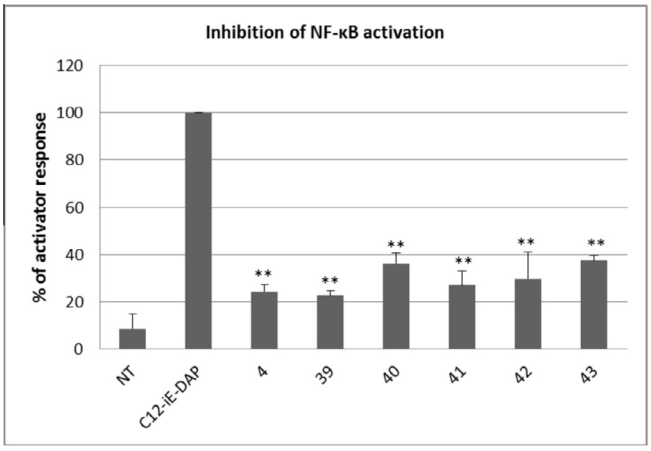


Figure 4. Inhibition of C12-iE-DAP-induced NF-κB transcriptional activity by compounds **4** and **39**–**43**. Secreted embryonic alkaline phosphatase (SEAP) activity was measured in HEK-Blue cells after pretreatment with NOD1 inhibitors (25 μM) for 1 h followed by stimulation with C12-iE-DAP (100 nM) for 18 h. Columns represent means of duplicates of three independent experiments. Error bars indicate ± SD; $^{**}p$ <0.01 vs C12-iE-DAP-treated cells.

The *N*-alkylated compound **9** exemplified another good starting point for further optimization, due to its promising inhibitory activity observed in the preliminary assay (RA = 29%). We therefore explored the SAR of the *N*-benzylated series of compounds, employing the well established bioisosteric replacement approach. By introducing classical and nonclassical bioisosteric replacements of the chlorine atom at the 4-position, we probed the novel chemical space with the aim of finding an optimal balance of steric, hydrophobic, electronic and hydrogen-bonding properties. The set of novel synthesized compounds **13**–**19** includes six compounds with different bioisosteric replacements for the chlorine atom and one compound in which the chlorine atom was simply removed. These bioisosteres of **9** were then screened for their NOD1-inhibitory activity (Fig. 5).

Replacement of the 4-Cl substituent with its fluorine counterpart, resulted in a significantly lower NOD1 inhibitory activity as exemplified by **14** (RA = 53%). A similarly lower value of activity was observed in the case of the removal of chlorine atom (compound **13** (RA = 58%)). On the other hand, compound **15**, with a methyl group at the 4-position, was the best of the series, exhibiting a high NOD1-inhibitory ability (RA = 24%). Introduction of the trifluoromethyl bioisosteric moiety, however, resulted in a lower degree of inhibition (compound **17** (RA = 46%)). Analogous results were obtained for the 4-iPr analog **16** (RA = 42%), the 4-Br analog **18** (RA = 45%) and the 4-cyclopropyl analog **19** (RA = 39%).

The SAR of the *N*-benzylated 2-iminoindoline structural type of compound, exemplified by hit compound **12** (RA = 44%), was next explored, using the bioisosteric replacement approach described above. Six new compounds **33**–**38** were prepared, five of them incorporating different bioisosteric replacements for the chlorine atom and one in which the chlorine atom was removed. As expected, bioisosteric replacement of the chlorine substituent resulted in complete loss of inhibitory activity for all but one of the compounds (Fig. 6).

The only exception was compound **33**, lacking a substituent at 4-position, which still retained weak NOD1-inhibitory activity (RA = 72%), although less than that of the parent compound. These results indicate that the chlorine atom is important for the activity of this series of compounds, since its replacement rendered the compounds **34**–**38** inactive.

The effect of the linker connecting the indole moiety to the aromatic ring was investigated. Comparison of the exhibited

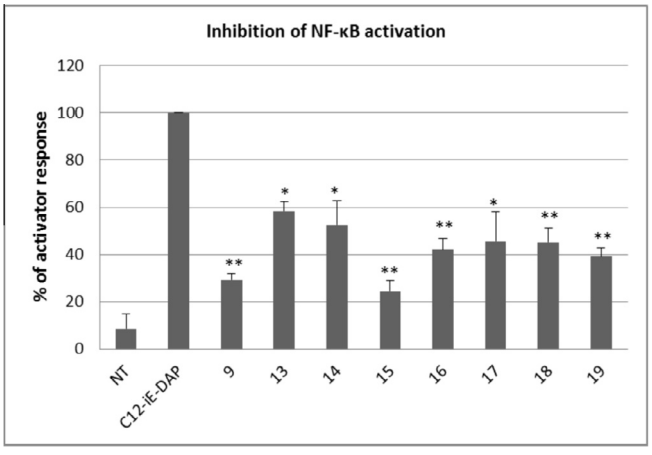


Figure 5. Effects of compounds **9** and **13**–**19** on the inhibition of C12-iE-DAP-induced NF-κB transcriptional activity. Secreted embryonic alkaline phosphatase (SEAP) activity was measured in HEK-Blue cells after pretreatment with NOD1 inhibitors (25 μM) followed by the addition of C12-iE-DAP (100 nM) and a subsequent incubation for 18 h. Columns represent means of duplicates of three independent experiments. Error bars indicate ± SD; $^{*}p$ <0.05 and $^{**}p$ <0.01 versus C12-iE-DAP-treated cells.

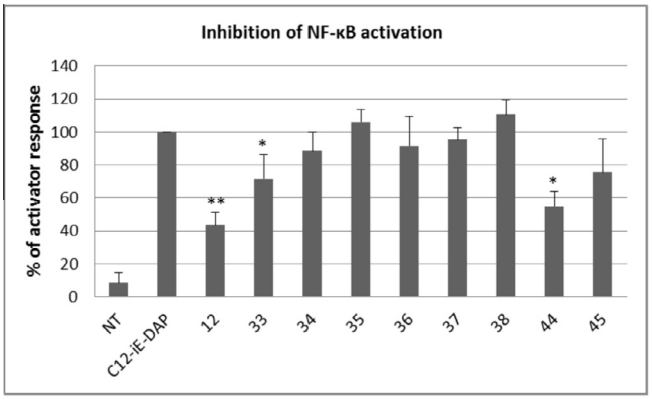


Figure 6. Inhibition of C12-iE-DAP-induced NF-κB transcriptional activity by compounds **12**, **33**–**38**, **44** and **45**. Secreted embryonic alkaline phosphatase (SEAP) activity was measured in HEK-Blue cells after pretreatment with NOD1 inhibitors (25 μM) followed by the addition of C12-iE-DAP (100 nM) and a subsequent incubation for 18 h. Columns represent means of duplicates of three independent experiments. Error bars indicate ± SD; $^{*}p$ <0.05 and $^{**}p$ <0.01 vs C12-iE-DAP-treated cells.

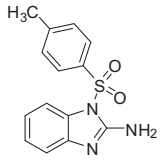
NOD1-inhibitory activities of compounds **4** (sulfonyl linker; RA = 24%), **44** (carbonyl linker; RA = 55%) and **15** (methylene linker; RA = 24%) showed that the nature of the linker is another important factor determining NOD1 inhibitory activity. While compounds **4** and **15**, that incorporate the sulfonyl and methylene linkers, displayed similar NOD1-inhibitory capacity, introduction of a carbonyl linker (compound **44**) significantly reduced NOD1-inhibitory activity. Finally, the fact that the free acid analog of **4**, compound **6**, exhibited no NOD1-inhibitory activity was presumed also to be the result of its unfavorable cell permeation capacity, being completely ionized under the pH of the assay. Replacement of the carboxylic acid function of **6** by a carboxamide moiety gave compound **45**, that displayed only weak NOD1-inhibitory activity (RA = 76%) further reinforcing the presumption.

2.3.3. Determination of IC_{50} of selected compounds and their selectivity versus NOD2

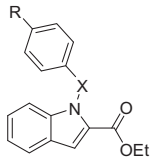
The new group of inhibitors was further investigated by selecting certain compounds for additional experiments in which their IC_{50} and dose dependence were determined (listed in Table 1). An analogous assay on the HEK-Blue NOD2 cell line was used as a counterscreen, in terms of ascertaining the compounds' selectivity profile, by measuring the effect on NOD2-dependent activation of NF-κB. The dose-dependence results corroborated those obtained with experiments carried out at a single concentration of 25 μM. The NOD1 and NOD2 inhibition effects of all the compounds tested are dose-dependent, following a non-linear semilogarithmic model. The reference NOD1 inhibitor, Noditinib-1 (**1**) (IC_{50} (NOD1) = 0.771 μM; IC_{50} (NOD2) = 54.9 μM) exhibits a submicromolar IC_{50} on NOD1 and is ~75-fold selective towards NOD2. These results are in agreement with previously reported activities.[31]

Importantly, these results reveal the nonselective nature of the indole scaffold in terms of inhibition of NOD1 and NOD2. The chemical class of *N*-sulfonylated indoles possessing the 2-ethoxycarbonyl moiety emerged as the most potent of the series, displaying similar potencies in the micromolar range toward NOD1 and NOD2. Compound **4** (IC_{50} (NOD1) = 5.74 μM; IC_{50} (NOD2) = 6.45 μM) was identified as the best of the series, possessing NOD1- and NOD2-inhibitory activities in the lower micromolar range. These results show that compound **4** is 7-fold less potent than Noditinib-1 (**1**) in terms of NOD1 inhibition and completely devoid of selective activity for NOD1 or NOD2 as opposed to Noditinib-1 (**1**). Further, the results provide some indication as to how the stereoelectronic nature of the substituent R at the 4-position of

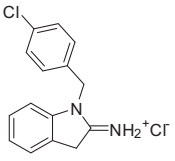
Table 1
Inhibition of NOD1 and NOD2 by selected compounds



1 (Noditinib-1)



compounds 4,15,39,41,42,44



12

Compd	R	X	IC_{50} NOD1 (μM)	IC_{50} NOD2 (μM)
1	—	—	0.771	54.9
4	CH_3	SO_2	5.74	6.45
39	F	SO_2	9.22	12.6
41	NO_2	SO_2	15.0	16.9
42	Cl	SO_2	19.8	23.4
44	CH_3	CO	16.0	24.6
15	CH_3	CH_2	7.78	9.04
12	—	—	15.7	>25

the phenyl moiety affects the bioactivity. Introduction of bulkier groups possessing electron-withdrawing properties have been shown, here and elsewhere,[31] to decrease inhibitory potency on both targets, as exemplified by the 4-chloro analog **42** (IC_{50} (NOD1) = 19.8 μM; IC_{50} (NOD2) = 23.4 μM) and the 4-nitro analog **41** (IC_{50} (NOD1) = 15.0 μM; IC_{50} (NOD2) = 16.9 μM). On the other hand, replacement of the 4-methyl group by a 4-fluoro group resulted in NOD-inhibitory activities (compound **39**; IC_{50} (NOD1) = 9.22 μM; IC_{50} (NOD2) = 12.6 μM) similar to those of the parent compound **4**. In general, the presence of an electron-donating group on the phenyl moiety resulted in compounds with higher potencies. The nature of the linker type is also seen to be important in determining the NOD1- and NOD2-inhibitory activities. While the sulfonyl- and methylene-incorporating analogs **4** and **15** displayed similar activities in NOD1 and NOD2 inhibition, their carbonyl-incorporating congener **44** exhibited ~3-fold lower potencies against the two targets. Finally, the only representative of the 2-iminoindoline class, compound **12**, exhibited an IC_{50} of 15.7 μM on NOD1, whereas its NOD2 IC_{50} was >25 μM. In summary, compounds **4** and **15** exhibit balanced dual activities of less than 10 μM on the two targets. Our SAR exploratory campaign has thus resulted in the identification of dual NOD1 and NOD2 inhibitors based on a novel scaffold.

3. Conclusions

In conclusion, therapeutic targeting of NOD1 is still largely unexplored due to the lack of information regarding the underlying mechanisms of NOD1 activation and its regulation. A new class of 2-ethoxycarbonylindoles has here been identified as providing potent dual inhibitors of NOD1/2-induced NF-κB transcriptional activation. This ligand-based work has established a deeper understanding of the structure–activity relationship with regard to the NOD1- and NOD2-inhibitory activities. The synthesized compounds described here provide not only new chemical tools for further development of NOD1 and NOD2 inhibitors but also valuable research tools for elucidating the role of these proteins in NOD-related diseases as well as in normal host-defense mechanisms.

