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Synthesis, Structure-Activity Relationship and Antiviral Activity of Indole-Containing Inhibitors of Flavivirus NS2B-NS3 protease

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Abstract

Zika virus belongs to the Flavivirus family of RNA viruses, which include other important human pathogens such as dengue and West Nile virus. There are no approved antiviral drugs for these viruses. The highly conserved NS2B-NS3 protease of Flavivirus is essential for the replication of these viruses and it is therefore a drug target. Compound screen followed by medicinal chemistry optimization yielded a novel series of 2,6-disubstituted indole compounds that are potent inhibitors of Zika virus protease (ZVpro) with IC_{50} values as low as 320 nM. The structure-activity relationships of these and related compounds are discussed. Enzyme kinetics studies show the inhibitor **66** most likely exhibited a non-competitive mode of inhibition. In addition, this series of ZVpro inhibitors also inhibit the NS2B-NS3 protease of dengue and West Nile virus with reduced potencies. The most potent compounds **66** and **67** strongly inhibited Zika virus replication in cells with EC_{68} values of 1–3 μM. These compounds are novel pharmacological leads for further drug development targeting Zika virus.

Graphical Abstract

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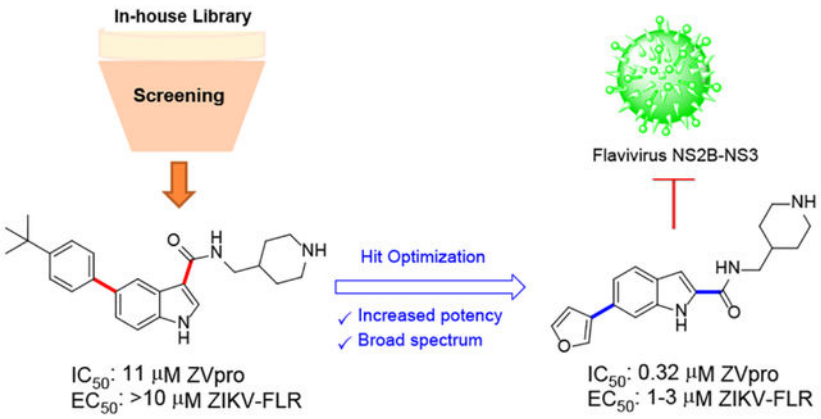
Competing interests

The authors declare no competing interests.



Keywords

Zika virus; Flavivirus; NS2B-NS3 protease; structure-activity relationship; antiviral

INTRODUCTION

Zika virus (ZIKV) belongs to the genus *Flavivirus* of the virus family *Flaviviridae*, which includes other important human pathogenic viruses such as yellow fever, dengue (DENV), West Nile virus (WNV)[1]. These viruses are transmitted among humans and other animal hosts by the bite of mosquitoes or another insect (such as ticks). ZIKV was first isolated in 1947 in the Zika Forest of Uganda in Africa[2]. ZIKV was found to cause only sporadic infection in humans in Africa and Asia[3, 4]. However, there have been three major ZIKV outbreaks in the Yap Island in the west Pacific Ocean in 2007 with several thousand cases[5, 6], in the French Polynesia in the central Pacific Ocean in 2013 with ~28,000 cases[78–10], and in Brazil in 2015–2016. The last one occurred in a much larger scale and quickly spread across Latin Americas and the southern United States with millions people infected[1112, 13]. ZIKV infection causes mild, self-healing symptoms in human, including fever, rashes and conjunctivitis (red eyes). However, it causes a 20-fold increase in the incidence of more serious neurological diseases, including Guillain-Barré syndrome[10, 14, 15] and microcephaly (small brain/head) in newborns[16, 1718]. More than 4,000 cases of microcephaly with suspected ZIKV involvement were reported in Brazil. Because the prognosis of these infants to have normal brain functions is low and their life expectancy is significantly shorter, this will cause huge negative impacts to these families and the society.

DENV is also a major human pathogen. It infects as many as 400 million people every year, with ~100 million showing symptoms including fever, headache, rash, conjunctivitis and pain in muscle and joints[19]. However, ~500,000 cases/year develop serious and possibly life-threatening Dengue hemorrhagic fever (DHF) or Dengue shock syndrome (DSS) with symptoms including bleeding, severe vomiting with blood, black stools and drowsiness. ~22,000 people (mostly children) die of DENV per year.

ZIKV and DENV contain a single stranded, positive-sense RNA with ~11,000 nucleotides, which encodes a viral polyprotein of ~3,450 amino acids in length[20, 21]. The polyprotein is cleaved by the viral NS2B-NS3 protease and several human proteases to produce functional proteins, including, from N- to C-terminus, structural proteins C, prM, E, non-structural NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. All of these proteins are necessary for the life cycle of the viruses. Given its essential role, the NS2B-NS3 proteases of ZIKV and DENV (ZVpro and DVpro) are promising drug targets[20]. A number of peptidomimetic compounds have been found to be potent inhibitors of ZVpro[22–25] and DVpro[20]. However, they did not show potent cellular and in vivo antiviral activity, presumably due to poor cell permeability or metabolic stability. Non-peptidic compounds were also found to be inhibitors[20, 26–31], but many of these compounds are relatively weak and their mode of inhibition as well as interaction with the protease is unknown. We recently reported a novel series of tri-substituted pyrazine-containing inhibitors of the proteases of ZIKV, DENV and WNV with IC_{50} values as low as 200 nM[32, 33]. These compounds also exhibited strong antiviral activities in cells and in a mouse model of ZIKV infection. Here, we report inhibitor discovery, chemical synthesis, structure-activity relationship (SAR) and other biochemical and cellular studies of a new series of 2,6-disubstituted indole-containing inhibitors (compounds **1**-**73**) of the proteases of Zika, dengue and West Nile virus.

RESULTS AND DISCUSSION

Chemical synthesis.

The general methods for synthesis of these indole compounds are shown in Scheme 1A–E. Methyl indole-3-carboxylate **74** was brominated to give compound **75**, which was hydrolyzed and subjected to an amide-formation reaction to produce the indole intermediate **76**. The target compounds **1** and **67** were obtained by a Suzuki coupling reaction and deprotection of the *tert*-butyloxycarbonyl (Boc) group (Scheme 1A). Next, treatment of 5- or 6-bromoindole **77** with trifluoroacetic anhydride (TFAA) gave compound **78**, which was hydrolyzed to give the corresponding indole-3-carboxylic acid. Amide formation reaction with a 4-amino-1-Boc-piperidine, or 4-aminomethyl-1-Boc-piperidine afforded 5- or 6-bromoindole-3-carboxamide **79**, which underwent a Suzuki coupling reaction followed by deprotection to produce compounds **2**, **7** and **14**-**32** (Scheme 1B). Compound **81** was prepared by a nucleophilic substitution reaction of compound **80** with Boc-protected 4-(3-bromopropyl)piperidine. Similar transformations of **81** yielded compound **3** (Scheme 1C). A Suzuki coupling reaction between 5-iodoindole and 4-*tert*-butylphenylboronic acid gave compound **83**, which was reacted with 4-bromobenzyl bromide to afford **84**. The target products **4** and **5** were obtained through a Suzuki coupling reaction of compound **84** (Scheme 1D). 5- or 6-bromoindole-carboxylic acid, obtained from its ester **85**, was reacted with a 4-amino-1-Boc-piperidine, or 4-aminomethyl-1-Boc-piperidine to give indole-2-carboxamide **86**, which underwent similar transformations to give compounds **6**, **8** and **33**-**66** (Scheme 1E).

For synthesis of the pyrrolo[2,3-*b*]pyridine compounds **9** and **70**-**73** (Scheme 2A), a Friedel-Crafts acylation between 6-bromo-1H-pyrrolo[2,3-*b*]pyridine **87** and trichloroacetyl chloride gave compound **88** with a 3-trichloroacetyl group, which was hydrolyzed and subjected

to an amide-forming and a Suzuki coupling reaction to afford, upon deprotection of the Boc group, the target compounds. 4-Bromobenzene-1,2-diamine **90** was subjected to a Suzuki coupling followed by a cyclization reaction with methyl 2,2,2-trichloroacetimidate to give benzimidazole **91** (Scheme 2B), whose trichloromethyl group was converted the corresponding carboxylic acid by hydrolysis. An amide-formation reaction followed by deprotection of Boc produced compound **10**. With a similar route to synthesize compound **1**, the benzofuran and benzothiophene analogs **11**-**13**, **68**, and **69** were prepared (Scheme 2C).

Inhibitor discovery and SAR studies.

A recombinant ZVpro protein, consisting of NS2B (residues 47–95) and NS3 (residues 1–170) of ZIKV connected with a Gly_4-Ser-Gly_4 linker, was expressed and purified for compound screening[32]. A fluorescence-based biochemical assay was used and monitored with the excitation wavelength at 360 nm and emission at 460 nm, using benzoyl-norleucine-lysine-lysine-arginine 7-amino-4-methylcoumarine (Bz-Nle-Lys-Lys-Arg-AMC) as the substrate[32]. ZVpro-mediated hydrolysis of the substrate causes a significant increase of the fluorescence signal, while an inhibitor can reduce it.

Using this assay, we performed screening of our in-house compound library, which consists of ~1,200 compounds synthesized for medicinal chemistry studies of other protein targets[32]. Indole-3-carboxamide compounds **1** and **2** with *para*-*tert*-butylphenyl substituent were found to be inhibitors of ZVpro with IC_{50} values of 4.5 and 11 μM (Table 1 and Figure S1). Compound **3** with an additional 1-substituent is less active (IC_{50} = 14.6 μM) than **2**, but compounds **4** and **5** without a 3-carboxamide group are inactive. These data suggest the importance of the 3-carboxamide group for inhibition of ZVpro and a 1-substituent might be disfavored.

Analogous compounds **6**-**13** with a *tert*-butylphenyl group were synthesized for SAR studies. Compound **7** bearing a *tert*-butylphenyl group inhibited ZVpro with an IC_{50} of 5.0 μM, exhibiting an improved activity as compared to compound **2** with a 5-substituent. Moving the 3-carboxamide group to the 2-position in compound **8** significantly enhanced the inhibitory activity with an IC_{50} of 1.3 μM. Comparing the activity of compound **8** with that of **6** also indicates that the 4-*tert*-butylphenyl group at the 6-position is more favorable. Compounds **9**-**13** with a different core, i.e., pyrrolo[2,3-*b*]pyridine, benzimidazole, benzothiophene and benzofuran, exhibited comparable activities with IC_{50} values of 2.1–9.1 μM.

Indole-3-carboxamide compounds **14**-**18** were synthesized with a variety of aryl group at the 5-position, including more electron-rich or -deficient phenyl groups than the *para*-*tert*-butylphenyl in compound **2**. However, none of these compounds are active against ZVpro (Table 2).

The substituent at the 6-position of the indole-3-carboxamides was next modified (Table 3). Replacement of the *tert*-butyl group with a smaller group with a different electronic property, such as -OMe, -Me, -F and -CN in compounds **19**-**24**, is disfavored, while changing to a hydrophobic and more bulky group, such as those in compounds **25** and **26** (IC_{50} = 4.2 and 3.1 μM), retained the inhibitory activity (as compared to compound

7 with an IC_{50} of 5.0 μM). Except for compound **27** (IC_{50} = 42 μM) with an additional -Cl substituent, replacing the 6-phenyl ring in compound **7** with a 3- or 4-pyridinyl ring significantly enhanced the inhibitory activity with compounds **28**-**31** showing IC_{50} values of 0.39–1.1 μM. Varying the linker length of the 3-carboxamide substituents for compounds **29**-**31** suggested that one -CH_2- (in the most active compound **29**) between the amide and the piperidine ring is optimal. Compound **32** (IC_{50} = 5.7 μM) with a pyrazole ring at the 6-position exhibited a similar activity to compound **7**.

Next, 5-Substituted indole-2-carboxamide compounds **33**-**46** (Table 4) were synthesized for SAR studies. As compared to compound **6** (IC_{50} = 7.0 μM), a pyridine ring at the 5-position for compounds **33** and **34** considerably reduces the inhibitory activity, while a more polar pyrazole or *para*-aminomethylphenyl group at this position in compound **35** and **36** can retain the inhibitory activity. It is of interest that a furan-3-yl or thiophen-3-yl ring in compounds **37** and **38** (IC_{50} = 0.87 and 1.1 μM) increases the inhibitory potency by ~8-fold. Replacing the furan ring with another cyclic moiety in compounds **39**-**43** (IC_{50} = 3.4–33 μM) reduced the activity. Compounds **44**-**46** with a cyano-substituted phenyl ring at the 5-position exhibited moderate to low activity against ZVpro with IC_{50} values of 9–40 μM.

SAR studies based on the indole-2-carboxamide compound **8** (IC_{50} = 1.3 μM) were performed to optimize its 6-substituent. Compound **47** without a 6-substituent showed a modest activity (IC_{50} = 24.6 μM), while compound **48** with a 6-phenyl substituent exhibited a ~2.5-fold activity increase with an IC_{50} of 9.4 μM. Its activity also shows the importance of the *para*-*tert*-butyl group in **8** for ZVpro inhibition. While replacing the *para*-*tert*-butyl with -F in compound **49** caused a sharply decreased activity, that with a -OCH_3 in compound **50** (IC_{50} = 1.0 μM) slightly enhanced the activity. Compound **51** having a -OCF_3 with comparable bulkiness is ~3-fold less active than **50**. Therefore, the descending activity for -OCH_3 ~ -*t*-Bu > -OCF_3 > -F suggests the importance of an electron-rich phenyl ring at the 6-position. Compounds **52** and **54** bearing a smaller, polar amino- or hydroxy-methyl group exhibited comparable activities to **51**, while compound **53** or **55** (IC_{50} = 3.2 or 45.6 μM) with a more electron-withdrawing or longer group, respectively, is less potent. Compound **56** (IC_{50} = 1.6 μM) with a more bulky phenoxyphenyl group can retain the inhibitory activity, but compounds **57** and **58** are 4–6-fold less active (than compound **8**), presumably due to more rigid bicyclic rings. Compounds **59**-**66** with several heterocyclic rings were investigated. Pyridine and two other 6-membered rings in compounds **59**-**63** with IC_{50} values of 2.2–27 μM are disfavored. 5-membered pyrazine- and furan-containing compounds **64** and **66** (IC_{50} = 0.99 and 0.32 μM, respectively) were found to possess enhanced inhibitory activities against ZVpro. Thiophene-containing compound **65** (IC_{50} = 6.4 μM) is significantly less active.

With the finding of potent ZVpro inhibitors such as compound **66**, SARs for related compounds **67**-**73** are discussed. Indole-3-carboxamide compound **67** with two furan-3-yl groups at both 5- and 6-positions showed a comparable inhibitory activity with an IC_{50} of 0.37 μM. Analogous benzofuran and pyrrolo[2,3-b]pyridine compounds **68**−**73** showed

similar SARs as their corresponding indole compounds (in Table 3), showing the core changes do not significantly affect the inhibitory activity.

Activity against dengue and West Nile virus proteases.

DENV and West Nile virus (WNV) also belong to the Flavivirus family and are important human pathogens. Given the high homology between ZVpro and DENV or WNV proteases[32], it is of interest to find whether the identified ZVpro inhibitors inhibit these two enzymes. Recombinant NS2B-NS3 proteases from DENV serotype-2 (DV2pro) and WNV (WVpro) were expressed and purified and similar fluorescence-based biochemical assays developed using our previous methods[32]. 16 selected ZVpro inhibitors with a wide range of activity against ZVpro were tested for their inhibitory activity against DV2pro and WVpro. As summarized in Table 7, indole-carboxamide based ZVpro inhibitors also inhibit activity of DV2pro and WVpro, but they are general 5->10-fold less potent, particularly for highly potent ZVpro inhibitors such as compounds **66** and **67**.

Enzyme kinetics.

Steady-state enzyme kinetic studies for the most potent compound **66** were performed to find its mode of inhibition against ZVpro and DV2pro. Initial velocities were determined in the presence of increasing concentrations of the inhibitor and substrate. IC_{50} values of compound **66** were then calculated and plotted against the substrate concentrations. As shown in Figure 1A, the IC_{50} values against DV2pro were found to be almost unchanged when the substrate concentration was increased from 0.5 to 50 μM (~0.05–5×K_m) and do not linearly increase according to the Cheng-Prusoff equation ($IC_{50} = K_i + K_i/K_m \times [S]$). These results show compound **66** is not a competitive inhibitor and more likely adopts a non-competitive mode of inhibition. Similarly, the IC_{50} values of **66** against ZVpro remain almost unchanged when the substrate concentration was increased from 0.5 to 10 μM (~0.03–0.7×K_m) (Figure 1B). It is noted that the IC_{50} values for the highest substrate concentration are slightly higher for both ZVpro and DV2pro, presumably because of the very high initial velocity of the enzyme catalyzed reaction in these conditions. The relatively slow reading speed of our microplate reader could miss the initial velocity and cause larger errors. Another possibility is that the inhibitor might adopt a mixed mode of inhibition at the high substrate concentrations. The results for ZVpro do not exclude a competitive mode of action. However, given the very high similarity between ZVpro and DV2pro in both sequence and structure[32], it is postulated that compound **66** inhibits ZVpro with the same mode of action.

Anti-ZIKV activity.

Anti-ZIKV activity was evaluated in human U87 glioma cells and monkey Vero cells. ZIKV replicates rapidly in these two cells, but does not cause cytopathic effects (CPE) in U87 cells[34]. However, ZIKV causes significant CPE and cell lysis (in ~5 days post-infection) in Vero cells which lack interferon-mediated defense[35]. This feature can be conveniently used to detect ZIKV in the cells. The passage-3 stock of the ZIKV FLR strain, which was isolated from the serum of a patient infected in Colombia in 2015[36], was used for the assay. 0.01 multiplicity of infection (MOI), as defined by the number of infectious viral particles

per cell, of ZIKV was added to a monolayer of cells. Upon virus attachment, cells were washed and incubated with increasing concentrations of a ZVpro inhibitor for 2 days. The supernatant containing newly generated viruses was subjected to an end-point dilution assay to determine the viral titer, which can be used to evaluate the anti-ZIKV activity of the compound[32].

Selected ZVpro inhibitors were first tested for their cytotoxicity against U87 cells (using MTT assay) and non-toxic compounds further evaluated for their antiviral activity. As shown in Table 8, all of these compounds did not inhibit growth of U87 cells at 10 μM. The most potent ZVpro inhibitor **66** was able to inhibit ZIKV replication in U87 cells by 68% (i.e., half-log reduction) at 1 μM in two independent experiments. Compound **67** with a comparable enzyme activity exhibited a reduced anti-ZIKV activity with an EC_{68} of 3 μM. In addition, as compared to compound **66**, compounds **72**, **30** and **31** with slightly to moderately reduced activity against ZVpro (IC_{50} = 0.45–1 μM) showed considerably reduced anti-ZIKV activity (EC_{68} = 10 μM). Compounds **73** and **64** did not inhibit ZIKV replication by 68% at 10 μM. These variable antiviral activities might be due to different cellular permeability of these compounds. Compound **56** with an enzyme IC_{50} of 1.6 μM exhibited a strong anti-ZIKV activity with an EC_{68} of 2.5 μM, which might be due to improved cellular uptake or off-target effects. Less potent inhibitors **26**, **54**, **51** and **25** (IC_{50} = 3.1–4.2 μM) did not show antiviral activity at 10 μM.

The most potent compound **66** also showed dose-dependent anti-ZIKV activity. It was able to inhibit ZIKV (FLR strain) replication in U87 cells by 68%, 90% and 99% (0.5, 1 and 2 log reduction) at the concentration of 1, 3 and 10 μM, respectively, in two independent experiments. These results suggest the potent ZVpro inhibitor **66** is a promising antiviral agent against ZIKV infection.