4. Experimental section

4.1. General

Chemicals were obtained from Acros, Aldrich Chemical Co., Molekula and Fluka, and used without further purification. C12-iE-DAP (a synthetic NOD1 agonist) and MDP (NOD2 agonist) were obtained from InvivoGen, Inc., (San Diego, CA); LPS was from Sigma. The NOD1 antagonist, Noditinib-1 (ML-130), was synthesized as described.[31] Analytical TLC was performed on Merck 60 F254 silica gel plates (0.25 mm), using visualization with ultraviolet light and ninhydrin. Column chromatography was carried out on silica gel 60 (particle size 240–400 mesh). Melting points were determined on a Reichert hot stage microscope and are uncorrected. ^1H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on a Bruker AVANCE III spectrometer in DMSO-d_6, $CDCl_3$, or MeOH-d_4 solution, with TMS as the internal standard. Spectra were assigned using gradient COSY and HSQC experiments. IR spectra were recorded on a Perkin–Elmer 1600 FT-IR spectrometer. Mass spectra were obtained using a VG-Analytical Autospec Q mass spectrometer. HPLC analyses were performed on an Agilent Technologies HP 1100 instrument with G1365B UV–VIS detector (210, 220 or 254 nm), using a Luna C18 column (4.6 × 150 mm) at a flow rate of 1 mL/min. The eluant was a mixture of 0.1% TFA in water (A) and acetonitrile (B). Method A: Gradient was 50% B to 80% B in 30 min; Method B: Gradient was 30% B to 80% B in 32 min; Method C: Gradient was 30% B to 80% B in 30 min. The purity of all pharmacologically investigated compounds was >95% as determined by RP-HPLC.

4.2. General procedures

4.2.1. N-sulfonylation of indole derivatives

Ethyl indole-2-carboxylate (**3**) (5 mmol) was dissolved in THF (15 mL); potassium *tert*-butoxide (7.5 mmol) was then added and the mixture stirred for 15 min at room temperature. Subsequently, the corresponding sulfonyl chloride (5.5 mmol) was added to the reaction mixture which was heated overnight at reflux. Upon completion of the reaction, which was monitored by TLC, the mixture was concentrated in vacuo and the residue was dissolved in dichloromethane (80 mL). The organic phase was washed with water (2 × 30 mL), dried with anhydrous Na_2SO_4 then concentrated in vacuo. The residue was purified by flash silica gel column chromatography (gradient elution; starting eluent: hexane/ethyl acetate 6:1 v/v) to afford pure *N*-sulfonylated compounds.

4.2.2. N-alkylation of indole derivatives

Ethyl indole-2-carboxylate (**3**) (5.0 mmol) was first dissolved in acetonitrile (40 mL); cesium carbonate (7.5 mmol) was then added and the mixture stirred for 15 min at room temperature. Subsequently, the corresponding alkyl bromide (5.5 mmol) was added to the reaction mixture which was heated overnight at reflux. Upon completion of the reaction, which was monitored by TLC, the mixture was concentrated in vacuo and the residue dissolved in dichloromethane (80 mL). The organic phase was washed with water (2 × 30 mL), dried with anhydrous Na_2SO_4 and then concentrated in vacuo to afford sufficiently pure *N*-alkylated products.

4.2.3. Alkaline hydrolysis

To a solution of ethyl ester (2 mmol) in ethanol was added 1 M KOH (4 mL) and the mixture stirred for 24 h at room temperature. Upon completion of the reaction which was monitored by TLC, the ethanol was evaporated from the mixture and the remaining water phase acidified with 1 M HCl to pH ~3. The obtained precipitate was filtered off and dried at 75 °C in an oven to afford compounds.

4.2.4. Curtius rearrangement

To a solution of carboxylic acid (1 mmol) in *tert*-butanol were added pulverized 4 Å molecular sieves (100 mg). The mixture was stirred at 30 °C. After 20 min triethylamine (1.5 equiv; 1.5 mmol) and diphenylphosphorazidate (1.5 equiv; 1.5 mmol) were added and the mixture was heated overnight at 82 °C. Upon

completion of the reaction, the mixture was concentrated in vacuo and the residue purified by flash silica gel column chromatography (gradient elution; starting eluent: hexane/ethyl acetate 6:1 v/v) to afford compounds.

4.2.5. Acidolytic cleavage of the Boc protecting group

To an ice-chilled stirred mixture of *tert*-butyl carbamates (0.3 mmol) in dioxane (5 mL) 4 M HCl in dioxane was added and the mixture allowed to warm to room temperature. After 3 h the reaction was complete and the solvent was evaporated in vacuo. The residue was washed three times with diethyl ether giving sufficiently pure products.

4.3. Characterization of compounds

4.3.1. Ethyl 1*H*-indole-2-carboxylate (3)

Synthesized from indole-2-carboxylic acid according to the standard procedure for acid-catalyzed esterification (150 mmol). Yellow amorphous solid, yield: 24,7 g (87%); mp 116–120 °C; lit. 122–125 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.45 (t, 3H, J = 7.2 Hz, –C*H*$_3$), 4.44 (q, 2H, J = 7.2 Hz, –C*H*$_2$CH$_3$), 7.16–7.20 (m, 1H, indole-*H*), 7.26–7.27 (m, 1H, indole-*H*), 7.33–7.37 (m, 1H, indole-*H*), 7.44–7.46 (m, 1H, indole-*H*), 7.71–7.73 (m, 1H, indole-*H*), 8.92 (s, 1H, indole-N*H*) ppm. MS (ESI): m/z (%) = 190.1 (M+H)$^+$. IR (ATR): ν = 3307, 1686, 1525, 1381, 1339, 1307, 1247, 1200, 1144, 1020, 820, 771, 743, 663, 607, 580 cm^{-1}. HRMS Calcd for $C_{11}H_{12}NO_2$ m/z: 190.0868 (M+H)$^+$, found 190.0865.

4.3.2. Ethyl 1-(4-toluenesulfonyl)-1*H*-indole-2-carboxylate (4)[55]

Synthesized from **3** (5 mmol) according to the General procedure for N-sulfonylation. Orange crystals, yield: 1,27 g (74%); mp 95–97 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.42 (t, 3H, J = 7.2 Hz, –C*H*$_3$), 2.40 (s, 3H, Ar–C*H*$_3$), 4.43 (q, 2H, J = 7.2 Hz, –C*H*$_2$–CH$_3$), 7.27–7.29 (m, 4H, indole-*H* and Ar–*H*), 7.34–7,35 (m, 1H, indole-*H*), 7.42–7.44 (m, 1H, indole-*H*), 7.57–7.59 (m, 1H, Ar–*H*), 7.92 (d, 2H, J = 8.4 Hz, Ar–*H*), 8.12–8.14 (m, H, Ar–*H*) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.86, 21.00, 61.77, 114.91, 116.43, 122.86, 124.43, 126.95, 127.15, 128.07, 129.93, 131.48, 134.15, 136.95, 145.51, 160.86 ppm. MS (ESI): m/z (%) = 344.1 (M+H)$^+$. IR (ATR): ν = 1729, 1366, 1337, 1264, 1191, 1175, 1147, 1129, 1092, 1042, 1017, 816, 758, 743, 676, 652, 579, 555 cm^{-1}. HPLC (254 nm): 100%, t_R = 24.66 min. HRMS Calcd for $C_{18}H_{18}NO_4S$ m/z: 344.0957 (M+H)$^+$, found 344.0954.

4.3.3. Ethyl 1-(4-toluenesulfonyl)-1*H*-indole-2-hydrazide (5)

Synthesized from **4** (2 mmol) according to the following procedure for hydrazinolysis. Orange crystals, yield: 546 mg (83%); mp 97–100 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.34 (s, 3H, Ar–C*H*$_3$), 4.56–4.59 (m, 2H, –NH–N*H*$_2$), 6.94 (s, 1H, indole-*H*), 7.26–7.28 (m, 1H, indole-*H*), 7.37–7.40 (m, 3H, indole-*H*), 7.62–7.64 (m, 1H, Ar–*H*), 7.94–7.97 (m, 1H, Ar–*H*), 8.06 (d, 2H, J = 6.2 Hz, Ar–*H*), 9.96 (s, 1H, –N*H*–NH$_2$) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 21.03, 69.75, 111.53, 114.23, 122.04, 123.86, 125.73, 127.42, 128.29, 129.80, 134.55, 135.52, 145.32, 160.51 ppm. MS (ESI): m/z (%) = 330.1 (M+H)$^+$. IR (ATR): ν = 2964, 1676, 1544, 1483, 1446, 1368, 1342, 1259, 1174, 1149, 1087, 1015, 926, 869, 796, 747, 669, 631, 581 cm^{-1}. HPLC (254 nm): 99.5%, t_R = 7.79 min. HRMS Calcd for $C_{16}H_{16}N_3O_3S$ m/z: 330.0912 (M+H)$^+$, found 330.0918. Anal. Calcd for $C_{16}H_{15}N_3O_3S \times 0.65C_2H_6O$ (%): C, 57.83; H, 5.30; N, 11.69. Found: C, 57.76; H, 5.26; N, 11.71.

4.3.4. 1-(4-Toluenesulfonyl)-1*H*-indole-2-carboxylic acid (6)[56]

Synthesized from **5** (1 mmol) according to the General procedure for alkaline hydrolysis. Pink crystals, yield: 274 mg (87%); mp 137–141 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.33 (s, 3H, Ar–C*H*$_3$), 6.38 (s, 1H, indole-*H*), 7.14–7.17 (m, 1H, indole-*H*), 7.32–7.34 (m, 2H, indole-*H*), 7.43–7.45 (m, 1H, indole-*H*), 7.85 (d, 1H, J = 7.8 Hz, Ar–*H*), 8.21 (d, 2H, J = 7.8 Hz, Ar–*H*), 8.35 (s, 1H, Ar–*H*), 11.76 (s, 1H, –COO*H*) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 21.03, 114.90, 115.45, 122.68, 124.24, 126.78, 127.09, 128.15, 129.85, 132.99, 134.45, 136.90, 145.34, 162.11 ppm. MS (ESI): m/z (%) = 316.1 (M+H)$^+$. IR (ATR): ν = 2841, 1703, 1369, 1203, 1177, 1151, 1130, 1086, 816, 744, 672, 624, 570, 560 cm^{-1}. HPLC (254 nm): 100%, t_R = 15.69 min. HRMS Calcd for $C_{16}H_{14}NO_4S$ m/z: 316.0644 (M+H)$^+$, found 316.0641. Anal. Calcd for $C_{16}H_{13}NO_4S$ (%): C, 60.94; H, 4.16; N, 4.44. Found: C, 60.66; H, 3.82; N, 4.51.

4.3.5. *tert*-Butyl (1-(4-toluenesulfonyl)-1*H*-indole-2-yl)carbamate (7)

Synthesized from **6** (1 mmol) according to the General procedure for Curtius rearrangement. Pink crystals, yield: 193 mg (50%); mp/°C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.49 (s, 9H, (–C*H*$_3$)$_3$), 2.26 (s, 2H, Ar–C*H*$_3$), 6.67 (s, 1H, –N*H*-CO), 7.08–7.12 (m, 3H, indole-*H* and Ar–*H*), 7.24–7.26 (m, 1H, indole-*H*), 7.55 (d, 2H, J = 8.4 Hz, indole-*H*), 7.90–7.92 (m, 1H, Ar–*H*), 8.45 (s, 1H, Ar–*H*) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 20.96, 27.93, 80.10, 102.94, 114.30, 120.49, 123.91, 124.01, 126.52, 128.84, 129.97, 133.13, 134.06, 145.39, 152.89 ppm. MS (ESI): m/z (%) = 387.1 (M+H)$^+$. IR (ATR): ν = 3384, 2977, 1733, 1597, 1513, 1453, 1336, 1297, 1243, 1213, 1144, 1086, 1019, 923, 850, 797, 743, 702, 633, 630, 571 cm^{-1}. HPLC (254 nm): 97.7%, t_R = 30.97 min. HRMS Calcd for $C_{20}H_{23}N_2O_4S$ m/z: 387.1379 (M+H)$^+$, found 387.1376.

4.3.6. 1-(4-Toluenesulfonyl)-indoline-2-imminium chloride (8)

Synthesized from **7** (0.5 mmol) according to the General procedure for acidolysis. Orange solidified oil, yield: 146 mg (90%); mp/°C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.43 (s, 3H, –Ar–C*H*$_3$), 3.23 (s, 2H, indoline-C*H*$_2$), 5.71–5.73 (m, 1H, indole-*H*), 6.81–6.83 (m, 3H, indole-*H*), 7.89–7.92 (m, 4H, Ar–*H*), 8.89 (s, 1H, indole-N*H*$_2$) ppm. MS (ESI): m/z (%) = 288.1 (M−Cl+H)$^+$. IR (ATR): ν = 2961, 2359, 2341, 1660, 1406, 1327, 1258, 1012, 791, 668, 560 cm^{-1}. HRMS Calcd for $C_{15}H_{16}N_2O_2S$ m/z: 288.0932 (M−Cl+H)$^+$, found 288.0938.