CONCLUSION

Zika, dengue and other Flavivirus species are important human pathogens. However, except for mosquito controls, there have been no effective antiviral drugs. There is therefore a pressing need to discover and develop novel small-molecule compounds targeting essential Flavivirus proteins such as the viral protease. Compound screening found di-substituted indole compounds **1** and **2** are a novel series of inhibitor of ZVpro. Iterative SAR and medicinal chemistry optimization were performed and found 2,6-disubstituted indole compounds, such as **66**, are potent ZVpro inhibitors with IC_{50} values as low as 320 nM. Changing to another aromatic core did not yield a better inhibitor. Activity optimization for the R3 or R5 group did not give more potent compounds. These ZVpro inhibitors also inhibit activity of homologous proteases of dengue and West Nile virus, but with considerably reduced potencies. The most potent inhibitor **66** exhibited strong antiviral activity against ZIKV with an EC_{68} value of 1 μM, showing it represents a novel lead for further developing antiviral drugs against Zika and other Flavivirus infections.

Experimental Section

All chemicals for synthesis were purchased from Alfa Aesar (Ward Hill, MA) or Aldrich (Milwaukee, WI). Unless otherwise stated, all solvents and reagents used as received. All reactions were performed using a Teflon-coated magnetic stir bar at the indicated temperature and were conducted under an inert atmosphere when stated. The identity of the synthesized compounds was characterized by ^{1}H and ^{13}C NMR on a Varian (Palo Alto, CA) 400-MR spectrometer and mass spectrometer (Shimadzu LCMS-2020). Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). The identity of the potent inhibitors was confirmed with high resolution mass spectra (HRMS) using an Agilent 6550 iFunnel quadrupole-time-of-flight (Q-TOF) mass spectrometer with electrospray ionization (ESI). The purities of the final compounds were determined to be >95% with a Shimadzu Prominence HPLC using a Zorbax C18 (or C8) column (4.6 × 250 mm) monitored by UV at 254 nm.

Chemical synthesis.

1. General procedure for the synthesis of compounds 1 and 67.—To a suspension of methyl 1*H*-indole-3-carboxylate (**74**) (4.0 g, 22.8 mmol) in acetic acid (30 mL) was added dropwise liquid Bromine (2.57 mL, 50.2 mmol) under an atmosphere of argon. The reaction stirred in dark for 3 days. The suspension was filtered, washed with hot ethanol, and dried to afford **75** (5 g, 66 %) as a grey solid. Methyl 5,6-dibromo-1H-indole-3-carboxylate (**75**).

To a solution of methyl 5,6-dibromo-1H-indole-3-carboxylate **75** (4.0 mmol) in MeOH/H_2O (3/1,v/v, 16 mL) was added slowly sodium hydroxide solution (20.0 mmol). The resulting mixture was heated at 50 °C for 5 h before it was cooled to room temperature. The solvent was removed in vacuo, and the white slurry was diluted with H_2O and acidified by HCl (3 N). It was filtered, washed with H_2O, and dried to give acid as a white solid. The crude product was used for the next step without further purification.

To a solution of the crude carboxylic acid (0.4 g, 1.25 mmol) and 4-aminomethyl-1-Boc-piperidine (1.5 mmol), or 4-amino-1-Boc-piperidine (1.5 mmol) in DMF (20 mL) was added N, N-diisopropylethylamine (0.33 mL, 1.88 mmol) and HATU (0.572 g, 1.5 mmol). The mixture was stirred for 12 h before it was quenched with H_2O. The mixture was extracted with ethyl acetate (3 × 30 mL) and the combined organic layers were washed with water and brine and dried over Na_2SO_4. The solvent was removed in vacuo to afford a crude oil, which was purified by column chromatography (silica gel, hexanes: ethyl acetate from 2:1 to 1:1) to afford **76a or 76b** as a withe solid.

Tert-butyl 4-((5,6-dibromo-1H-indole-3-carboxamido)methyl)piperidine-1-carboxylate (**76b**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.17 (s, 1H), 8.02 (t, J = 34.8 Hz, 3H), 7.65 (s, 1H), 7.51 (s, 1H), 7.04 (s, 1H), 4.11 – 4.06 (m, 2H), 3.33 (s, 2H), 2.68 (s, 2H), 1.88 – 1.65 (m, 3H), 1.42 (s, 9H), 1.22 – 1.10 (m, 2H).

Compound **76a** or **76b** (0.1 mmol), aryl boronic acid (0.12 mmol), tetrakis(triphenylphosphine)palladium (5.8 mg, 0.005 mmol), and sodium carbonate (21 mg, 0.2 mmol) in *p*-dioxane/H_2O (5/1, v/v, 6.0 mL) were placed in a sealed tube. The mixture was degassed and heated to 90 °C for 16 h. The reaction was then cooled and quenched with brine (10 mL). The product was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with water and brine and dried over Na_2SO_4. The solvent was removed *in vacuo* to give a crude oil, which was purified by column chromatography (silica gel, hexanes: ethyl acetate from 1:1 to 1:2) to afford the corresponding coupling product.

To a solution of this intermediate (0.08 mmol) in DCM (2 mL) was added dropwise HCl (0.2 mL, 4 N in *p*-dioxane) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 4 h. The solvent was removed *in vacuo* to afford the final product **1** or **67** as a hydrochloric salt.

5,6-Bis(4-(tert-butyl)phenyl)-N-(piperidin-4-yl)-1H-indole-3-carboxamide Hydrochloride (**1**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 8.64 (brs, 2H), 8.14 (d, *J* = 2.8 Hz, 1H), 8.07 (s, 1H), 7.94 (d, *J* = 7.6 Hz, 1H), 7.36 (s, 1H), 7.20 (d, *J* = 8.4 Hz, 4H), 7.01 (t, *J* = 8.4 Hz, 4H), 4.02 (s, 1H), 3.21 (s, 2H), 2.99 (t, *J* = 11.2 Hz, 2H), 1.97 (d, *J* = 13.6 Hz, 2H), 1.71 (dd, *J* = 23.2, 11.6 Hz, 2H), 1.26 – 1.15 (m, 18H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0, 148.2, 148.0, 139.7, 139.3, 135.6, 134.5, 133.2, 129.45, 129.38, 129.0, 125.6, 124.45, 124.36, 122.8, 110.4, 92.4, 43.5, 42.4, 34.1, 31.2, 31.1, 28.6. MS (ESI) $[M+H]^+$ 508.3.

5,6-Di(furan-3-yl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride (**67).** ^{1}H NMR (400 MHz, D_2O) δ 8.38 (s, 1H), 7.68 (s, 2H), 7.31 (s, 2H), 7.20 (s, 1H), 7.17 (s, 2H), 6.15 (s, 2H), 3.35 (d, *J* = 11.6 Hz, 2H), 3.18 (s, 2H), 2.85 (t, *J* = 11.6 Hz, 2H), 1.91 – 1.79 (m, 3H), 1.36 (dd, *J* = 22.8, 11.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 142.8, 142.6, 139.7, 139.6, 135.7, 128.8, 126.5, 126.1, 125.6, 125.3, 123.9, 122.4, 112.6, 112.2, 112.0, 110.5, 43.2, 42.9, 34.0, 26.4. MS (ESI) $[M+H]^+$ 390.2.

2. General procedure for the synthesis of compound 2, 7, and 14–32.—To a solution of 5- or 6-halogen substituted indole **77** (3 mmol) in DMF (10 mL) was added TFAA (626 μL, 4.5 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 2 h. Then the reaction was quenched with H_2O. A lot of yellow solid precipitated from the solvent. It was filtered to get the intermediate **78**, which was used directly for the next step.

The intermediate **78** was added to 20% NaOH solution. The resulting mixture was stirred at 50 °C for 2 days. After the starting material was consumed, the mixture was quenched with 1 N HCl solution. White solid precipitated from the solvent. It was filtered to give the corresponding carboxylic acid which was used for the next step without purification. The preparation of the final compounds **2, 7,** and **14**–**32** followed the same procedure as described for compound **1**.

5-(4-(tert-butyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide **(2).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 – 11.63 (m, 1H), 8.84 (d, *J* = 12.0 Hz, 1H), 8.54 (d, *J* = 12.0 Hz, 1H), 8.35 (s, 1H), 8.10–8.01 (m, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.51 – 7.30 (m,

4H), 3.23 (d, *J* = 12.4 Hz, 2H), 3.15 (t, *J* = 6.0 Hz, 2H), 2.80 (q, *J* = 12.0 Hz, 2H), 1.80 (d, *J* = 12.0 Hz, 3H), 1.36 (d, *J* = 12.4 Hz, 2H), 1.29 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 149.2, 139.3, 136.0, 133.1, 128.9, 127.1, 126.8, 126.0, 121.6, 119.2, 112.6, 111.2, 43.6, 43.3, 34.6, 34.4, 31.6, 26.8. MS (ESI) $[M+H]^+$ 390.3.

6-(4-(tert-butyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride. **(7).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (s, 1H), 8.59 (s, 1H), 8.28 (s, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 8.01 (dd, *J* = 7.6, 4.4 Hz, 2H), 7.64 – 7.53 (m, 3H), 7.52 – 7.42 (m, 2H), 7.37 (dd, *J* = 8.4, 1.6 Hz, 1H), 3.25 (d, *J* = 12.0 Hz, 2H), 3.16 (t, *J* = 6.0 Hz, 2H), 2.83 (d, *J* = 12.0 Hz, 2H), 1.81 (d, *J* = 13.2 Hz, 3H), 1.35–1.29 (m, 11H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 149.6, 138.7, 137.2, 134.6, 128.7, 126.8, 126.1, 125.8, 121.7, 120.1, 110.9, 109.9, 43.6, 43.4, 34.6, 34.4, 31.6, 26.8. MS (ESI) $[M+H]^+$ 390.3.

5-(4-cyanophenyl)-N-(piperidin-4-yl)-1H-indole-3-carboxamide Hydrochloride **(14).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H), 8.94 (s, 2H), 8.47 (s, 1H), 8.21 (d, *J* = 2.0 Hz, 1H), 8.07 (d, *J* = 7.2 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.52 (q, *J* = 8.0 Hz, 2H), 4.06 (s, 1H), 3.30 (d, *J* = 12.4 Hz, 2H), 2.99 (s, 2H), 1.99 (d, *J* = 12.0 Hz, 2H), 1.77 (dd, *J* = 24.0, 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 146.8, 136.7, 133.2, 131.3, 129.7, 127.9, 127.4, 121.7, 120.3, 119.5, 113.1, 111.2, 109.3, 44.1, 42.6, 28.9. MS (ESI) $[M+H]^+$ 345.4.

N-(piperidin-4-yl)-5-(3,4,5-trimethoxyphenyl)-1H-indole-3-carboxamide Hydrochloride **(15).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H), 8.87 (s, 2H), 8.34 (s, 1H), 8.16 (d, *J* = 4.0 Hz, 1H), 8.00 (d, *J* = 7.2 Hz, 1H), 7.53 – 7.30 (m, 2H), 6.85 (s, 2H), 4.05 (s, 1H), 3.84 (s, 6H), 3.68 (s, 3H), 3.30 (d, *J* = 12.0 Hz, 2H), 2.98 (d, *J* = 8.0 Hz, 2H), 1.98 (d, *J* = 8.0 Hz, 2H), 1.76 (dd, *J* = 22.0, 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.6, 153.5, 138.2, 136.9, 136.0, 133.6, 129.3, 127.1, 121.9, 119.5, 112.5, 110.9, 104.6, 60.5, 56.4, 44.0, 42.7, 29.0. MS (ESI) $[M+H]^+$ 410.6.

5-(4-fluorophenyl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride (**16).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 8.69 (s, 1H), 8.39 (s, 1H), 8.33 (d, *J* = 1.6 Hz, 1H), 8.07–8.04 (m, 2H), 7.65 – 7.62 (m, 2H), 7.48 (d, *J* = 12.0 Hz, 1H), 7.39 (d, *J* = 8.0, 1H), 7.26 (t, *J* = 8.0 Hz, 2H), 3.29 – 3.19 (m, 2H), 3.16 (d, *J* = 12.4 Hz, 2H), 2.81 (d, *J* = 12.0 Hz, 2H), 1.81 (d, *J* = 12.0 Hz, 3H), 1.35 (q, *J* = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 161.8 (d, *J* = 242.9 Hz), 138.7, 136.1, 132.3, 129.0, 128.9, 127.1, 121.6, 119.5, 116.0 (d, *J* = 21.1 Hz), 112.7, 111.3, 43.6, 43.4, 34.4, 26.8. MS (ESI) $[M+H]^+$ 352.1.

5-(2,4-difluorophenyl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride **(17).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.69 (s, 1H), 8.72 (s, 1H), 8.41 (s, 1H), 8.25 (d, *J* = 1.6 Hz, 1H), 8.07 (d, *J* = 4.0 Hz, 2H), 7.56 – 7.48 (m, 2H), 7.34 – 7.24 (m, 2H), 7.18–7.13 (m, 1H), 3.23 (d, *J* = 12.4 Hz, 2H), 3.14 (t, *J* = 6.0 Hz, 2H), 2.80 (q, *J* = 11.6 Hz, 2H), 1.79 (d, *J* = 12.8 Hz, 3H), 1.34 (q, *J* = 13.2, 12.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.0, 162.8 (d, *J* = 12.0 Hz), 160.4 (d, *J* = 11.7 Hz), 158.2 (d, *J* = 12.2 Hz), 136.0, 132.5 (dd, *J* = 9.7, 4.8 Hz), 128.9, 126.9, 126.8, 123.34, 121.9 (d, *J* = 2.4 Hz), 112.4, 112.4, 112.2, 111.2, 105.0, 104.8, 104.5, 55.3, 43.4, 34.4, 26.8. MS (ESI) $[M+H]^+$ 370.6.

5-(3-formylphenyl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride **(18).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.74 (s, 1H), 10.09 (s, 1H), 8.82 (d, J = 12.0 Hz, 1H), 8.53 (d, J = 12.0 Hz, 1H), 8.47 (s, 1H), 8.16 – 8.08 (m, 3H), 8.02 – 7.98 (m, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.67 (t, J = 8.0 Hz, 1H), 7.54 – 7.49(m, 2H), 3.23 (d, J = 16.0 Hz, 2H), 3.16 (t, J = 8.0 Hz, 2H), 2.85 – 2.79 (m, 2H), 1.81 (d, J = 12.0 Hz, 3H), 1.37 (d, J = 16.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.6, 164.7, 142.6, 136.9, 136.1, 132.8, 131.5, 129.9, 128.8, 127.7, 127.5, 126.9, 121.2, 119.4, 112.6, 111.0, 66.4, 55.0, 43.0, 34.1, 26.5. MS (ESI) $[M+H]^+$ 362.2.

N-(piperidin-4-ylmethyl)-6-(3,4,5-trimethoxyphenyl)-1H-indole-3-carboxamide Hydrochloride. **(19)**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.63 (d, J = 4.0 Hz, 1H), 8.92 (d, J = 12.0 Hz, 1H), 8.62 (d, J = 12.0 Hz, 1H), 8.14 (d, J = 8.4 Hz, 1H), 8.11–8.07 (m, 2H), 7.64 (d, J = 1.6 Hz, 1H), 7.40 (dd, J = 8.4, 1.6 Hz, 1H), 6.89 (s, 2H), 3.84 (s, 6H), 3.66 (s, 3H), 3.23 (d, J = 12.0 Hz, 2H), 3.14 (t, J = 6.0 Hz, 2H), 2.87–2.76 (m, 2H), 1.81 (d, J = 12.0 Hz, 3H), 1.37 (q, J = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 153.6, 137.6, 137.1, 134.9, 128.9, 126.0, 121.6, 120.3, 110.9, 110.2, 104.6, 66.8, 60.5, 56.4, 43.3, 34.4, 26.8. MS (ESI) $[M+H]^+$ 424.8.

6-(4-fluorophenyl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride **(20).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H), 8.82 (s, 1H), 8.53 (s, 1H), 8.15 (d, J = 8.4 Hz, 1H), 8.07 (d, J = 2.8 Hz, 2H), 7.68 (dd, J = 8.4, 5.6 Hz, 2H), 7.61 (s, 1H), 7.36 (d, J = 9.6 Hz, 1H), 7.25 (t, J = 8.0 Hz, 2H), 3.23 (d, J = 12.0 Hz, 2H), 3.15 (t, J = 6.0 Hz, 2H), 2.80 (d, J = 11.2 Hz, 2H), 1.81 (d, J = 11.2 Hz, 3H), 1.46 – 1.27 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.0, 161.9 (d, J = 243.2 Hz), 138.1 (d, J = 2.9 Hz), 137.1, 133.6, 129.0 (d, J = 8.0 Hz), 128.9, 125.9, 121.9, 120.1, 116.1 (d, J = 21.2 Hz), 110.9, 110.1, 109.9, 43.6, 43.4, 34.4, 26.8. MS (ESI) $[M+H]^+$ 352.6.

6-(2-fluorophenyl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride **(21).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.71 (s, 1H), 8.79 (s, 1H), 8.50 (s, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.09 (d, J = 4.0 Hz, 2H), 7.65 – 7.49 (m, 3H), 7.45 – 7.32 (m, 1H), 7.28 (dd, J = 13.6, 6.0 Hz, 2H), 3.23 (d, J = 12.0 Hz, 2H), 3.16 (t, J = 6.0 Hz, 2H), 2.90 – 2.67 (m, 2H), 1.81 (d, J = 12.0 Hz, 3H), 1.36 (q, J = 10.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.0, 159.6 (d, J = 245.1 Hz), 136.6, 132.5 (d, J = 2.7 Hz), 131.9 (d, J = 9.8 Hz), 131.4 (d, J = 3.4 Hz), 129.6 (d, J = 13.0 Hz), 129.3 (d, J = 6.3 Hz), 129.1 (d, J = 3.1 Hz), 129.1, 128.1, 126.5, 126.1, 125.3 (d, J = 3.4 Hz), 121.5, 121.0 (d, J = 70.1 Hz), 116.5 (d, J = 22.8 Hz), 112.6 (d, J = 3.6 Hz), 110.9, 110.5 (d, J = 92.3 Hz), 66.8, 55.3, 34.4, 26.8. MS (ESI) $[M+H]^+$ 352.1.

N-(piperidin-4-ylmethyl)-6-(p-tolyl)-1H-indole-3-carboxamide Hydrochloride **(22).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 8.74 (d, J = 9.6 Hz, 1H), 8.44 (d, J = 12.0 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.10 – 7.95 (m, 2H), 7.61 (s, 1H), 7.53 (d, J = 7.6 Hz, 2H), 7.36 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 7.6 Hz, 2H), 3.23 (d, J = 11.6 Hz, 2H), 3.15 (s, 2H), 2.80 (q, J = 12.0 Hz, 2H), 2.30 (s, 3H), 1.80 (d, J = 12.0 Hz, 3H), 1.35 (q, J = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.2, 138.6, 137.2, 136.4, 134.6, 129.9, 128.8, 126.9, 125.7, 121.7, 119.9, 110.9, 109.8, 66.8, 43.4, 34.4, 26.8, 21.1. MS (ESI) $[M+H]^+$ 348.9.

6-(4-(aminomethyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride **(23).**

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.77 (s, 1H), 8.98 (s, 1H), 8.69 (s, 1H), 8.47 (s, 3H), 8.20 (d, J = 8.0 Hz, 1H), 8.14 – 8.12 (m, 2H), 7.74 (d, J = 8.0 Hz, 2H), 7.68 (s, 1H), 7.57 (d, J = 8.0 Hz, 2H), 7.44 (dd, J = 8.0, 4.0 Hz, 1H), 4.06 (s, 2H), 3.26 (d, J = 12.0 Hz, 2H), 3.18 (t, J = 6.0 Hz, 2H), 2.83 (q, J = 12.0 Hz, 2H), 1.84 (d, J = 12.0 Hz, 3H), 1.41 (q, J = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.0, 141.7, 137.2, 133.9, 132.8, 129.9, 129.1, 127.3, 126.3, 121.9, 120.1, 110.9, 110.2, 43.6, 43.3, 42.4, 34.4, 26.8. MS (ESI) $[M+H]^+$ 363.2.