4.3.7. Ethyl 1-(4-chlorobenzyl)-1*H*-indole-2-carboxylate (9)[57]

Synthesized from **3** (5 mmol) according to the General procedure for N-alkylation. Yellow crystals, yield: 1,21 g (77%); mp 98–100 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.29 (t, 3H, J = 7.2 Hz, –C*H*$_3$), 4.29 (q, 2H, J = 7.2 Hz, –C*H*$_2$CH$_3$), 5.85 (s, 2H, Ar–C*H*$_2$), 7.03 (d, 2H, J = 8.0 Hz, Ar–*H*), 7.17 (t, 1H, J = 8.0 Hz, Ar–*H*), 7.31–7.39 (m, 4H, indole-*H* and Ar–*H*), 7.58 (dd, 1H, J = 7.2 Hz, J = 0.4 Hz, indole-*H*), 7.76 (d, 1H, J = 8.0 Hz, indole-*H*) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 14.07, 46.49, 60.48, 110.77, 111.19, 120.92, 122.53, 125.35, 125.52, 127.05, 128.08, 128.45, 131.60, 137.51, 138.95, 161.16 ppm. MS (ESI): m/z (%) = 314.1 (M+H)$^+$. IR (ATR): ν = 1709, 1517 1488, 1456, 1410, 1259, 1219, 1182, 1139, 1117, 1012, 825, 798, 760, 742 cm^{-1}. HPLC (254 nm): 95.1%, t_R = 30.90 min. HRMS Calcd for $C_{18}H_{17}ClNO_2$ m/z: 314.0948 (M+H)$^+$, found 314.0951.

4.3.8. 1-(4-Chlorobenzyl)-1*H*-indole-2-carboxylic acid (10)[57]

Synthesized from **9** (3 mmol) according to the General procedure for alkaline hydrolysis. White crystals, yield: 846 mg (99%); mp 198–202 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 5.87 (s, 2H, Ar–C*H*$_2$), 7.03–7.05 (m, 2H, Ar–*H*), 7.12–7.15 (m, 1H, Ar–*H*), 7.28–7.34 (m, 4H, indole-*H* and Ar–*H*), 7.53–7.56 (m, 1H, indole-*H*), 7.70–7.72 (m, 1H, indole-*H*), 13.00 (s, 1H, –COOH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 14.39, 56.35, 56.76, 62.27, 69.94, 115.20, 115.35, 116.20, 116.56, 123.31, 124.84, 127.55, 128.57, 128.75, 129.99, 131.96, 133.94, 137.30, 161.49, 164.30, 166.51 ppm. MS (ESI): m/z (%) = 284.0 (M−H)$^-$. IR (ATR): ν = 2690, 1675, 1520, 1485, 1459, 1438, 1269, 1206, 1164, 1183,

1089, 1014, 898, 840, 825, 803, 747, 582, 564 cm^{-1}. HPLC (254 nm): 97.7%, t_R = 20.40 min. HRMS Calcd for $C_{16}H_{11}ClNO_2$ *m/z*: 284.0478 $(M-H)^-$, found 284.0490.

4.3.9. *tert*-Butyl (1-(4-chlorobenzyl)-1*H*-indole-2-yl)carbamate (11)

Synthesized from **10** (1 mmol) according to the General procedure for Curtius rearrangement. Orange solidified oil, yield: 171 mg (48%); mp/°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.34 (s, 9H, $(-CH_3)_3$), 5.36 (s, 2H, Ar–CH_2), 6.36 (s, 1H, indole-*H*), 6.97–7.05 (m, 2H, Ar–*H*), 7.32–7.36 (m, 4H, indole-*H* and Ar–*H*), 7.46–7.52 (m, 2H, indole-*H*), 9.36 (s, 1H, –N*H*–CO–) ppm. MS (ESI): *m/z* (%) = 357.1 $(M+H)^+$. IR (ATR): ν = 2910, 2166, 1684, 1588, 1486, 1368, 1335, 1298, 1269, 1202, 1180, 1156, 1093, 1057, 1024, 1010, 940, 762, 686, 597, 557 cm^{-1}. HRMS Calcd for $C_{20}H_{22}ClN_2O_2$ *m/z*: 357.1370 $(M+H)^+$, found 357.1361. Anal. Calcd for $C_{20}H_{21}ClN_2O_2$ (%): C, 67.32; H, 5.93; N, 7.85. Found: C, 67.22; H, 5.72; N, 8.15.

4.3.10. 1-(4-Chlorobenzyl)-indoline-2-imminium chloride (12)

Synthesized from **11** (0.5 mmol) according to the General procedure for acidolysis. White amorphous solid, yield: 57 mg (39%); mp 128–129 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 4.37 (s, 2H, –indoline-CH_2–), 5.31 (s, 2H, Ar–CH_2), 7.14 (d, 1H, *J* = 7.6 Hz, Ar–*H*), 7.23 (t, 1H, *J* = 7.4 Hz, indoline-*H*), 7,33 (t, 1H, *J* = 7.4 Hz, indoline-*H*), 7.40 (d, 2H, *J* = 8.8 Hz, Ar–*H*), 7.41–7.52 (m, 3H, Ar–*H* and indoline-*H*), 10.07 (s, 1H, indoline-NH_2), 10.49 (s, 1H, indol–N*H*–CH_2–Ar) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 35.92, 45.04, 111.01, 124.53, 124.75, 126.26, 127.95, 128.72, 129.11, 132.58, 132.93, 143.30, 170.25 ppm. MS (ESI): *m/z* (%) = 257.0 $(M-Cl-H)^-$. IR (ATR): ν = 2910, 1692, 1604, 1494, 1465, 1434, 1368, 1098, 885, 812, 799, 749, 663, 642, 598, 560 cm^{-1}. HPLC (254 nm): 97.4%, t_R = 6.91 min. HRMS Calcd for $C_{15}H_{13}ClN_2$ *m/z*: 223.1235 $(M-Cl-H)^-$, found 223.1231. Anal. Calcd for $C_{15}H_{14}Cl_2N_2$ (%): C, 61.45; H, 4.81; N, 9.55. Found: C, 61.70; H, 4.52; N, 9.35.

4.3.11. Ethyl 1-benzyl-1*H*-indole-2-carboxylate (13)[57]

Synthesized from **3** (5 mmol) according to the General procedure for N-alkylation. Yellow crystals, yield: 1.06 g (76%); mp 53–55 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.39 (t, 3H, *J* = 7.2 Hz, $-CH_3$), 4.36 (q, 2H, *J* = 7,2 Hz, $-CH_2CH_3$), 5.88 (s, 2H, Ar–CH_2), 7.07–7.08 (m, 2H, Ar–*H*), 7.17–7.40 (m, 6H, indole-*H* and Ar–*H*), 7.43 (d, 1H, *J* = 0.8 Hz, indole-*H*), 7.73–7.75 (m, 1H, indole-*H*) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 14.07, 47.05, 60.42, 110.63, 111.30, 120.80, 122.47, 125.22, 125.51, 126.18, 127.00, 127.18, 128.44, 138.45, 139.03, 161.22 ppm. MS (ESI): *m/z* (%) = 280.1 $(M+H)^+$. IR (ATR): ν = 1704, 1513, 1457, 1350, 1315, 1251, 1191, 1136, 1095, 1025, 811, 743, 423, 964, 605, 593, 569 cm^{-1}. HPLC (254 nm): 100%, t_R = 27.94 min. HRMS Calcd for $C_{18}H_{18}NO_2$ *m/z*: 280.1338 $(M+H)^+$, found 280.1339.

4.3.12. Ethyl 1-(4-fluorobenzyl)-1*H*-indole-2-carboxylate (14)[58]

Synthesized from **3** (5 mmol) according to the General procedure for N-alkylation. Yellow crystals, yield: 1.17 g (79%); mp 78–80 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.29 (t, 3H, *J* = 7.2 Hz, $-CH_3$), 4.30 (q, 2H, *J* = 7.2 Hz, $-CH_2CH_3$), 5.84 (s, 2H, Ar–CH_2), 7.09–7.16 (m, 5H, indole-*H* and Ar–*H*), 7.31–7.35 (m, 1H, indole-*H*), 7.38 (d, 1H, *J* = 0.8 Hz, indole-*H*), 7.59–7.62 (m, 1H, indole-*H*), 7.72–7.74 (m, 1H, indole-*H*) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 14.08, 46.38, 60.47, 110.74, 111.26, 115.14, 115.35, 120.87, 122.51, 125.30, 125.52, 127.04, 128.25, 128.33, 134.59, 134.62, 138.92, 159.98, 161.21, 162.39 ppm. MS (ESI): *m/z* (%) = 298.1 $(M+H)^+$. IR (ATR): ν = 1675, 1501, 1456, 1365, 1311, 1248, 1145, 1088, 1053, 1013, 936, 887, 827, 791, 736, 685, 632, 600, 574, 555 cm^{-1}. HPLC (254 nm): 100%, t_R = 27.99 min. HRMS Calcd for $C_{18}H_{17}FNO_2$ *m/z*: 298.1243 $(M+H)^+$, found 298.1238.

4.3.13. Ethyl 1-(4-methylbenzyl)-1*H*-indole-2-carboxylate (15)

Synthesized from **3** (5 mmol) according to the General procedure for N-alkylation. Pale yellow oil, yield: 1.14 g (78%); mp/°C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.29 (t, 3H, *J* = 7.2 Hz, $-CH_3$), 2.21 (s, 3H, Ar–CH_3), 4.29 (q, 2H, *J* = 7.2 Hz, $-CH_2CH_3$), 5.81 (s, 2H, Ar–CH_2), 6.92 (d, 2H, *J* = 8.4 Hz, Ar–*H*), 7.06 (d, 2H, *J* = 7.6 Hz, Ar–*H*), 7.13 (dt, 1H, *J* = 8.0 Hz, *J* = 0.8 Hz, Ar–*H*), 7.30 (dt, 1H, *J* = 8.0 Hz, *J* = 1.2 Hz, Ar–*H*), 7.36 (d, 1H, *J* = 0.8 Hz, Ar–*H*), 7.56 (dd, 1H, *J* = 8.4 Hz, *J* = 0.8 Hz, Ar–*H*), 7.71 (d, 1H, *J* = 8.0 Hz, Ar–*H*) ppm. MS (ESI): *m/z* (%) = 294.1 $(M+H)^+$. IR (ATR): ν = 2980, 1704, 1516, 1480, 1456, 1410, 1376, 1351, 1319, 1245, 1183, 1163, 1138, 1118, 1093, 1025, 1012, 799, 767, 748 cm^{-1}. HPLC (254 nm): 97.3%, t_R = 30.40 min. HRMS Calcd for $C_{19}H_{20}NO_2$ *m/z*: 294.1494 $(M+H)^+$, found 294.1491.

4.3.14. Ethyl 1-(4-isopropylbenzyl)-1*H*-indole-2-carboxylate (16)

Synthesized from **3** (5 mmol) according to the General procedure for N-alkylation. Beige crystals, yield: 1.32 g (82%); mp 56–60 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.12 (d, 6H, *J* = 6.8 Hz, $(CH_3)_2CH$), 1.29 (t, 3H, *J* = 7.2 Hz, $-CH_3$), 2.76–2.83 (m, 1H, $(CH_3)_2$-*CH*), 4.29 (q, 2H, *J* = 7.2 Hz, $-CH_2CH_3$), 5.82 (s, 2H, Ar–CH_2), 6.95 (d, 2H, *J* = 8.0 Hz, Ar–*H*), 7.11–7.16 (m, 3H, Ar–*H*), 7.31 (dt, 1H, *J* = 7.6 Hz, *J* = 0.8 Hz, Ar–*H*), 7.36 (d, 1H, *J* = 0.4 Hz, Ar–*H*), 7.58 (d, 1H, *J* = 8.0 Hz, Ar–*H*), 7.72 (d, 1H, *J* = 8.0 Hz, Ar–*H*) ppm. MS (ESI): *m/z* (%) = 322.2 $(M+H)^+$. IR (ATR): ν = 2956, 2929, 1699, 1514, 1477, 1459, 1441, 1414, 1351, 1319, 1276, 1261, 1246, 1189, 1164, 1138, 1122, 1098, 1050, 1026, 1010, 813, 766, 746 cm^{-1}. HPLC (254 nm): 93.6%, t_R = 29.42 min. HRMS Calcd for $C_{21}H_{24}NO_2$ *m/z*: 322.1807 $(M+H)^+$, found 322.1798.

4.3.15. Ethyl 1-(4-trifluoromethylbenzyl)-1*H*-indole-2-carboxylate (17)[59]

Synthesized from **3** (5 mmol) according to the General procedure for N-alkylation. White crystals, yield: 1.2 g (66%); mp 48–52 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.27 (t, 3H, *J* = 7.2 Hz, $-CH_3$), 4.27 (q, 2H, *J* = 7.2 Hz, $-CH_2CH_3$), 5.95 (s, 2H, Ar–CH_2), 7.15–7.19 (m, 3H, Ar–*H*), 7.33 (dt, 1H, *J* = 6.8 Hz, *J* = 1.2 Hz, indole-*H*), 7.41 (d, 1H, *J* = 0.8 Hz, indole-*H*), 7.57 (dd, 1H, *J* = 8.4 Hz, *J* = 0.8 Hz, indole-*H*), 7.65 (d, 2H, *J* = 8.0 Hz, indole-*H*), 7.75 (d, 1H, *J* = 8.0 Hz, indole-*H*) ppm. MS (ESI): *m/z* (%) = 348.1 $(M+H)^+$. IR (ATR): ν = 2981, 2359, 2337, 1703, 1620, 1519, 1482, 1459, 1414, 1355, 1321, 1261, 1200, 1159, 1141, 1106, 1065, 1018, 900, 821, 742, 754, 731, 642 cm^{-1}. HPLC (254 nm): 100%, t_R = 29.26 min. HRMS Calcd for $C_{19}H_{17}F_3NO_2$ *m/z*: 348.1211 $(M+H)^+$, found 348.1205.