6-(4-cyanophenyl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride (**24).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.86 (s, 1H), 9.02 (d, J = 8.0 Hz, 1H), 8.73 (d, J = 12.0 Hz, 1H), 8.22 (d, J = 12.0 Hz, 1H), 8.20 – 8.08 (m, 2H), 7.88 (s, 3H), 7.76 (s, 1H), 7.47 (d, J = 8.4 Hz, 1H), 3.23 (d, J = 12.0 Hz, 2H), 3.16 (s, 2H), 2.80 (q, J = 10.4 Hz, 2H), 1.81 (d, J = 11.6 Hz, 3H), 1.48 – 1.24 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 146.2, 137.1, 133.2, 132.4, 129.8, 127.9, 127.1, 122.1, 120.1, 119.5, 111.0, 110.8, 109.5, 43.7, 43.3, 34.4, 26.8. MS (ESI) $[M+H]^+$ 359.5.

6-([1,1'-biphenyl]-4-yl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride **(25).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.71 (s, 1H), 8.99 (d, J = 11.0 Hz, 1H), 8.70 (d, J = 8.0 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 2.8 Hz, 2H), 7.88 (s, 1H), 7.73 (d, J = 7.2 Hz, 3H), 7.65 (d, J = 7.6 Hz, 1H), 7.62 – 7.43 (m, 5H), 7.36 (dd, J = 8.0 Hz, 6.0 Hz, 1H), 3.32 – 3.07 (m, 4H), 2.80 (q, J = 12.0 Hz, 2H), 1.81 (d, J = 11.2 Hz, 3H), 1.39 (q, J = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 142.3, 141.3, 140.8, 137.2, 134.5, 129.9, 129.4, 129.1, 127.9, 127.3, 126.4, 126.2, 125.6, 121.9, 120.3, 110.9, 110.4, 66.8, 43.3, 34.4, 26.8. MS (ESI) $[M+H]^+$ 410.6.

6-(4-(piperidin-1-ylmethyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride **(26).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 2H), 10.86 (s, 2H), 9.11 (d, J = 9.2 Hz, 1H), 8.82 (d, J = 12.0 Hz, 1H), 8.28 – 8.05 (m, 2H), 7.74–7.67 (m, 3H), 7.43 (d, J = 8.4 Hz, 1H), 4.25 – 4.19 (m, 9H), 3.28–3.15 (m, 4H), 2.92 – 2.65 (m, 3H), 1.97 – 1.53 (m, 5H), 1.51 – 1.16 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.0, 142.5, 137.2, 133.7, 132.5, 129.3, 128.5, 127.3, 126.4, 121.9, 120.1, 110.9, 110.3, 58.9, 51.9, 43.7, 43.2, 34.4, 26.8, 22.5, 21.9. MS (ESI) $[M+H]^+$ 431.3.

6-(6-chloropyridin-3-yl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride **(27).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.09 (s, 1H), 8.81 (d, J = 8.0 Hz, 2H), 8.74 (s, 1H), 8.67 (s, 1H), 8.42 – 8.24 (m, 4H), 8.12 (d, J = 11.2 Hz, 2H), 7.74 (d, J = 8.0 Hz, 1H), 4.03 (s, 1H), 3.30 (d, J = 12.0 Hz, 2H), 2.98 (d, J = 12.0 Hz, 2H), 1.98 (d, J = 11.2 Hz, 3H), 1.88 – 1.63 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.2, 156.6, 142.6, 136.9, 131.8, 129.4, 128.2, 123.6, 122.5, 120.5, 112.5, 111.0, 44.1, 42.8, 28.9. MS (ESI) $[M+H]^+$ 369.9.

N-(piperidin-4-ylmethyl)-6-(pyridin-3-yl)-1H-indole-3-carboxamide Hydrochloride **(28).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (s, 1H), 9.25 (s, 1H), 8.98 (s, 1H), 8.88 (d, J = 8.4 Hz, 1H), 8.81 (d, J = 4.0 Hz, 1H), 8.71 (d, J = 12.0 Hz, 1H), 8.27 (d, J = 8.4 Hz, 1H), 8.22 (dd, J = 12.0, 4.0 Hz, 2H), 8.08 (dd, J = 8.0, 4.0 Hz, 1H), 7.92 (s, 1H), 7.58 (d, J = 8.4 Hz, 1H),

3.22 (d, J = 12.4 Hz, 2H), 3.15 (t, J = 6.0 Hz, 2H), 2.79 (q, J = 12.0 Hz, 2H), 1.80 (d, J = 12.0 Hz, 3H), 1.38 (q, J = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.8, 143.2, 140.2, 140.1, 136.9, 130.4, 127.8, 127.7, 127.6, 122.4, 120.1, 111.5, 111.1, 43.7, 43.3, 34.4, 26.8. MS (ESI) $[M+H]^+$ 335.2.

N-(piperidin-4-ylmethyl)-6-(pyridin-4-yl)-1H-indole-3-carboxamide Hydrochloride **(29).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 1H), 8.93 (s, 1H), 8.86 (d, J = 8.0 Hz, 2H), 8.67 (s, 1H), 8.42 (d, J = 4.0 Hz, 2H), 8.36 – 8.05 (m, 4H), 7.77 (d, J = 8.0 Hz, 1H), 3.23 (d, J = 11.2 Hz, 2H), 3.17 (s, 2H), 2.80 (d, J = 12.0 Hz, 2H), 1.82 (d, J = 11.6 Hz, 3H), 1.39 (d, J = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.6, 157.2, 141.9, 136.9, 131.7, 129.5, 127.9, 123.7, 122.5, 120.4, 112.6, 111.3, 66.8, 43.28, 34.4, 26.8. MS (ESI) $[M+H]^+$ 335.7.

N-(piperidin-4-yl)-6-(pyridin-4-yl)-1H-indole-3-carboxamide Hydrochloride **(30).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 1H), 8.99 (s, 2H), 8.85 (d, J = 4.0 Hz, 2H), 8.46 – 8.34 (m, 3H), 8.31 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.10 (s, 1H), 7.75 (d, J = 8.6 Hz, 1H), 4.06 (s, 1H), 3.30 (d, J = 12.4 Hz, 2H), 2.99 (s, 2H), 1.97 (d, J = 11.6 Hz, 2H), 1.79 (q, J = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.1, 156.5, 142.6, 136.9, 131.9, 129.5, 128.1, 123.6, 122.5, 120.4, 112.5, 111.1, 44.0, 42.6, 28.9. MS (ESI) $[M+H]^+$ 321.6.

N-(2-(piperidin-4-yl)ethyl)-6-(pyridin-4-yl)-1H-indole-3-carboxamide Hydrochloride **(31).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 8.81 (s, 1H), 8.54 (s, 1H), 8.17 (d, J = 8.0 Hz, 1H), 8.08 (t, J = 5.6 Hz, 2H), 7.72 (d, J = 8.0 Hz, 2H), 7.66 (s, 1H), 7.53 (d, J = 8.0 Hz, 2H), 7.41 (d, J = 8.0 Hz, 1H), 4.03 (d, J = 5.6 Hz, 2H), 3.23 (d, J = 12.0 Hz, 2H), 3.15 (t, J = 6.0 Hz, 2H), 2.81 (q, J = 12.0 Hz, 2H), 1.81 (d, J = 12.0 Hz, 3H), 1.36 (q, J = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.4, 156.5, 142.7, 136.9, 131.4, 129.3, 128.1, 123.6, 122.5, 120.3, 112.5, 111.5, 43.6, 36.2, 36.1, 31.3, 28.7. MS (ESI) $[M+H]^+$ 349.4.

N-(piperidin-4-ylmethyl)-6-(1H-pyrazol-3-yl)-1H-indole-3-carboxamide Hydrochloride **(32).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.49 (s, 1H), 8.68 (s, 1H), 8.39 (s, 1H), 8.17 – 7.86 (m, 5H), 7.56 (s, 1H), 7.34 (d, J = 8.0 Hz, 1H), 3.24 (d, J = 12.0 Hz, 2H), 3.14 (t, J = 4.0 Hz, 2H), 2.81 (q, J = 12.0 Hz, 2H), 1.80 (d, J = 11.2 Hz, 3H), 1.39 – 1.29 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 137.1, 130.9, 128.1, 127.2, 125.0, 122.6, 121.7, 119.2, 111.1, 108.2, 43.6, 43.4, 34.4, 26.8. MS (ESI) $[M+H]^+$ 324.2.

3. Synthesis of compound 3—To a solution of **80** (483.4 mg, 1 mmol) in DMF (10 mL) were added NaH (84 mg, 2.1 mmol). The resulting mixture was stirred at room temperature for 0.5 h. Then *tert*-butyl 4-(3-bromopropyl)piperidine-1-carboxylate (336.9 mg, 1.1 mmol) was added to the mixture. The mixture was stirred for 14 h before it was quenched with NH_4Cl aq. The mixture was extracted with ethyl acetate (3 × 10 mL) and the combined organic layers were washed with water and brine and dried over Na_2SO_4. The volatiles were removed in vacuum to afford a crude oil, which was purified by column chromatography (silica gel, hexanes: ethyl acetate = 3:1) to afford a white solid **81**, which was used directly for next step.

The above intermediate **81** (70.9 mg, 0.1 mmol), (4-(*tert*-butyl)phenyl)boronic acid (21.4 mg, 0.12 mmol), tetrakis(triphenylphosphine)palladium (5.8 mg, 0.005 mmol), and sodium

carbonate (21 mg, 0.2 mmol) in Toluene/DMF/EtOH/H_2O (5/1/0.8/1 mL) were placed in a sealed tube. The mixture was degassed and heated to 80 °C for 14 h. The reaction was then cooled and quenched with brine (5 mL). The product was extracted with ethyl acetate (3 × 15 mL) and the combined organic layers were washed with water and brine and dried over Na_2SO_4. The volatiles were removed *in vacuo* to give a crude oil, which was purified by column chromatography (silica gel, hexanes: ethyl acetate from 1:1 to 1:2) to afford the corresponding coupling product.

To a solution of this intermediate in DCM (2 mL) was added dropwise HCl (0.2 mL, 4 N in *p*-dioxane) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 1 h. The volatiles were removed *in vacuo* to afford an the final product as a hydrochloric salt.

5-(4-(tert-butyl)phenyl)-1-(3-(piperidin-4-yl)propyl)-N-(piperidin-4-ylmethyl)-1H-indole-3-carboxamide Hydrochloride **(3).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (s, 2H), 8.65 (s, 2H), 8.38 (d, J = 4.0 Hz, 1H), 8.13 (s, 1H), 8.10 (t, J = 4.0 Hz, 1H), 7.62 – 7.58 (m, 3H), 7.47 (d, J = 8.0 Hz, 3H), 4.22 (t, J = 6.0 Hz, 2H), 3.54 (s, 2H), 3.27 – 3.18 (m, 6H), 2.87–2.76 (m, 4H), 1.85 – 1.73 (m, 8H), 1.41–1.19 (m, 16H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.8, 149.4, 139.1, 135.9, 133.4, 131.9, 127.4, 126.9, 126.1, 121.6, 119.5, 111.2, 66.8, 43.7, 43.5, 43.3, 34.6, 34.4, 33.0, 32.9, 31.62, 28.7, 27.1, 26.8. MS (ESI) $[M+H]^+$ 515.4.

4. General procedure for the synthesis of 4 and 5.—A mixture of 5-iodoindole **82** (500 mg, 2.06 mmol) 4-tert-Butylphenylboronic acid (403 mg, 2.26 mmol), tetrakis(triphenylphosphine)palladium (119 mg, 0.103 mmol), and sodium carbonate (435 mg, 4.10 mmol) in *p*-dioxane/H_2O (4/1, v/v, 15 mL) were placed in a sealed tube. The mixture was degassed and heated to 80 °C for 16 h. The reaction was then cooled and quenched with brine (20 mL). The product was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with water and brine and dried over Na_2SO_4. The volatiles were removed *in vacuum* to give a crude oil, which was purified by column chromatography (silica gel, hexanes: ethyl acetate from 5:1 to 2:1) to afford **83** (415 mg, 81%).

To a solution of **83** (400 mg, 1.60 mmol) in DMF (6 mL) was added 4-bromobenzyl bromide (480 mg, 1.92 mmol) and potassium carbonate (332 mg, 2.4 mmol). The resulting mixture was stirred for 16 h, quenched with H_2O, and extracted with ethyl acetate (3 × 15 mL). the combined organic layers were washed with water and brine and dried over Na_2SO_4. The volatiles were removed *in vacuum* to give a crude oil, which was purified by column chromatography (silica gel, hexanes: ethyl acetate from 8:1 to 2:1) to afford **84** (612 mg, 91%).

A mixture of **84** (1.0 mmol), furan-3-ylboronic acid or pyridin-4-ylboronic acid (1.2 mmol), tetrakis(triphenylphosphine)palladium (0.10 mmol), and sodium carbonate (2.0 mmol) in *p*-dioxane/H_2O (4/1, v/v, 15 mL) were placed in a sealed tube. The mixture was degassed and heated to 80 °C for 16 h. The reaction was then cooled and quenched with brine (20 mL). The product was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with water and brine and dried over Na_2SO_4. The volatiles were

removed *in vacuum* to give a crude oil, which was purified by column chromatography to afford **4** or **5**.

5-(4-(Tert-butyl)phenyl)-1-(4-(furan-3-yl)benzyl)-1H-indole Hydrochloride **(4).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.69 (s, 1H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.47 – 7.40 (m, 6H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 3H), 6.66 (s, 1H), 6.60 (d, *J* = 2.8 Hz, 1H), 5.34 (s, 2H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.3, 143.9, 139.7, 138.7, 136.3, 135.9, 133.2, 132.0, 129.4, 128.9, 127.5, 127.1, 126.4, 126.1, 125.7, 121.8, 119.5, 110.0, 108.9, 102.2, 50.2, 34.6, 31.6. MS (ESI) $[M+H]^+$ 406.8.

5-(4-(Tert-butyl)phenyl)-1-(4-(pyridin-4-yl)benzyl)-1H-indole Hydrochloride **(5).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 2H), 7.87 (s, 1H), 7.61 – 7.52 (m, 4H), 7.49 – 7.40 (m, 5H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.27 – 7.21 (m, 2H), 7.20 – 7.15 (m, 1H), 6.63 (d, *J* = 2.0 Hz, 1H), 5.40 (s, 2H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.3, 149.2, 147.7, 139.5, 138.6, 137.5, 135.7, 133.1, 129.2, 128.8, 127.5, 127.4, 127.0, 125.6, 121.7, 121.5, 119.4, 109.8, 102.3, 49.9, 34.4, 31.4. MS (ESI) $[M+H]^+$ 417.2.

5. General Procedure for the synthesis of compounds 6, 8, and 33−66.—To a solution of 5, or 6-bromoindole (1.8 g, 6.72 mmol) in THF/H_2O (12/4 mL) was added slowly sodium hydroxide solution (806.0 mg, 20.2 mmol). The resulting mixture was heated at 50 °C for 5 h before it was cooled to room temperature. The solvent was removed in vacuo, and the white slurry was diluted with H_2O and acidified by HCl (3 N). It was filtered, washed with H_2O, and dried to give acid as a white solid. The crude product was used for the next step without further purification.

To a solution of the crude acid (1.5 g) and 4-aminomethyl-1-Boc-piperidine (1.47 g, 6. 88 mmol), or 4-amino-1-Boc-piperidine (1.38 g, 6.89 mmol) in DMF (20 mL) was added N, N-diisopropylethylamine (1.84 mL, 12.5 mmol) and HATU (2. 85 g, 7.5 mmol). The mixture was stirred for 12 h before it was quenched with H_2O. The mixture was extracted with ethyl acetate (3 × 30 mL) and the combined organic layers were washed with water and brine and dried over Na_2SO_4. The volatiles were removed in vacuo to afford a crude oil, which was purified by column chromatography (silica gel, hexanes: ethyl acetate from 1.5:1 to 1:1) to afford **86**.

Compound **86** (0.2 mmol), aryl boronic acid or aryl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.24 mmol), tetrakis(triphenylphosphine)palladium (11.6 mg, 0.01 mmol), and sodium carbonate (42 mg, 0.4 mmol) in *p*-dioxane/H_2O (5/1, v/v, 6 mL) were placed in a sealed tube. The mixture was degassed and heated to 100 °C for 16 h. The reaction was then cooled and quenched with brine (5 mL). The product was extracted with ethyl acetate (3 × 15 mL) and the combined organic layers were washed with water and brine and dried over Na_2SO_4. The volatiles were removed *in vacuum* to give a crude oil, which was purified by column chromatography to afford the corresponding coupling product.

To a solution of this intermediate (0.15 mmol) in DCM (3 mL) was added dropwise HCl (0.2 mL, 4 N in *p*-dioxane) at 0 °C. The reaction mixture was warmed to room temperature and

stirred for 6 h. The volatiles were removed *in vacuum* to afford an oil, which was triturated in diether ether and solidified to give the final product as a hydrochloric salt.

5-(4-(Tert-butyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(6).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 9.05 (s, 1H), 8.83 – 8.69 (m, 2H), 7.84 (s, 1H), 7.58 (d, J = 8.4 Hz, 2H), 7.52 – 7.40 (m, 4H), 7.21 (s, 1H), 3.30 – 3.16 (m, 4H), 2.82 (dd, J = 21.6, 10.0 Hz, 2H), 1.83 (d, J = 12.8 Hz, 3H), 1.42 (dd, J = 22.8, 10.0 Hz, 2H), 1.31 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.2, 148.8, 138.6, 135.9, 134.0, 132.4, 126.4, 125.6, 124.1, 122.8, 119.1, 112.7, 103.2, 43.2, 42.8, 34.2, 33.8, 31.2, 26.3. MS (ESI) $[M+H]^+$ 390.2.

6-(4-(Tert-butyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(8).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (t, J = 6.0 Hz, 1H), 7.66 – 7.61 (m, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.4 Hz, 1H), 7.11 (s, 1H), 3.27 – 3.17 (m, 4H), 2.82 (t, J = 11.6 Hz, 2H), 1.81 (d, J = 11.6 Hz, 3H), 1.28 (s, 11H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.3, 149.6, 138.5, 137.1, 135.9, 132.2, 126.6, 126.5, 125.9, 122.1, 119.6, 110.0, 102.7, 43.6, 43.1, 34.4, 33.9, 31.4, 26.4; MS (ESI) $[M+H]^+$ 390.2.

N-(Piperidin-4-ylmethyl)-5-(pyridin-3-yl)-1H-indole-2-carboxamide Hydrochloride **(33).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.97 (s, 1H), 9.24 (s, 1H), 9.08 (s, 1H), 8.92 – 8.73 (m, 4H), 8.20 (s, 1H), 8.09 – 7.99 (m, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.31 (s, 1H), 3.27 – 3.21 (m, 4H), 2.83 (dd, J = 24.8, 13.1 Hz, 2H), 1.84 (d, J = 12.8 Hz, 3H), 1.43 (dd, J = 23.2, 11.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.3, 142.4, 140.5, 140.4, 140.2, 137.3, 133.7, 128.2, 127.3, 126.0, 123.0, 121.4, 113.8, 103.9, 44.0, 43.2, 34.2, 26.7. MS (ESI) $[M+H]^+$ 335.8.

N-(Piperidin-4-ylmethyl)-5-(pyridin-4-yl)-1H-indole-2-carboxamide Hydrochloride **(34).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 8.89 – 8.77 (m, 4H), 8.63 – 8.52 (m, 1H), 8.44 (s, 1H), 8.38 (d, J = 5.6 Hz, 2H), 7.87 (d, J = 8.8 Hz, 1H), 7.62 (d, J = 8.8 Hz, 1H), 7.34 (s, 1H), 3.26 – 3.23 (m, 4H), 2.84 (dd, J = 20.4, 10.4 Hz, 2H), 1.84 (d, J = 12.8 Hz, 3H), 1.40 (dd, J = 23.2, 11.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.8, 156.0, 142.4, 138.1, 133.7, 127.8, 126.0, 122.8, 122.8, 122.5, 113.5, 103.9, 43.6, 42.8, 33.8, 26.3. MS (ESI) $[M+H]^+$ 335.2.