4.3.16. Ethyl 1-(4-bromobenzyl)-1*H*-indole-2-carboxylate (18)

Synthesized from **3** (5 mmol) according to the General procedure for N-alkylation. Pale yellow crystals, yield: 1.6 g (87%); mp 94–97 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.28 (t, 3H, *J* = 7.2 Hz, $-CH_3$), 4.28 (q, 2H, *J* = 7.2 Hz, $-CH_2CH_3$), 5.82 (s, 2H, Ar–CH_2), 6.94 (d, 2H, *J* = 8.4 Hz, Ar–*H*), 7.15 (t, 1H, *J* = 8.0 Hz, Ar–*H*), 7.32 (dd, 1H, *J* = 7.2 Hz, *J* = 1.2 Hz, indole-*H*), 7.38 (d, 1H, *J* = 0.8 Hz, indole-*H*), 7.47 (d, 2H, *J* = 8.04 Hz, indole-*H*), 7.57 (dd, 1H, *J* = 8.8 Hz, *J* = 0.8 Hz, indole-*H*), 7.73 (d, 1H, *J* = 8.0 Hz, indole-*H*) ppm. MS (ESI): *m/z* (%) = 358.0 $(M+H)^+$. IR (ATR): ν = 2982, 1709, 1517, 1481, 1457, 1440, 1410, 1352, 1259, 1249, 1196, 1181, 1163, 1141, 1120, 1096, 1069, 1027, 1009, 826, 794, 748, 740 cm^{-1}. HPLC (254 nm): 100%, t_R = 29.56 min. HRMS Calcd for $C_{18}H_{17}BrNO_2$ *m/z*: 358.0443 $(M+H)^+$, found 358.0453.

4.3.17. Ethyl 1-(4-cyclopropylbenzyl)-1*H*-indole-2-carboxylate (19)

Synthesized from **18** according to the following procedure for Suzuki cross-coupling.[47] To a stirred solution of **18** (3 mmol) in a mixture of toluen (10 mL) and water (600 μL), cyclopropylboronic

acid (3.9 mmol), tricyclohexylphosphine (0.3 mmol) and K_3PO_4 (10.5 mmol) were added and the mixture was bubbled with argon for 5 min. After removing the dissolved oxygen $Pd(OAc)_2$ (0.15 mmol) was added and the mixture was heated to 100 °C for three h and then cooled to room temperature. Water (30 mL) was added to the reaction mixture, then it was extracted with ethyl acetate (2 × 40 mL). The organic phase was washed with brine (30 mL), dried with anhydrous Na_2SO_4 and then concentrated in vacuo to afford the crude residue. This product was additionally purified using flash column chromatography (gradient elution; starting eluent: hexane/ethyl acetate 9:1 v/v) to afford sufficiently pure **19** as pale yellow oil, yield: 651 mg (68%); mp/. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.57–0.60 (m, 2H, –CH_2CH_2–), 0.86–0.91 (m, 2H, –CH_2CH_2–), 1.31 (t, 3H, J = 7.2 Hz, –CH_3), 1.81–1.85 (m, 1H, $(CH_2)_2CH$), 4.30 (q, 2H, J = 7.2 Hz, –CH_2CH_3), 5.81 (s, 2H, Ar–CH_2), 6.92 (d, 2H, J = 8.4 Hz, Ar–*H*), 6.96 (d, 2H, J = 8.0 Hz, Ar–*H*), 7.14 (t, 1H, J = 7.6 Hz, Ar–*H*), 7.31 (t, 1H, J = 8.0 Hz, Ar–*H*), 7.37 (s, 1H, Ar–H), 7.58 (d, 1H, J = 8.8 Hz, Ar–*H*), 7.72 (d, 1H, J = 8.0 Hz, Ar–*H*) ppm. MS (ESI): m/z (%) = 320.2 $(M+H)^+$. IR (ATR): ν = 2981, 2603, 2360, 2342, 1705, 1673, 1614, 1518, 1482, 1457, 1442, 1429, 1408, 1350, 1319, 1246, 1187, 1162, 1137, 1120, 1093, 1072, 1046, 1011, 897, 801, 765, 746 cm^{-1}. HPLC (254 nm): 95.5%, t_R = 29.58 min. HRMS Calcd for $C_{21}H_{22}NO_2$ m/z: 320.1651 $(M+H)^+$, found 320.1646.

4.3.18. 1-Benzyl-1*H*-indole-2-carboxylic acid (20)[57]

Synthesized from **13** (3 mmol) according to the General procedure for alkaline hydrolysis. White crystals, yield: 723 mg (96%); mp 198–200 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 5.89 (s, 2H, Ar–CH_2), 7.02–7.04 (m, 2H, Ar–*H*), 7.11–7.15 (m, 1H, Ar–*H*), 7.18–7.22 (m, 1H, indole-*H*), 7.24–7.29 (m, 3H, indole-1*H* and Ar–*H*), 7.30–7.33 (m, 1H, indole-*H*), 7.54 (dd, 1H, J = 8.6 Hz, J = 0.8 Hz, indole-*H*), 7.70–7.72 (m, 1H, indole-*H*), 12.99 (s, 1H, –COOH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 46.84, 110.33, 111.26, 120.60, 122.33, 124.84, 125.58, 126.25, 126.95, 128.21, 128.42, 138.64, 138.88, 162.91 ppm. MS (ESI): m/z (%) = 252.1 $(M+H)^+$. IR (ATR): ν = 2870, 1665, 1514, 1484, 1456, 1423, 1354, 1322, 1266, 1202, 1133, 885, 816, 745, 732, 719, 694, 583, 567 cm^{-1}. HRMS Calcd for $C_{16}H_{14}NO_2$ m/z: 252.1025 $(M+H)^+$, found 252.1015.

4.3.19. 1-(4-Fluorobenzyl)-1*H*-indole-2-carboxylic acid (21)[58]

Synthesized from **14** (3 mmol) according to the General procedure for alkaline hydrolysis. White crystals, yield: 702 mg (87%); mp 176–180 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 5.86 (s, 2H, Ar–CH_2), 7.09–7.15 (m, 5H, indole-1*H* and Ar–*H*), 7.28–7.33 (m, 2H, indole-1*H* and Ar–*H*), 7.58 (dd, 1H, J = 8.4 Hz, J = 0.8 Hz, indole-*H*), 7.71 (d, 1H, J = 7.8 Hz, indole-*H*), 13.01 (s, 1H, –COOH) ppm. MS (ESI): m/z (%) = 268.1 $(M-H)^-$. IR (ATR): ν = 2528, 1674, 1607, 1512, 1483, 1460, 1445, 1425, 1354, 1318, 1268, 1206, 117, 1134, 1091, 1016, 898, 826, 805, 768, 739, 615, 573 cm^{-1}. HPLC (254 nm): 100%, t_R = 18.04 min. HRMS Calcd for $C_{16}H_{11}FNO_2$ m/z: 268.0774 $(M-H)^-$, found 268.0786.

4.3.20. 1-(4-Methylbenzyl)-1*H*-indole-2-carboxylic acid (22)[60]

Synthesized from **15** (3 mmol) according to the General procedure for alkaline hydrolysis. Beige solid, yield: 724 mg (91%); mp 159–163 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.21 (s, 3H, Ar–CH_3), 5.82 (s, 2H, Ar–CH_2), 6.93 (d, 2H, J = 8.4 Hz, Ar–*H*), 7.05 (d, 2H, J = 7.6 Hz, Ar–*H*), 7.11 (dt, 1H, J = 8.0 Hz, J = 0.8 Hz, Ar–*H*), 7.27 (dt, 1H, J = 8.0 Hz, J = 1.2 Hz, Ar–*H*), 7.30 (d, 1H, J = 0.8 Hz, Ar–*H*), 7.52 (dd, 1H, J = 8.4 Hz, J = 0.8 Hz, Ar–*H*), 7.68 (d, 1H, J = 8.0 Hz, Ar–*H*), 12.97 (s, 1H, –COOH) ppm. MS (ESI): m/z (%) = 264.1 $(M-H)^-$. IR (ATR): ν = 2938, 2603, 2361, 2343, 1673, 1614, 1522, 1483, 1456, 1443, 1426, 1354, 1323, 1269, 1206, 1139, 1118, 895, 820, 789, 749, 733 cm^{-1}. HRMS Calcd for $C_{17}H_{15}NO_2$ m/z: 264.1025 $(M-H)^-$, found 264.1030.

4.3.21. 1-(4-Isopropylbenzyl)-1*H*-indole-2-carboxylic acid (23)

Synthesized from **16** (3 mmol) according to the General procedure for alkaline hydrolysis. Beige amorphous solid, yield: 783 mg (89%); mp 218–221 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.14 (d, 6H, J = 7.2 Hz, $(CH_3)_2CH$), 2.77–2.84 (m, 1H, $(CH_3)_2CH$), 5.84 (s, 2H, Ar–CH_2), 6.97 (d, 2H, J = 8.4 Hz, Ar–*H*), 7.11–7.15 (m, 3H, Ar–*H*), 7.29 (dt, 1H, J = 7.2 Hz, J = 1.2 Hz, Ar–*H*), 7.32 (d, 1H, J = 0.8 Hz, Ar–*H*), 7.56 (dd, 1H, J = 8.4 Hz, J = 0.8 Hz, Ar–*H*), 7.70 (d, 1H, J = 8.0 Hz, Ar–*H*), 12.99 (s, 1H, –COOH) ppm. MS (ESI): m/z (%) = 292.1 $(M-H)^-$. IR (ATR): ν = 2960, 2870, 1678, 1522, 1484, 1456, 1445, 1428, 1349, 1266, 1247, 1197, 1136, 745, 735, 727 cm^{-1}. HRMS Calcd for $C_{19}H_{18}NO_2$ m/z: 292.1338 $(M-H)^-$, found 292.1331.

4.3.22. 1-(4-Trifluoromethylbenzyl)-1*H*-indole-2-carboxylic acid (24)[59]

Synthesized from **17** (3 mmol) according to the General procedure for alkaline hydrolysis. Pale yellow amorphous solid, yield: 948 mg (99%); mp 180–184 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 5.97 (s, 2H, Ar–CH_2), 6.97 (d, 2H, J = 8.8 Hz, Ar–*H*), 7.14 (dt, 1H, J = 7.6 Hz, J = 1.2 Hz, Ar–*H*), 7.18 (d, 2H, J = 7.6 Hz, Ar–*H*), 7.29 (dt, 1H, J = 7.6 Hz, J = 1.2 Hz, Ar–*H*), 7.35 (d, 1H, J = 0.8 Hz, Ar–*H*), 7.53 (dd, 1H, J = 8.4 Hz, J = 0.8 Hz, Ar–*H*), 7.64 (d, 2H, J = 8.0 Hz, Ar–*H*), 7.72 (d, 1H, J = 8.0 Hz, Ar–*H*), 13.03 (s, 1H, –COOH) ppm. MS (ESI): m/z (%) = 318.1 $(M-H)^-$. IR (ATR): ν = 2879, 2605, 2526, 2362, 2343, 1671, 1619, 1520, 1484, 1458, 1446, 1429, 1352, 1321, 1265, 1203, 1160, 1139, 1109, 1065, 1015, 920, 897, 851, 835, 822, 771, 752, 738, 647 cm^{-1}. HRMS Calcd for $C_{17}H_{11}F_3NO_2$ m/z: 318.0742 $(M-H)^-$, found 318.0748.

4.3.23. 1-(4-Bromobenzyl)-1*H*-indole-2-carboxylic acid (25)

Synthesized from **18** (3 mmol) according to the General procedure for alkaline hydrolysis. Dirty white amorphous solid, yield: 908 mg (92%); mp 176–180 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 5.84 (s, 2H, Ar–CH_2), 6.97 (d, 2H, J = 8.8 Hz, Ar–*H*), 7.13 (dt, 1H, J = 7.6 Hz, J = 0.8 Hz, Ar–*H*), 7.29 (dt, 1H, J = 7.6 Hz, J = 1.2 Hz, Ar–*H*), 7.33 (d, 1H, J = 8.4 Hz, Ar–*H*), 7.47 (d, 2H, J = 8.4 Hz, Ar–*H*), 7.53 (dd, 1H, J = 8.4 Hz, J = 1.2 Hz, Ar–*H*), 7.71 (d, 1H, J = 7.6 Hz, Ar–*H*), 13.02 (s, 1H, –COOH) ppm. MS (ESI): m/z (%) = 328.0 $(M-H)^-$. IR (ATR): ν = 2929, 2604, 2360, 2342, 1672, 1615, 1519, 1483, 1459, 1440, 1429, 1321, 1266, 1207, 1164, 1138, 1072, 1011, 826, 800, 747, 736 cm^{-1}. HRMS Calcd for $C_{16}H_{11}BrNO_2$ m/z: 327.9973 $(M-H)^-$, found 327.9979.

4.3.24. 1-(4-Cyclopropylbenzyl)-1*H*-indole-2-carboxylic acid (26)

Synthesized from **19** (3 mmol) according to the General procedure for alkaline hydrolysis. White crystals, yield: 274 mg (94%); mp 182–186 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.14 (d, 6H, J = 7.2 Hz, $(CH_3)_2CH$), 2.77–2.84 (m, 1H, $(CH_3)_2CH$), 5.84 (s, 2H, Ar–CH_2), 6.97 (d, 2H, J = 8.4 Hz, Ar–*H*), 7.11–7.15 (m, 3H, Ar–*H*), 7.29 (dt, 1H, J = 7.2 Hz, J = 1.2 Hz, Ar–*H*), 7.32 (d, 1H, J = 0.8 Hz, Ar–*H*), 7.56 (dd, 1H, J = 8.4 Hz, J = 0.8 Hz, Ar–*H*), 7.70 (d, 1H, J = 8.0 Hz, Ar–*H*), 12.99 (s, 1H, –COOH) ppm. MS (ESI): m/z (%) = 290.1 $(M-H)^-$. IR (ATR): ν = 3021, 2600, 2530, 1670, 1615, 1518, 1483, 1459, 1442, 1428, 1357, 1321, 1270, 1199, 1165, 1138, 1102, 1047, 1017, 897, 846, 826, 807, 766, 747, 733 cm^{-1}. HRMS Calcd for $C_{19}H_{16}NO_2$ m/z: 290.1181 $(M-H)^-$, found 290.1189.

4.3.25. *tert*-Butyl (1-benzyl-1*H*-indole-2-yl)carbamate (27)

Synthesized from **20** (1 mmol) according to the General procedure for Curtius rearrangement. Brown amorphous solid, yield: 242 mg (75%); mp 147–151 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.44 (s, 9H, $(-CH_3)_3$), 5.37 (s, 2H, Ar–CH_2), 6.35 (s, 1H, indole-*H*), 6.96–7.04 (m, 4H, Ar–*H*), 7.18–7.22 (m, 4H, indole-*H*), 7.44–7.46 (m, 1H, indole-*H*), 9.36 (s, 1H, –N*H*–CO–) ppm. ^{13}C NMR

(DMSO-d_6, 100 MHz): δ = 27.96, 44.95, 79.52, 93.42, 109.81, 119.41, 119.45, 120.37, 126.48, 126.91, 126.99, 128.37, 133.64, 134.40, 138.04, 153.40 ppm. MS (ESI): m/z (%) = 323.2 $(M+H)^+$. IR (ATR): ν = 3284, 1693, 1553, 1460, 1395, 1369, 1334, 1313, 1148, 1060, 775, 746, 732, 709, 692 cm^{-1}. HRMS Calcd for $C_{20}H_{23}N_2O_2$ m/z: 323.1760 $(M+H)^+$, found 323.1747. Anal. Calcd for $C_{20}H_{22}N_2O_2$ (%): C, 74.51; H, 6.88; N, 8.69. Found: C, 74.34; H, 6.65; N, 8.56.

4.3.26. *tert*-Butyl (1-(4-methylbenzyl)-1*H*-indole-2-yl)carbamate (28)

Synthesized from **22** (1 mmol) according to the General procedure for Curtius rearrangement. Brown amorphous solid, yield: 195 mg (58%); mp 147–151 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.44 (s, 9H, (–CH_3)$_3$), 2.22 (s, 3H, Ar–CH_3), 5.30 (s, 2H, Ar–CH_2), 6.32 (s, 1H, indole-*H*), 6.93–7.01 (m, 4H, Ar–*H*), 7.06 (d, 2H, J = 8.0 Hz, Ar–*H*), 7.26 (dd, 1H, J = 8.0 Hz, J = 0.8 Hz, Ar–*H*), 7.33 (dd, 1H, J = 8.4 Hz, J = 1.2 Hz, Ar–*H*), 7.42–7.51 (m, 2H, Ar–H), 9.33 (s, 1H, –N*H*–CO–) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 20.57, 27.96, 44.74, 79.51, 109.86, 119.38, 120.31, 126.53, 128.90, 133.60, 134.34, 134.98, 136.12, 153.41 ppm. MS (ESI): m/z (%) = 337.2 $(M+H)^+$. IR (ATR): ν = 3258, 3174, 3050, 3018, 2981, 2927, 2360, 2342, 2168, 1692, 1612, 1590, 1578, 1554, 1514, 1481, 1462, 1393, 1369, 1333, 1312, 1251, 1204, 1181, 1150, 1107, 1060, 1011, 965, 909, 891, 838, 810, 772, 761, 751, 730, 688, 658, 644 cm^{-1}. HRMS Calcd for $C_{21}H_{25}N_2O_2$ m/z: 337.1916 $(M+H)^+$, found 337.1912. Anal. Calcd for $C_{21}H_{24}N_2O_2 \times 0.25CH_2Cl_2$ (%): C, 71.36; H, 6.90; N, 7.83. Found: C, 71.70; H, 6.42; N, 8.29.

4.3.27. *tert*-Butyl(1-(4-isopropylbenzyl)-1*H*-indole-2-yl)carbamate (29)

Synthesized from **23** (1 mmol) according to the General procedure for Curtius rearrangement. Brown amorphous solid, yield: 186 mg (51%); mp 127–131 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.13 (d, 6H, J = 7.2 Hz, (CH_3)$_2$CH), 1.43 (s, 9H, (–CH_3)$_3$), 2.76–2.83 (m, 1H, (CH$_3$)$_2$C*H*), 5.31 (s, 2H, Ar–CH_2), 6.32 (s, 1H, indole-*H*), 6.94–7.02 (m, 4H, Ar–*H*), 7.12 (d, 2H, J = 8.0 Hz, Ar–*H*), 7.28–7.35 (m, 1H, Ar–*H*), 7.44 (d, 1H, J = 7.2 Hz, Ar–*H*), 9.35 (s, 1H, –N*H*–CO–) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 23.81, 27.96, 33.02, 79.51, 109.83, 119.39, 125.25, 126.27, 126.51, 134.35, 135.42, 147.13. 153.39 ppm. MS (ESI): m/z (%) = 365.2 $(M+H)^+$. IR (ATR): ν = 3222, 3140, 3050, 2979, 2956, 2928, 2866, 2360, 2342, 2169, 1689, 1613, 1580, 1560, 1511, 1489, 1465 1449, 1395, 1372, 1354, 1336, 1316, 1259, 1182, 1153, 1105, 1062, 1053, 1015, 997, 966, 941, 920, 892, 842, 815, 778, 765, 746, 734, 717, 688, 669, 630, 607 cm^{-1}. HRMS Calcd for $C_{23}H_{29}N_2O_2$ m/z: 365.2229 $(M+H)^+$, found 365.2219. Anal. Calcd for $C_{23}H_{28}N_2O_2 \times 0.3H_2O$ (%): C, 74.68; H, 7.79; N, 7.57. Found: C, 74.05; H, 7.04; N, 7.46.

4.3.28. *tert*-Butyl (1-(4-trifluoromethylbenzyl)-1*H*-indole-2-yl)-carbamate (30)

Synthesized from **24** (1 mmol) according to the General procedure for Curtius rearrangement. Beige amorphous solid, yield: 215 mg (55%); mp 146–150 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.40 (s, 9H, (–CH_3)$_3$), 5.47 (s, 2H, Ar–CH_2), 6.38 (s, 1H, indole-*H*), 6.98–7.04 (m, 2H, Ar–*H*), 7.17 (d, 2H, J = 8.0 Hz, Ar–*H*), 7.26–7.28 (m, 1H, Ar–*H*), 7.32–7.35 (m, 1H, Ar–*H*), 7.46–7.51 (m, 2H, Ar–*H*), 7.65 (d, 2H, J = 8.0 Hz, Ar–*H*), 9.36 (s, 1H, –N*H*–CO–) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 27.89, 44.60, 79.57, 109.66, 119.54, 119.67, 120.61, 125.30, 125.34, 127.05, 129.66, 130.40, 133.66, 134.33, 142.95, 153.34 ppm. MS (ESI): m/z (%) = 391.2 $(M+H)^+$. IR (ATR): ν = 3293, 2989, 2972, 2934, 2361, 2342, 2170, 1701, 1620, 1586, 1561, 1514, 1489, 1458, 1422, 1394, 1369, 1360, 1325, 1264, 1247, 1211, 1152, 1117, 1067, 1018, 999, 967, 920, 829, 815, 801, 769, 742, 729, 719, 684, 643, 619 cm^{-1}. HRMS Calcd for $C_{21}H_{22}F_3N_2O_2$ m/z: 391.1633 $(M+H)^+$, found 391.1631. Anal. Calcd for $C_{21}H_{21}F_3N_2O_2 \times 0.05H_2O$ (%): C, 64.46; H, 5.44; N, 7.16. Found: C, 64.18; H, 5.50; N, 7.49.

4.3.29. *tert*-Butyl (1-(4-bromobenzyl)-1*H*-indole-2-yl)carbamate (31)

Synthesized from **25** (1 mmol) according to the General procedure for Curtius rearrangement. Beige amorphous solid, yield: 244 mg (61%); mp 132–136 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.43 (s, 9H, (–CH_3)$_3$), 5.34 (s, 2H, Ar–CH_2), 6.35 (s, 1H, indole-*H*), 6.95–7.01 (m, 4H, Ar–*H*), 7.26 (d, 1H, J = 8.4 Hz, Ar–*H*), 7.32–7.35 (m, 1H, Ar–*H*), 7.44–7.48 (m, 3H, Ar–*H*), 9.34 (s, 1H, –N*H*–CO–) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 27.94, 44.38, 79.58, 109.73, 119.48, 119.58, 120.06, 120.51, 128.67, 130.41, 131.26, 131.37, 131.56, 133.59, 134.29, 137.51, 153.37 ppm. MS (ESI): m/z (%) = 401.1 $(M+H)^+$. IR (ATR): ν = 3221, 3136, 3080, 3019, 2979, 2928, 2360, 2342, 2170, 1685, 1578, 1561, 1510, 1487, 1463, 1442, 1392, 1368, 1335, 1313, 1259, 1216, 1152, 1106, 1070, 1058, 1011, 996, 968, 943, 918, 845, 806, 778, 763, 744, 727, 688, 670, 645 cm^{-1}. HRMS Calcd for $C_{20}H_{22}BrN_2O_2$ m/z: 401.0865 $(M+H)^+$, found 401.0874. Anal. Calcd for $C_{20}H_{21}BrN_2O_2$ (%): C, 59.86; H, 5.27; N, 6.98. Found: C, 59.78; H, 4.97; N, 7.02.

4.3.30. *tert*-Butyl (1-(4-cyclopropylbenzyl)-1*H*-indole-2-yl)carbamate (32)

Synthesized from **26** (1 mmol) according to the General procedure for Curtius rearrangement. Ochre amorphous solid, yield: 203 mg (56%); mp 123–127 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.55–0.57 (m, 2H, –CH_2CH$_2$–), 0.85–0.91 (m, 2H, –CH_2CH$_2$–), 1.42 (s, 9H, (–CH_3)$_3$), 1.80–1.82 (m, 1H, (CH$_2$)$_2$C*H*), 5.28 (s, 2H, Ar–CH_2), 6.31 (s, 1H, indole-*H*), 6.92–7.45 (m, 8H, Ar–*H*), 9.34 (s, 1H, –N*H*–CO–) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 9.27, 14.67, 27.97, 44.69, 79.53, 107.11, 109.85, 109.90, 119.38, 120.32, 125.32, 126.52, 126.89, 130.41, 133.61, 134.36, 134.90, 142.49, 153.39, 166.07 ppm. MS (ESI): m/z (%) = 363.2 $(M+H)^+$. IR (ATR): ν = 3217, 3135, 3080, 3009, 2976, 2927, 2360, 2341, 2170, 1687, 1613, 1578, 1559, 1504, 1463, 1443, 1399, 1364, 1336, 1316, 1254, 1215, 1152, 1106, 1059, 1046, 1015, 995, 966, 941, 920, 901, 889, 845, 831, 812, 798, 777, 763, 745, 729, 712, 667, 623, 606 cm^{-1}. HRMS Calcd for $C_{23}H_{27}N_2O_2$ m/z: 363.2073 $(M+H)^+$, found 363.2068. Anal. Calcd for $C_{23}H_{26}N_2O_2 \times 0.75H_2O$ (%): C, 73.47; H, 7.37; N, 7.45. Found: C, 73.09; H, 6.94; N, 7.72.