N-(Piperidin-4-ylmethyl)-5-(1H-pyrazol-4-yl)-1H-indole-2-carboxamide Hydrochloride **(35).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.62 (s, 1H), 9.01 (d, J = 8.4 Hz, 1H), 8.80 – 8.63 (m, 2H), 8.17 (s, 2H), 7.83 (s, 1H), 7.42 (dd, J = 25.6, 8.4 Hz, 2H), 7.12 (s, 1H), 3.29 – 3.13 (m, 4H), 2.80 (dd, J = 22.4, 11.2 Hz, 2H), 1.81 (d, J = 12.0 Hz, 3H), 1.39 (dd, J = 24.4, 12.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.6, 135.8, 132.6, 130.6, 127.9, 126.0, 124.3, 123.1, 122.4, 118.0, 113.0, 103.1, 43.9, 43.2, 34.2, 26.7. MS (ESI) $[M+H]^+$ 324.4.

5-(4-(Aminomethyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(36).**

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.74 (s, 1H), 9.00 (s, 1H), 8.80 – 8.64 (m, 2H), 8.46 (s, 3H), 7.91 (s, 1H), 7.73 (d, J = 8.0 Hz, 2H), 7.56 (d, J = 8.0 Hz, 2H), 7.51 (s, 2H), 7.22 (s, 1H), 4.05 (s, 2H), 3.23 (dd, J = 16.4, 10.0 Hz, 4H), 2.88 – 2.77 (m, 2H), 1.91 – 1.74 (m, 3H),

1.41 (dd, *J* = 23.6, 11.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.1, 141.5, 136.1, 132.6, 132.1, 131.5, 129.5, 127.7, 126.8, 122.7, 119.4, 112.8, 103.2, 43.5, 42.8, 41.9, 33.8, 26.3. MS (ESI) $[M+H]^+$ 363.3.

5-(4-(Furan-3-yl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(37).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 8.87 (s, 1H), 8.71 (s, 1H), 8.58 (s, 1H), 8.23 (s, 1H), 7.92 (s, 1H), 7.76 (s, 1H), 7.73 – 7.62 (m, 4H), 7.57 – 7.47 (m, 2H), 7.21 (s, 1H), 7.01 (s, 1H), 3.26 – 3.19 (m, 4H), 2.91 – 2.78 (m, 2H), 1.84 (d, *J* = 12.0 Hz, 3H), 1.40 (dd, *J* = 24.0, 11.6 Hz, 2H). MS (ESI) $[M+H]^+$ 400.2.

N-(Piperidin-4-ylmethyl)-5-(4-(thiophen-3-yl)phenyl)-1H-indole-2-carboxamide Hydrochloride **(38).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 8.73 (s, 1H), 8.65 (s, 1H), 8.41 (s, 1H), 7.91 (s, 1H), 7.89 (s, 1H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.63 (dd, *J* = 4.8, 2.8 Hz, 1H), 7.58 (d, *J* = 4.8 Hz, 1H), 7.50 (dd, *J* = 19.2, 8.4 Hz, 2H), 7.18 (s, 1H), 3.21 (d, *J* = 7.2 Hz, 4H), 2.82 (dd, *J* = 22.0, 10.8 Hz, 2H), 1.81 (d, *J* = 10.8 Hz, 3H), 1.36 (dd, *J* = 23.2, 10.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.6, 141.6, 140.4, 136.5, 133.6, 132.8, 132.1, 128.1, 127.5, 126.9, 126.5, 123.0, 121.1, 119.5, 113.2, 110.0, 103.4, 43.9, 43.3, 34.2, 26.7. MS (ESI) $[M+H]^+$ 416.2.

5-(4-(Piperidin-1-ylmethyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(39).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.74 (s, 1H), 10.60 (s, 1H), 8.97 (d, *J* = 11.6 Hz, 1H), 8.74 (t, *J* = 5.6 Hz, 1H), 8.68 (d, *J* = 10.0 Hz, 1H), 7.94 (s, 1H), 7.76 (d, *J* = 7.6 Hz, 2H), 7.67 (d, *J* = 7.6 Hz, 2H), 7.53 (d, *J* = 4.0 Hz, 2H), 7.23 (s, 1H), 4.27 (d, *J* = 4.0 Hz, 2H), 3.36 – 3.19 (m, 8H), 2.91 – 2.76 (m, 4H), 1.87 – 1.75 (m, 6H), 1.69 (d, *J* = 11.6 Hz, 1H), 1.45 – 1.35 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.1, 142.3, 136.2, 132.6, 132.0, 131.3, 129.1, 127.8, 126.8, 122.7, 119.6, 114.9, 112.8, 103.2, 58.6, 51.5, 43.5, 42.8, 33.8, 26.3, 22.2, 21.4. MS (ESI) $[M+H]^+$ 430.3.

5-(4-(Piperidin-1-yl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(40).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.75 (s, 1H), 9.01 (brs, 1H), 8.81 – 8.66 (m, 2H), 7.96 – 7.75 (m, 5H), 7.54 – 7.45 (m, 2H), 7.22 (s, 1H), 3.49 (s, 4H), 3.21 (dd, *J* = 15.2, 9.2 Hz, 4H), 2.80 (dd, *J* = 22.4, 11.2 Hz, 2H), 1.98 (s, 3H), 1.81 (d, *J* = 12.8 Hz, 4H), 1.66 (s, 2H), 1.40 (q, *J* = 11.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.1, 136.2, 132.7, 130.7, 127.9, 127.7, 122.7, 121.7, 119.7, 112.9, 111.9, 109.6, 103.3, 43.6, 42.8, 33.8, 26.3, 23.11, 21.1. MS (ESI) [M+H]+ 417.3.

N-(Piperidin-4-ylmethyl)-5-(4-(pyrrolidin-1-yl)phenyl)-1H-indole-2-carboxamide Hydrochloride **(41).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.63 (s, 1H), 8.99 (d, *J* = 9.6 Hz, 1H), 8.69 (t, *J* = 5.6 Hz, 2H), 7.78 (s, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.44 (s, 2H), 7.16 (s, 1H), 6.99 (s, 2H), 3.37 (s, 4H), 3.25 – 3.16 (m, 4H), 2.81 (dd, *J* = 23.2, 12.0 Hz, 2H), 2.01 (s, 4H), 1.81 (d, *J* = 12.0 Hz, 3H), 1.39 (dd, *J* = 24.4, 12.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.2, 135.6, 132.3, 132.0, 127.7, 127.4, 127.3, 122.5, 118.4, 112.6, 103.0, 43.5, 42.8, 33.8, 26.3, 24.5. MS (ESI) [M+H]+ 403.2.

5-(4-Morpholinophenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(42).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H), 8.99 (d, *J* = 11.2 Hz, 1H), 8.76 – 8.63 (m, 2H), 7.84 (s, 1H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.46 (s, 2H), 7.35 (d, *J* = 6.8 Hz, 2H), 7.18

(s, 1H), 3.88 (s, 4H), 3.31 (s, 4H), 3.26 – 3.16 (m, 4H), 2.81 (dd, *J* = 22.8, 11.6 Hz, 2H), 1.81 (d, *J* = 12.4 Hz, 3H), 1.39 (q, *J* = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 161.2, 135.9, 132.4, 131.5, 127.7, 127.5, 122.6, 118.9, 117.9, 112.7, 103.1, 65.2, 50.6, 43.5, 42.8, 33.8, 26.3. MS (ESI) [M+H]+ 419.5.

5-(4-(Piperazin-1-yl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(43).** ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.64 (s, 1H), 9.35 (s, 2H), 9.00 (brs, 1H), 8.71 (d, *J* = 6.0 Hz, 2H), 7.79 (s, 1H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.44 (s, 2H), 7.16 (d, *J* = 2.0 Hz, 1H), 7.05 (d, *J* = 8.8 Hz, 2H), 3.39 (d, *J* = 5.2 Hz, 4H), 3.20 (s, 8H), 2.80 (q, *J* = 11.2 Hz, 2H), 1.81 (d, *J* = 12.8 Hz, 3H), 1.45 – 1.33 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 161.2, 148.6, 135.7, 133.2, 132.3, 131.9, 127.7, 127.3, 122.5, 118.4, 116.5, 112.6, 103.1, 45.6, 43.5, 42.8, 42.5, 33.8, 26.3. MS (ESI) [M+H]+ 418.2.

5-(4-Cyanophenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride (**44).** ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.79 (s, 1H), 8.87 – 8.76 (m, 1H), 8.72 (t, *J* = 4.8 Hz, 1H), 8.55 – 8.43 (m, 1H), 8.03 (s, 1H), 7.94 – 7.86 (m, 4H), 7.56 (dd, *J* = 22.2, 8.8 Hz, 2H), 7.24 (s, 1H), 3.23 (d, *J* = 7.2 Hz, 4H), 2.84 (dd, *J* = 23.2, 12.0 Hz, 2H), 1.84 (d, *J* = 12.0 Hz, 3H), 1.45 – 1.34 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 161.0, 145.9, 136.6, 132.8, 132.7, 130.1, 127.7, 127.4, 122.7, 120.3, 119.1, 113.0, 108.8, 103.3, 43.5, 42.8, 33.8, 26.3. MS (ESI) $[M+H]^+$ 359.6.

5-(4-Cyano-2-methylphenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(45).** ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.75 (s, 1H), 8.93 (s, 1H), 8.75 – 8.60 (m, 2H), 7.76 (s, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.58 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.20 – 7.13 (m, 2H), 3.21 (dd, *J* = 16.0, 10.0 Hz, 4H), 2.81 (dd, *J* = 22.4, 11.2 Hz, 2H), 2.27 (s, 3H), 1.81 (d, *J* = 12.4 Hz, 3H), 1.44 – 1.32 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 161.1, 147.4, 136.9, 135.8, 133.7, 132.6, 131.2, 130.9, 129.6, 127.1, 124.4, 121.6, 119.1, 112.2, 109.3, 103.0, 43.5, 42.8, 33.8, 26.3, 20.1. MS (ESI) [M+H]+ 373.4.