4.3.31. 1-Benzyl-indoline-2-imminium chloride (33)

Synthesized from **27** (0.5 mmol) according to the General procedure for acidolysis. White amorphous solid, yield: 106 mg (82%); mp 247–250 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 4.38 (s, 2H, –indoline-CH_2–), 5.38 (s, 2H, Ar–CH_2), 7.14 (d, 1H, J = 8.0 Hz, Ar–*H*), 7.19–7.21 (m, 1H, indoline-*H*), 7.33–7.37 (m, 6H, indoline-*H* and Ar–*H*), 7.49 (d, 1H, J = 7.2 Hz, indoline-*H*), 10.36 (s, 1H, –NH_2), 10.71 (s, 1H, –NH_2) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 35.89, 45.57, 111.10, 124.54, 124.72, 126.23, 127.03, 127.95, 128.81, 133.74, 143.41, 170.23 ppm. MS (ESI): m/z (%) = 223.1 $(M-Cl)^+$. IR (ATR): ν = 2943, 1681, 1601, 1493, 1468, 1443, 1376, 891, 746, 731, 709, 694, 662, 580 cm^{-1}. HPLC (254 nm): 100%, t_R = 4.24 min. HRMS Calcd for $C_{15}H_{15}N_2$ m/z: 223.1235 $(M-Cl)^+$, found 223.1231. Anal. Calcd for $C_{15}H_{15}N_2Cl \times 0.1HCl$ (%): C, 68.66; H, 5.80; N, 10.68. Found: C, 68.95; H, 5.40; N, 10.57.

4.3.32. 1-(4-Methylbenzyl)-indoline-2-imminium chloride (34)

Synthesized from **28** (0.5 mmol) according to the General procedure for acidolysis. Dark brown amorphous solid, yield: 59 mg (43%); mp 70–74 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.27 (s, 3H, Ar–CH$_3$), 4.36 (s, 2H, –indoline-CH_2–), 5.25 (s, 2H, Ar–CH_2), 6.93 (d, 1H, J = 7.6 Hz, Ar–*H*), 7.06 (d, 1H, J = 7.6 Hz, Ar–*H*), 7.13 (d, 1H, J = 7.6 Hz, Ar–*H*), 7.19 (d, 2H, J = 7.6 Hz, Ar–*H*), 7.26 (d, 2H,

J = 7.6 Hz, Ar–H), 7.30–7.34 (m, 1H, Ar–H), 7.49 (dd, 1H, J = 7.2 Hz, J = 0.8 Hz, Ar–H), 10.04 (s, 1H, –NH_2), 10.46 (s, 1H, –NH_2) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 37.81, 44.83, 58.03, 103.47, 111.61, 126.93, 127.01, 127.60, 128.98, 129.37, 143.76, 147.43, 169.41 ppm. MS (ESI): m/z (%) = 237.1 $(M-Cl)^+$. IR (ATR): ν = 2973, 2922, 2169, 1698, 1589, 1515, 1482, 1463, 1392, 1365, 1264, 1203, 1179, 1155, 1111, 1058, 1022, 1010, 965, 780, 739, 688 cm^{-1}. HPLC (254 nm): 94.1%, t_R = 6.72 min. HRMS Calcd for $C_{16}H_{17}N_2$ m/z: 237.1392 $(M-Cl)^+$, found 237.1388.

4.3.33. 1-(4-Isopropylbenzyl)-indoline-2-imminium chloride (35)

Synthesized from **29** (0.5 mmol) according to the General procedure for acidolysis. Brown amorphous solid, yield: 86 mg (57%); mp 82–86 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.17 (d, 6H, J = 7.2 Hz, $(CH_3)_2$CH), 2.81–2.90 (m, 1H, $(CH_3)_2CH$), 4.37 (s, 2H, –indoline-CH_2–), 5.31 (s, 2H, Ar–CH_2), 6.95–7.00 (m, 1H, Ar–H), 7.12–7.36 (m, 6H, Ar–H), 7.50 (d, 1H, J = 7.6 Hz, Ar–H), 10.27 (s, 1H, –NH_2), 10.63 (s, 1H, –NH_2) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 24.28, 33.57, 36.35, 45.80, 111.62, 125.21, 126.71, 127.20, 127.63, 128.47, 131.62, 143.94, 148.70, 161.46, 170.58 ppm. MS (ESI): m/z (%) = 265.2 $(M-Cl)^+$. IR (ATR): ν = 2960, 2929, 2169, 1698, 1610, 1514, 1481, 1464, 1420, 1365, 1242, 1206, 1157, 1112, 1056, 1019, 965, 739, 689 cm^{-1}. HPLC (254 nm): 94.7%, t_R = 10.59 min. HRMS Calcd for $C_{15}H_{13}ClN_2$ m/z: 265.1705 $(M-Cl)^+$, found 265.1712.

4.3.34. 1-(4-Trifluoromethylbenzyl)-indoline-2-imminium chloride (36)

Synthesized from **30** (0.5 mmol) according to the General procedure for acidolysis. Beige amorphous solid, yield: 65 mg (40%); mp 87–91 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 4.39 (s, 2H, –indoline-CH_2–), 5.51 (s, 2H, Ar–CH_2), 7.14 (d, 1H, J = 8.0 Hz, Ar–H), 7.22 (t, 1H, J = 7.2 Hz, Ar–H), 7.33 (t, 1H, J = 7.6 Hz, Ar–H), 7.52 (d, 1H, J = 7.2 Hz, Ar–H), 7.61 (d, 2H, J = 8.0 Hz, Ar–H), 7.77 (d, 2H, J = 8.0 Hz, Ar–H), 10.44 (s, 1H, –NH_2), 10.74 (s, 1H, –NH_2) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 36.46, 45.79, 111.40, 125.12, 125.29, 126.12, 126.16, 126.74, 128.31, 128.52, 139.19, 143.81, 171.00 ppm. MS (ESI): m/z (%) = 291.1 $(M-Cl)^+$. IR (ATR): ν = 2978, 2173, 1701, 1481, 1464, 1420, 1393, 1367, 1323, 1247, 1159, 1121, 1066, 1018, 968, 741 cm^{-1}. HPLC (254 nm): 97.9%, t_R = 8.62 min. HRMS Calcd for $C_{16}H_{14}F_3N_2$ m/z: 291.1109 $(M-Cl)^+$, found 291.1104. Anal. Calcd for $C_{16}H_{14}F_3N_2 \times 0.1$HCl (%): C, 58.17; H, 4.30; N, 8.48. Found: C, 58.18; H, 4.06; N, 8.32.

4.3.35. 1-(4-Bromobenzyl)-indoline-2-imminium chloride (37)

Synthesized from **31** (0.5 mmol) according to the General procedure for acidolysis. Light brown amorphous solid, yield: 77 mg (46%); mp 78–82 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 4.35 (s, 2H, –indoline-CH_2–), 5.33 (s, 2H, Ar–CH_2), 7.13 (d, 2H, J = 8.0 Hz, Ar–H), 7.21 (t, 1H, J = 7.6 Hz, Ar–H), 7.30–7.35 (m, 3H, Ar–H), 7.49 (d, 1H, J = 7.6 Hz, Ar–H), 7.59 (d, 2H, J = 8.4 Hz, Ar–H), 10.28 (s, 1H, –NH_2), 10.63 (s, 1H, –NH_2) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 36.43, 45.54, 111.51, 114.46, 121.63, 125.10, 125.25, 126.73, 128.48, 129.83, 132.17, 133.74, 143.79, 170.85 ppm. MS (ESI): m/z (%) = 301.0 $(M-Cl)^+$. IR (ATR): ν = 2968, 2924, 2169, 1697, 1604, 1591, 1566, 14871463, 1392, 1366, 1261, 1204, 1152, 1112, 1070, 1010, 966, 781, 739, 687 cm^{-1}. HPLC (254 nm): 96.1%, t_R = 7.50 min. HRMS Calcd for $C_{15}H_{14}BrN_2$ m/z: 301.0340 $(M-Cl)^+$, found 301.0333. Anal. Calcd for $C_{15}H_{14}BrClN_2 \times 0.25$HCl (%): C, 51.96; H, 4.14; N, 8.08. Found: C, 52.27; H, 3.87; N, 7.69.

4.3.36. 1-(4-Cyclopropylbenzyl)-indoline-2-imminium chloride (38)

Synthesized from **32** (0.5 mmol) according to the General procedure for acidolysis. Light brown amorphous solid, yield: 52 mg (35%); mp 62–66 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.62–0.66 (m, 2H, –CH_2CH_2–), 0.91–0.96 (m, 2H, –CH_2CH_2–), 1.86–1.93 (m, 1H, $(CH_2)_2CH$), 4.36 (s, 2H, –indoline-CH_2–), 5.28 (s, 2H, Ar–CH_2), 7.08 (d, 2H, J = 8.4 Hz, Ar–H), 7.16 (d, 1H, J = 7.6 Hz, Ar–H), 7.21 (dt, 1H, J = 7.6 Hz, J = 0.8 Hz, Ar–H), 7.26 (d, 2H, J = 8.4 Hz, Ar–H), 7.33 (dt, 1H, J = 8.0 Hz, J = 1.2 Hz, Ar–H), 7.49 (d, 1H, J = 6.8 Hz, Ar–H), 10.22 (s, 1H, –NH_2), 10.59 (s, 1H, –NH_2) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 10.01, 36.36, 44.69, 111.65, 125.02, 125.20, 126.18, 126.70, 127.62, 128.44, 131.01, 143.89, 144.18, 170.58 ppm. MS (ESI): m/z (%) = 263.2 $(M-Cl)^+$. IR (ATR): ν = 2973, 2926, 2169, 1698, 1605, 1590, 1487, 1463, 1392, 1366, 1261, 1204, 1153, 1111, 1010, 966, 781, 739, 687 cm^{-1}. HPLC (254 nm): 91.3%, t_R = 8.82 min. HRMS Calcd for $C_{18}H_{19}N_2$ m/z: 263.1548 $(M-Cl)^+$, found 263.1541. Anal. Calcd for $C_{18}H_{14}ClN_2 \times 0.3$HCl (%): C, 69.80; H, 6.28; N, 9.04. Found: C, 70.12; H, 5.60; N, 8.87.

4.3.37. Ethyl 1-((4-fluorophenyl)sulfonyl)-1*H*-indole-2-carboxylate (39)

Synthesized from **3** (5 mmol) according to the General procedure for N-sulfonylation. Orange crystals, yield: 1.00 g (58%); mp 95–98 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.43 (t, 3H, J = 7.2 Hz, –CH_3), 4.43 (q, 2H, J = 7.2 Hz, –CH_2CH_3), 7.15–7.20 (m, 3H, indole-H and Ar–H), 7.32–7.34 (m, 1H, indole-H), 7.45–7.47 (m, 1H, indole-H), 7.59–7.60 (m, 1H, Ar–H), 8.09–8.11 (m, 3H, Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.85, 61.80, 114.91, 116.78, 116.98, 117.01, 123.00, 124.54, 127.36, 127.98, 130.29, 130.39, 131.29, 133.51, 133.54, 137.06, 160.67, 163.99, 166.52 ppm. MS (ESI): m/z (%) = 348.1 $(M+H)^+$. IR (ATR): ν = 1726, 1589, 1495, 1366, 1337, 1265, 1176, 1149, 1127, 1088, 920, 861, 846, 818, 757, 711, 682, 655, 579, 554 cm^{-1}. HPLC (254 nm): 100%, t_R = 23.62 min. HRMS Calcd for $C_{17}H_{15}FNO_4S$ m/z: 348.0706 $(M+H)^+$, found 348.0706. Anal. Calcd for $C_{17}H_{14}FNO_4S$ (%): C, 58.78; H, 4.06; N, 4.03. Found: C, 58.88; H, 3.62; N, 4.05.

4.3.38. Ethyl 1-((4-methoxyphenyl)sulfonyl)-1*H*-indole-2-carboxylate (40)

Synthesized from **3** (5 mmol) according to the General procedure for N-sulfonylation. Yellow crystals, yield: 1.38 g (77%); mp 95–97 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.42 (t, 3H, J = 7.2 Hz, –CH_3), 2.40 (s, 3H, Ar–CH_3), 4.43 (q, 2H, J = 7.2 Hz, –CH_2–CH_3), 7.27–7,29 (m, 4H, indole-H and Ar–H), 7.34–7,35 (m, 1H, indole-H), 7.42–7.44 (m, 1H, indole-H), 7.57–7.59 (m, 1H, Ar–H), 7.93–7.85 (m, 2H, Ar–H), 8.12–8.14 (m, H, Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 14.39, 56.35, 62.28, 115.21, 115.35, 116.57, 123.32, 124.85, 127.56, 128.57, 128.74, 129.99, 131.96, 137.29, 161.49, 164.30 ppm. MS (ESI): m/z (%) = 360.1 $(M+H)^+$. IR (ATR): ν = 1722, 1594, 1497, 1365, 1336, 1311, 1265, 1149, 1087, 1017, 838, 804, 745, 677, 629 cm^{-1}. HPLC (254 nm): 100%, t_R = 23.13 min. HRMS Calcd for $C_{18}H_{18}NO_5S$ m/z: 360.0906 $(M+H)^+$, found 360.0905.

4.3.39. Ethyl 1-((4-nitrophenyl)sulfonyl)-1*H*-indole-2-carboxylate (41)

Synthesized from **3** (5 mmol) according to the General procedure for N-sulfonylation. Yellow crystals, yield: 1.3 g (68%); mp 104–108 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.39 (t, 3H, J = 7.2 Hz, –CH_3), 4.38 (q, 2H, J = 7.2 Hz, –CH_2–CH_3), 7.31–7.35 (m, 1H, Ar–H), 7.47–7.51 (m, 1H, Ar–H), 7.61 (td, 1H, J = 7.6 Hz, J = 1.2 Hz, Ar–H), 8.15 (dd, 1H, J = 8.8 Hz, J = 0.8 Hz, Ar–H), 8.23 (d, 2H, J = 9.2 Hz, Ar–H), 8.32 (d, 2H, J = 9.2 Hz, Ar–H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.16, 62.13, 115.20, 118.09, 122.96, 124.16, 124.71, 127.73, 128.11, 128.71, 131.47, 138.40, 144.38, 150.49, 160.76 ppm. MS (ESI): m/z (%) = 375.1 $(M+H)^+$. IR (ATR): ν = 1726, 1536, 1352, 1334, 1178, 1151, 1128, 1089, 1039, 1014,

850, 740, 683, 662, 615 cm^{-1}. HPLC (254 nm): 100%, t_R = 24.04 min. HRMS Calcd for $C_{17}H_{15}N_2O_6S$ *m/z*: 375.0651 $(M+H)^+$, found 375.0659.

4.3.40. Ethyl 1-((4-chlorophenyl)sulfonyl)-1*H*-indole-2-carboxylate (42)[61]

Synthesized from **3** (5 mmol) according to the General procedure for N-sulfonylation. Brown amorphous solid, yield: 1.1 g (63%); mp 86–90 °C. ^1H NMR ($CDCl_3$, 400 MHz): δ = 1.42 (t, 3H, *J* = 7.2 Hz, –C*H*$_3$), 4.43 (q, 2H, *J* = 7.2 Hz, –C*H*$_2$–CH$_3$), 7.22 (d, 1H, *J* = 0.8 Hz, Ar–H), 7.31 (dt, 1H, *J* = 8.0 Hz, *J* = 0.8 Hz, Ar–H), 7.44–7.48 (m, 3H, Ar–*H*), 7.34–7,35 (m, 1H, indole-*H*), 7.42–7.44 (m, 1H, indole-*H*), 7.59 (d, 1H, *J* = 7.6 Hz, Ar–H), 8.02 (d, 2H, *J* = 7.6 Hz, Ar–H), 8.13 (dd, 1H, *J* = 8.4 Hz, *J* = 0.8 Hz, Ar–H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.17, 62.05, 115.27, 117.23, 122.70, 124.37, 127.28, 128.19, 128.89, 129.28, 131.71, 137.07, 138.15, 161.15 ppm. MS (ESI): *m/z* (%) = 364.0 $(M+H)^+$. IR (ATR): *v* = 1625, 1477, 1396, 1319, 1249, 1195, 1176, 1149, 1095, 1011, 757, 664, 620, 574 cm^{-1}. HPLC (254 nm): 100%, t_R = 26.58 min. HRMS Calcd for $C_{17}H_{15}NO_4SCl$ *m/z*: 364.0410 $(M+H)^+$, found 364.0415.

4.3.41. Ethyl 1-((4-trifluoromethylphenyl)sulfonyl)-1*H*-indole-2-carboxylate (43)

Synthesized from **3** (5 mmol) according to the General procedure for N-sulfonylation. Ochre amorphous solid, yield: 1.6 g (80%); mp 62–64 °C. ^1H NMR ($CDCl_3$, 400 MHz): δ = 1.41 (t, 3H, *J* = 7.2 Hz, –C*H*$_3$), 4.43 (q, 2H, *J* = 7.2 Hz, –C*H*$_2$–CH$_3$), 7.27 (d, 1H, *J* = 0.8 Hz, Ar–H), 7.32 (dt, 1H, *J* = 8.0 Hz, *J* = 0.8 Hz, Ar–H), 7.48 (dt, 1H, *J* = 8.8 Hz, *J* = 1.2 Hz, Ar–H), 7.59 (dd, 1H, *J* = 7.6 Hz, *J* = 0.8 Hz, Ar–H), 7.76 (d, 2H, *J* = 8.4 Hz, Ar–H), 8.19 (dd, 1H, *J* = 8.4 Hz, *J* = 0.8 Hz, Ar–H), 8.24 (d, 2H, *J* = 8.8 Hz, Ar–H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.08, 62.06, 115.23, 117.61, 122.83, 124.50, 126.10, 126.14, 126.18, 127.49, 127.98, 128.16, 128.42, 131.63, 138.30, 142.32, 160.96 ppm. MS (ESI): *m/z* (%) = 398.1 $(M+H)^+$. IR (ATR): *v* = 3306, 1720, 1685, 1527, 1367, 1340, 1311, 1248, 1172, 1140, 1061, 1016, 918, 841, 771, 736, 714, 665, 603 cm^{-1}. HPLC (254 nm): 100%, t_R = 27.66 min. HRMS Calcd for $C_{18}H_{15}NO_4SF_3$ *m/z*: 398.0674 $(M+H)^+$, found 398.0682.

4.3.42. Ethyl 1-(4-toluoyl)-1*H*-indole-2-carboxylate (44)

Synthesized from **3** according to the following procedure for N-acylation. Ethyl indole-2-carboxylate (5 mmol) was first dissolved in THF (15 mL) then potassium *tert*-butoxide (7.5 mmol) was added and the mixture was stirred for 15 min at room temperature. Subsequently, toluoyl chloride (5.5 mmol) was added to the reaction mixture which was heated overnight at reflux. Upon completion of the reaction, which was monitored by TLC, the mixture was concentrated in vacuo and the residue was dissolved in dichloromethane (80 mL). The organic phase was washed with water (2 × 30 mL), dried with anhydrous Na_2SO_4 and then concentrated in vacuo. The residue was purified by flash silica gel column chromatography (gradient elution; starting eluent: hexane/ethyl acetate 6:1 v/v) to afford pure **44**. White amorphous solid, yield: 937 mg (61%); mp/°C. ^1H NMR ($CDCl_3$, 400 MHz): δ = 1.14 (t, 3H, *J* = 7.2 Hz, –C*H*$_3$), 2.44 (s, 3H, Ar–C*H*$_3$), 4.04 (q, 2H, *J* = 7.2 Hz, –C*H*$_2$–CH$_3$), 7.27–7.33 (m, 3H, Ar–H), 7.39–7.43 (m, 2H, Ar–H), 7.65 (d, 2H, *J* = 8.4 Hz, Ar–H), 7.73 (dd, 1H, *J* = 8.0 Hz, *J* = 1.2 Hz, Ar–H), 7.77 (dd, 1H, *J* = 8.4 Hz, *J* = 0.8 Hz, Ar–H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 13.92, 21.76, 61.31, 113.96, 115.31, 122.53, 123.29, 127.03, 127.19, 129.49, 129.64, 131.06, 132.77, 138.82, 144.23, 161.06, 169.11 ppm. MS (ESI): *m/z* (%) = 308.1 $(M+H)^+$. IR (ATR): *v* = 1714, 1608, 1541, 1444, 1371, 1306, 1229, 1200, 1154, 1093, 1015, 957, 913, 888, 832, 790, 750, 605 cm^{-1}. HPLC (254 nm): 100%, t_R = 26.52 min. HRMS Calcd for $C_{19}H_{18}NO_3$ *m/z*: 308.1287 $(M+H)^+$, found 308.1278.

4.3.43. Ethyl 1-(4-toluenesulfonyl)-1*H*-indole-2-carboxamide (45)

Synthesized from **6** (1 mmol) according to the following amidation procedure. White crystals, yield: 182 mg (58%); mp 218–220 °C. ^1H NMR (DMSO-d_6, 400 MHz): δ = 2.34 (s, 3H, Ar–C*H*$_3$), 6.98 (s, 1H, Ar–H), 7.27 (t, 1H, *J* = 7.2 Hz, Ar–H), 7.37–7.40 (m, 3H, Ar–*H*), 7.61 (d, 1H, *J* = 7.6 Hz, Ar–H), 7.72 (s, 1H, Ar–*H*), 7.94 (dd, 1H, *J* = 8.4 Hz, *J* = 0.8 Hz, Ar–H), 8.02 (d, 1H, *J* = 8.4 Hz, Ar–H), 8.22 (s, 1H, Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 21.52, 111.72, 114.91, 122.52, 124.37, 126.18, 127.88, 128.89, 130.25, 135.13, 136.17, 136.76, 145.69, 163.20 ppm. MS (ESI): *m/z* (%) = 315.1 $(M+H)^+$. IR (ATR): *v* = 3426, 3146, 1697, 1671, 1595, 1552, 1450, 1395, 1361, 1307, 1234, 1187, 1169, 1139, 1086, 1015, 1012, 910, 841, 808, 746, 703, 675, 660, 632 cm^{-1}. HPLC (254 nm): 99.5%, t_R = 11.94 min. HRMS Calcd for $C_{16}H_{15}N_2O_3S$ *m/z*: 315.0803 $(M+H)^+$, found 315.0801.

4.4. Cell culture (HEK-Blue NOD1 and NOD2 cells)

HEK-Blue NOD1 and NOD2 cells (Invivogen) were cultured in DMEM medium (Sigma) with 10 % heat-deactivated FBS (Gibco), 2 mM L-glutamine (Sigma), 50 U/mL penicillin (Sigma), 50 ng/mL streptomycin (Sigma), 4.5 g glucose (Sigma) and 100 µg/mL Normocin (Invivogen) for 2 passages. All subsequent passages were cultured in the medium supplemented with 30 µg/mL Blasticidin (Invivogen) and 100 µg/mL Zeocin (Invivogen). The experiments were carried out on passages 7–12.

4.5. Metabolic activity assay

The tested compounds were dissolved in DMSO and further diluted in culture medium to a desired final concentration, so that the final concentration of DMSO did not exceed 0.1%. HEK-Blue NOD1 cells (1×10^4 cells/well) were seeded in triplicates on 96 well plates in 100 µL culture medium and treated with 25 µM concentration of each compound or with the corresponding volume of vehicle—0.1% DMSO (control cells). After 24 h, metabolic activity was measured using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega, Madison/WI, USA), in accordance with the manufacturer's instructions. Three biological replicates were performed for each experiment.

4.6. Measurement of NF-κB transcriptional activity (HEK-Blue™ Detection)

The tested compounds were dissolved in DMSO and further diluted in culture medium to a desired final concentration, so that the final concentration of DMSO did not exceed 0.1%. For screening for potential NOD1 inhibitors, HEK-Blue NOD1 cells (5×10^5 cells/mL) were assayed in duplicate for NF-κB transcriptional activity after treatment with 25 µM NOD1 inhibitors or Noditinib-1 for 1 h, followed by the addition of 100 nM C12-iE-DAP and subsequent incubation for 18 h (37 °C, 5% CO_2, 100% humidity). The control cells (NT) were only treated with the corresponding volume of vehicle—0.1% DMSO. For determining IC_{50}, HEK-Blue NOD1 and NOD2 cells (5×10^5 cells/mL) were assayed in duplicates for NF-κB transcriptional activity after 1 h pre-treatment with 0.625–25 µM NOD1 inhibitors or Noditinib-1, followed by the addition of 100 nM C12-iE-DAP or 2 µM MDP, respectively, and subsequent incubation for 18 h. SEAP activity was determined spectrophotometrically as absorbance at 620 nm on a Tecan Safire 2 microplate reader (Reading, UK). Assays were performed in three biological replicates.

4.7. Statistics

All experiments were performed at least twice, with average values expressed as means ± SD. IC_{50} values of NOD1/2 inhibition

were calculated by a non-linear regression model using Graph Pad Prism 6 software. Statistical significance was determined with the Dunnet multiple comparison test. Differences were considered significant for p <0.05 and highly significant for p <0.01.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.08.044.

References and notes

1. Caruso, R.; Warner, N.; Inohara, N.; Nuñez, G. *Immunity* **2014**, *41*, 898.
2. Philpott, D. J.; Sorbara, M. T.; Robertson, S. J.; Croitoru, K.; Girardin, S. E. *Nat. Rev. Immunol.* **2014**, *14*, 9.
3. Hanson, P. I.; Whiteheart, S. W. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 519.
4. Ammelburg, M.; Frickey, T.; Lupas, A. N. *J. Struct. Biol.* **2006**, *156*, 2.
5. Proell, M.; Riedl, S. J.; Fritz, J. H.; Rojas, A. M.; Schwarzenbacher, R. *PLoS One* **2008**, *3*, e2119.
6. Mo, J.; Boyle, J. P.; Howard, C. B.; Monie, T. P.; Davis, B. K.; Duncan, J. A. *J. Biol. Chem.* **2012**, *287*, 23057.
7. Askari, N.; Correa, R. G.; Zhai, D.; Reed, J. C. *J. Biotechnol.* **2012**, *157*, 75.
8. Jakopin, Ž. *J. Med. Chem.* **2014**, *57*, 6897.
9. Girardin, S. E.; Boneca, I. G.; Carneiro, L. A. M.; Antignac, A.; Jéhanno, M.; Viala, J.; Tedin, K.; Taha, M. K.; Labigne, A.; Zähringer, U.; Coyle, A. J.; DiStefano, P. S.; Bertin, J.; Sansonetti, P. J.; Philpott, D. J. *Science* **2003**, *300*, 1584.
10. Chamaillard, M.; Hashimoto, M.; Horie, Y.; Masumoto, J.; Qiu, S.; Saab, L.; Ogura, Y.; Kawasaki, A.; Fukase, K.; Kusumoto, S.; Valvano, M. A.; Foster, S. J.; Mak, T. W.; Nuñez, G.; Inohara, N. *Nat. Immunol.* **2003**, *4*, 702.
11. Girardin, S. E.; Jéhanno, M.; Mengin-Lecreulx, D.; Sansonetti, P. J.; Alzari, P. M.; Philpott, D. J. *J. Biol. Chem.* **2005**, *280*, 38648.
12. a) Girardin, S. E.; Travassos, L. H.; Hervé, M.; Blanot, D.; Boneca, I. G.; Philpott, D. J.; Sansonetti, P. J.; Mengin-Lecreulx, D. *J. Biol. Chem.* **2003**, *278*, 41702; b) Inohara, N.; Ogura, Y.; Fontalba, A.; Gutierrez, O.; Pons, F.; Crespo, J.; Fukase, K.; Inamura, S.; Kusumoto, S.; Hashimoto, M.; Foster, S. J.; Moran, A. P.; Fernandez-Luna, J. L.; Nuñez, G. *J. Biol. Chem.* **2003**, *278*, 5509.
13. Dzierzbicka, K.; Wardowska, A.; Trzonkowski, P. *Curr. Med. Chem.* **2011**, *18*, 2438.
14. Correa, R. G.; Milutinovic, S.; Reed, J. C. *Biosci. Rep.* **2012**, *608*, 597.
15. Moreira, L. O.; Zamboni, D. S. *Front. Immunol.* **2012**, *3*, 328.
16. Travassos, L. H.; Carneiro, L. A.; Ramjeet, M.; Hussey, S.; Kim, Y. G.; Magalhães, J. G.; Yuan, L.; Soares, F.; Chea, E.; Le Bourhis, L.; Boneca, I. G.; Allaoui, A.; Jones, N. L.; Nuñez, G.; Girardin, S. E.; Philpott, D. J. *Nat. Immunol.* **2010**, *11*, 55.
17. Shaw, P. J.; Barr, M. J.; Lukens, J. R.; McGargill, M. A.; Chi, H.; Mak, T. W.; Kanneganti, T. D. *Immunity* **2011**, *34*, 75.
18. Nishio, H.; Kanno, S.; Onoyama, S.; Ikeda, K.; Tanaka, T.; Kusuhara, K.; Fujimoto, Y.; Fukase, K.; Sueishi, K.; Hara, T. *Arterioscler. Thromb. Vasc. Biol.* **2011**, *31*, 1093.
19. Rosenzweig, H. L.; Galster, K. T.; Planck, S. R.; Rosenbaum, J. T. *Invest. Ophthalmol. Visual Sci.* **2009**, *50*, 1746.
20. Cardenas, I.; Mulla, M. J.; Myrtolli, L.; Sfakianaki, A. K.; Norwitz, E. R.; Tadesse, S.; Guller, S.; Abrahamas, V. M. *J. Immunol.* **2011**, *187*, 980.
21. Schertzer, J. D.; Tamrakar, A. K.; Magalhaes, J. G.; Pereira, S.; Bilan, P. J.; Fullerton, M. D.; Liu, Z.; Steinberg, G. R.; Giacca, A.; Philpott, D. J.; Klip, A. *Diabetes* **2011**, *60*, 2206.
22. Zhao, L.; Hu, P.; Zhou, Y.; Purohit, J.; Hwang, D. *Am. J. Physiol. Endocrinol. Metab.* **2011**, *301*, E587.
23. Jiang, H.; Najmeh, S.; Berube, J.; Leone, A.; Savage, P.; Giannias, B.; Bourdeau, F.; Rousseau, S.; Park, M.; Ferri, L. E. *Cancer Res.* **2015**, *75*, 3162.
24. Ogura, Y.; Inohara, N.; Benito, A.; Chen, F. F.; Yamaoka, S.; Nuñez, G. *J. Biol. Chem.* **2001**, *276*, 4812.
25. Inohara, N.; Koseki, T.; del Peso, L.; Hu, Y.; Yee, C.; Chen, S.; Carrio, R.; Merino, J.; Liu, D.; Ni, J.; Núñez, G. *J. Biol. Chem.* **1999**, *274*, 14560.
26. Agnihotri, G.; Ukani, R.; Malladi, S. S.; Warshakoon, H. J.; Balakrishna, R.; Wang, X.; David, S. A. *J. Med. Chem.* **2011**, *54*, 1490.
27. Jakopin, Ž.; Gobec, M.; Kodela, J.; Hazdovac, T.; Mlinarič-Raščan, I.; Sollner, M. *Eur. J. Med. Chem.* **2013**, *69*, 232.
28. Magnuson, G.; Khan, P.; Yuan, H.; Brown, B.; Divlianska, D. B.; Stonich, D.; Peddibhotla, S.; Su, Y.; Dad, S.; Sergienko, E.; Chung, T. D. Y.; Roth, G. P.; Wimer, C.; Diaz, P.; Correa, R. G.; Reed, J. C. *High Throughput Screening Assays for NOD1 Inhibitors—Probe 1*; Probe Reports from the NIH Molecular Libraries Program: Bethesda (MD), 2010.
29. Magnuson, G.; Khan, P.; Yuan, H.; Brown, B.; Divlianska, D. B.; Stonich, D.; Peddibhotla, S.; Su, Y.; Dad, S.; Sergienko, E.; Chung, T. D. Y.; Roth, G. P.; Wimer, C.; Diaz, P.; Correa, R. G.; Reed, J. C. *High Throughput Screening Assays for NOD1 Inhibitors—Probe 2*; Probe Reports from the NIH Molecular Libraries Program: Bethesda (MD), 2010.
30. Rickard, D. J.; Sehon, C. A.; Kasparcova, V.; Kallal, L. A.; Haile, P. A.; Zeng, X.; Montoute, M. N.; Poore, D. D.; Li, H.; Wu, Z.; Eidam, P. M.; Emery, J. G.; Marquis, R. W.; Gough, P. J.; Bertin, J. *PLoS One* **2014**, *9*, e96737.
31. Khan, P. M.; Correa, R. G.; Divlianska, D. B.; Peddibhotla, S.; Sessions, E. H.; Magnuson, G.; Brown, B.; Suyama, E.; Yuan, H.; Mangravita-Novo, A.; Vicchiarelli, M.; Su, Y.; Vasile, S.; Smith, L. H.; Diaz, P. W.; Reed, J. C.; Roth, G. P. *ACS Med. Chem. Lett.* **2011**, *2*, 780.
32. Correa, R. G.; Khan, P. M.; Askari, N.; Zhai, D.; Gerlic, M.; Brown, B.; Magnuson, G.; Spreafico, R.; Albani, S.; Sergienko, E.; Diaz, P. W.; Roth, G. P.; Reed, J. C. *Chem. Biol.* **2011**, *18*, 825.
33. Jakopin, Ž.; Corsini, E.; Gobec, M.; Mlinarič-Raščan, I.; Sollner, M. *Eur. J. Med. Chem.* **2011**, *46*, 3762.
34. Jakopin, Ž.; Gobec, M.; Mlinarič-Raščan, I.; Sollner, M. *J. Med. Chem.* **2012**, *55*, 6478.
35. Jakopin, Ž. *Tetrahedron Lett.* **2015**, *56*, 504.
36. Jakopin, Ž. *Curr. Med. Chem.* **2013**, *20*, 2068.
37. Gobec, M.; Mlinarič-Raščan, I.; Sollner, M. *Eur. J. Med. Chem.* **2016**, *116*, 1.
38. Langdon, S. R.; Ertl, P.; Brown, N. *Mol. Inf.* **2010**, *29*, 366.
39. de Sa Alvers, F. R.; Barreiro, E. J. *Mini Rev. Med. Chem.* **2009**, *9*, 782.
40. Beaulieu, P. L.; Gillard, J.; Bykowski, D.; Brochu, C.; Dansereau, N.; Duceppe, J. S.; Hache, B.; Jakalian, A.; Lagace, L.; LaPlante, S.; McMercher, G.; Moreau, E.; Perreault, S.; Stammers, T.; Thauvette, L.; Warrington, J.; Kukolj, G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4987.
41. Silvestri, R.; De Martino, G.; La Regina, G.; Artico, M.; Massa, S.; Vargiu, L.; Mura, M.; Loi, A. G.; Marceddu, T.; La Colla, P. *J. Med. Chem.* **2003**, *46*, 2482.
42. Lebel, H.; Leogane, O. *Org. Lett.* **2005**, *7*, 4107.
43. Katritzky, A. R.; Widyan, K.; Kirichenko, K. *J. Org. Chem.* **2007**, *72*, 5802.
44. Jia, G.; Lown, J. W. *Bioorg. Med. Chem.* **2000**, *8*, 1607.
45. Murakami, Y.; Watanabe, T.; Sakai, M.; Yokoyama, Y. *Chem. Pharm. Bull.* **1988**, *36*, 3732.
46. Fink, D. M. *Synlett* **2004**, 2394.
47. Wallace, D. J.; Chen, C. *Tetrahedron Lett.* **2002**, *43*, 6987.
48. Kettle, J. G.; Faull, A. W.; Barker, A. J.; Davies, D. H.; Stone, M. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 405.
49. Pozdnev, V. F. *Tetrahedron Lett.* **1995**, *36*, 7115.
50. Golubeva, G. A.; Portnov, Y. N.; Kost, A. N. *Chem. Heterocycl. Compd.* **1973**, *9*, 471.
51. Nakagawa, M.; Yamaguchi, H.; Hino, T. *Tetrahedron Lett.* **1970**, *11*, 4035.
52. Mangette, J. E.; Chen, X.; Krishnamoorthy, R.; Vellekoop, A. S.; Csakai, A. J.; Camara, F.; Paquette, W. D.; Wang, H.-J.; Takahashi, H.; Fleck, R.; Roth, G. P. *Tetrahedron Lett.* **2011**, *52*, 1292.
53. Hino, T.; Nakagawa, M.; Hashizume, T.; Yamaji, N.; Miwa, Y. *Tetrahedron Lett.* **1970**, *11*, 2205.
54. Kost, A. N.; Portnov, Y. N.; Golubeva, G. A.; Popova, A. G.; Mushket, B. *Chem. Heterocycl. Compd.* **1980**, *16*, 917.
55. Sayyad, M.; Nanaji, Y.; Ghorai, M. K. *J. Org. Chem.* **2015**, *80*, 12659.
56. Takeda, T.; Harada, S.; Nishida, A. *Org. Lett.* **2015**, *17*, 5184.
57. Sindac, J. A.; Barraza, S. J.; Dobry, C. J.; Xiang, J.; Blakely, P. K.; Irani, D. N.; Keep, R. F.; Miller, D. J.; Larsen, S. D. *J. Med. Chem.* **2013**, *56*, 9222.
58. Breteche, A.; Duflos, M.; Dassonville, A.; Nourrisson, M.-R.; Brelet, J.; Le Baut, G.; Grimaud, N.; Petit, J.-Y. *J. Enzyme Inhib. Med. Chem.* **2002**, *17*, 415.
59. Hopkins, C. R.; O'Neil, S. V.; Laufersweiler, M. C.; Wang, Y.; Pokross, M.; Mekel, M.; Evdokimov, A.; Walter, R.; Kontoyianni, M.; Petrey, M. E.; Sabatakos, G.; Roesgen, J. T.; Richardson, E.; Demuth, T. P., Jr. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5659.
60. Elliott, J. D.; Leber, J. D.; Thompson, S. K.; Halbert, S. M., PCT Int. Appl. 1996, WO 9618393 A1 19960620.
61. Ragno, R.; Coluccia, A.; La Regina, G.; De Martino, G.; Piscitelli, F.; Lavecchia, A.; Novellino, E.; Bergamini, A.; Ciaprini, C.; Sinistro, A.; Maga, G.; Crespan, E.; Artico, M.; Silvestri, R. *J. Med. Chem.* **2006**, *49*, 3172.