5-(3-Cyanophenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(46).** ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.79 (s, 1H), 9.01 (s, 1H), 8.83 – 8.66 (m, 2H), 8.15 (s, 1H), 8.04 (d, *J* = 7.6 Hz, 1H), 8.01 (s, 1H), 7.75 (d, *J* = 7.6 Hz, 1H), 7.64 (t, *J* = 7.6 Hz, 1H), 7.55 (dd, *J* = 21.2, 8.4 Hz, 2H), 7.24 (s, 1H), 3.23 (dd, *J* = 14.4, 9.2 Hz, 4H), 2.83 (dd, *J* = 23.2, 11.2 Hz, 2H), 1.83 (d, *J* = 12.4 Hz, 3H), 1.42 (dd, *J* = 22.0, 10.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 161.0, 142.5, 136.4, 132.8, 131.4, 130.0, 130.04, 130.00, 129.9, 127.7, 122.6, 120.0, 119.0, 113.0, 112.0, 103.3, 43.6, 42.8, 33.8, 26.3. MS (ESI) [M+H]+ 359.2.

N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(47)**. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.69 (s, 1H), 8.69 (s, 1H), 7.65 – 7.53 (m, 2H), 7.16 (d, *J* = 10.8 Hz, 2H), 3.32 – 3.15 (m, 4H), 2.83 (t, *J* = 12.0 Hz, 2H), 1.82 (d, *J* = 12.0 Hz, 3H), 1.36 (dd, *J* = 24.4, 12.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 161.2, 137.2, 132.5, 126.2, 123.6, 123.1, 116.3, 114.9, 102.9, 43.7, 43.0, 33.9, 26.4. MS (ESI) $[M+H]^+$ 258.5.

6-Phenyl-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(48)**. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.71 (s, 1H), 8.95 (d, *J* = 10.4 Hz, 1H), 8.73 – 8.57 (m, 2H), 7.67

(d, *J* = 8.4 Hz, 1H), 7.64 – 7.58 (m, 3H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.31 (t, *J* = 7.6 Hz, 2H), 7.15 (s, 1H), 3.21 (dd, *J* = 15.6, 9.6 Hz, 4H), 2.81 (dd, *J* = 22.0, 11.2 Hz, 2H), 1.81 (d, *J* = 12.0 Hz, 3H), 1.48 – 1.30 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 161.1, 141.2, 137.0, 135.7, 132.4, 128.9, 126.9, 126.8, 126.5, 121.9, 119.3, 110.0, 102.6, 43.5, 42.8, 33.8, 26.3. MS (ESI) $[M+H]^+$ 334.2.

6-(4-Fluorophenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(49)**. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.69 (s, 1H), 8.64 (s, 2H), 8.33 (s, 1H), 7.72 – 7.64 (m, 3H), 7.61 (s, 1H), 7.36 – 7.25 (m, 3H), 7.16 (s, 1H), 3.25 – 3.19 (m, 4H), 2.85 (dd, *J* = 21.6, 10.4 Hz, 2H), 1.84 (d, *J* = 11.2 Hz, 3H), 1.37 (dd, *J* = 24.4, 12.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 162.8, 161.1, 160.4, 137.79, 137.76, 137.0, 134.7, 132.6, 128.7, 128.6, 126.5, 122.0, 119.3, 115.8, 115.6, 110.1, 102.7, 43.6, 42.8, 33.8, 26.3. MS (ESI) $[M+H]^+$ 352.1.

6-(4-Methoxyphenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(50)**. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.67 (s, 1H), 9.02 (s, 1H), 8.70 (s, 2H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.61 – 7.53 (m, 3H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.16 (s, 1H), 7.03 (d, *J* = 7.2 Hz, 2H), 3.80 (s, 3H), 3.33 – 3.09 (m, 4H), 2.83 (d, *J* = 9.6 Hz, 2H), 1.83 (d, *J* = 12.0 Hz, 3H), 1.41 (d, *J* = 11.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 161.1, 158.5, 137.1, 135.4, 133.6, 132.2, 127.8, 126.0, 121.8, 119.1, 114.4, 109.4, 102.6, 55.2, 43.5, 42.8, 33.8, 26.3. MS (ESI) $[M+H]^+$ 364.7.

N-(Piperidin-4-ylmethyl)-6-(4-(trifluoromethoxy)phenyl)-1H-indole-2-carboxamide Hydrochloride **(51)**. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.78 (s, 1H), 9.03 (d, *J* = 9.6 Hz, 1H), 8.81 – 8.68 (m, 2H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.62 (s, 1H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.17 (s, 1H), 3.20 (dd, *J* = 13.6, 8.4 Hz, 4H), 2.79 (dd, *J* = 22.0, 11.6 Hz, 2H), 1.80 (d, *J* = 12.0 Hz, 3H), 1.39 (dd, *J* = 22.8, 10.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 161.1, 147.4, 140.6, 136.9, 136.4, 134.1, 132.8, 131.7, 128.6, 126.8, 123.2, 122.1, 121.5, 119.6, 119.3, 118.9, 112.3, 110.3, 102.6, 43.6, 42.8, 33.8, 26.3. MS (ESI) $[M+H]^+$ 418.2.

6-(4-(aminomethyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(52)**. ^{1}H NMR (400 MHz, D_2O) δ 7.82 – 7.66 (m, 3H), 7.62 (s, 1H), 7.48 (d, *J* = 7.2 Hz, 2H), 7.37 (d, *J* = 7.6 Hz, 1H), 7.00 (s, 1H), 4.17 (s, 2H), 3.42 (d, *J* = 11.6 Hz, 2H), 3.20 (s, 2H), 2.93 (t, *J* = 12.0 Hz, 2H), 1.93 (d, *J* = 13.6 Hz, 3H), 1.52 – 1.33 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 163.3, 141.5, 136.9, 136.0, 131.3, 130.8, 129.3, 127.5, 126.7, 122.4, 119.8, 110.0, 103.8, 43.9, 43.6, 42.6, 33.5, 26.0. MS (ESI) $[M+H]^+$ 363.5.

6-(4-Carbamoylphenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(53)**. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.77 (s, 1H), 8.92 (d, *J* = 7.6 Hz, 1H), 8.71 (s, 1H), 8.62 (d, *J* = 8.0 Hz, 1H), 8.02 (s, 1H), 7.95 (d, *J* = 8.0 Hz, 2H), 7.78 – 7.60 (m, 4H), 7.39 (d, *J* = 8.8 Hz, 2H), 7.17 (s, 1H), 3.30 – 3.12 (m, 4H), 2.81 (dd, *J* = 22.4, 11.2 Hz, 2H), 1.81 (d, *J* = 12.0 Hz, 3H), 1.38 (d, *J* = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 167.6, 161.1, 143.9, 137.0, 134.6, 132.8, 132.5, 128.2, 126.9, 126.4, 122.1, 119.3, 110.4, 102.6, 43.5, 42.8, 33.8, 26.3. MS (ESI) $[M+H]^+$ 377.4.

6-(4-(Hydroxymethyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(54)**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (s, 1H), 9.01 (d, J = 11.2 Hz, 1H), 8.76 – 8.68 (m, 2H), 7.71 – 7.63 (m, 3H), 7.61 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 7.6 Hz, 2H), 7.34 (d, J = 8.4 Hz, 1H), 7.18 (s, 1H), 4.54 (s, 2H), 3.28 – 3.21 (m, 4H), 2.83 (dd, J = 22.8, 11.6 Hz, 2H), 1.84 (d, J = 11.6 Hz, 3H), 1.45 – 1.38 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.1, 141.2, 139.6, 137.0, 135.6, 132.4, 129.5, 127.1, 126.4, 121.9, 119.3, 109.9, 102.6, 62.6, 43.5, 42.8, 33.8, 26.3. MS (ESI) $[M+H]^+$ 364.2.

6-(4-(2-Hydroxyethyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(55)**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (s, 1H), 9.05 (brs, 1H), 8.73 (brs, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.62 (s, 1H), 7.55 (d, J = 8.0 Hz, 2H), 7.31 (t, J = 8.4 Hz, 4H), 7.18 (s, 1H), 4.71 (t, J = 4.8 Hz, 1H), 3.67 – 3.59 (m, 2H), 3.28 – 3.18 (m, 4H), 2.87 – 2.79 (m, 2H), 2.76 (t, J = 6.8 Hz, 2H), 1.83 (d, J = 12.0 Hz, 3H), 1.42 (dd, J = 26.0, 14.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.1, 138.9, 138.3, 137.1, 135.7, 132.3, 129.5, 126.6, 126.4, 121.9, 119.3, 109.8, 102.6, 62.2, 62.1, 43.4, 42.7, 33.8, 26.3. MS (ESI) $[M+H]^+$ 378.2.

6-(4-Phenoxyphenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(56)**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.74 (s, 1H), 9.02 (d, J = 12.4 Hz, 1H), 8.73 (t, J = 6.0 Hz, 2H), 7.73 – 7.56 (m, 4H), 7.47 – 7.37 (m, 2H), 7.34 (dd, J = 8.4, 1.6 Hz, 1H), 7.20 – 7.13 (m, 2H), 7.12 – 6.99 (m, 4H), 3.23 (dd, J = 15.2, 9.2 Hz, 4H), 2.83 (dd, J = 22.0, 11.2 Hz, 2H), 1.84 (d, J = 12.4 Hz, 3H), 1.41 (dd, J = 24.8, 13.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.1, 156.6, 155.9, 137.0, 136.5, 135.0, 132.4, 130.1, 128.3, 126.4, 123.6, 122.0, 119.2, 119.0, 118.8, 109.8, 102.6, 43.5, 42.8, 33.8, 26.3. MS (ESI) $[M+H]^+$ 426.2.

6-(4-(Piperazin-1-yl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(57)**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.63 (s, 1H), 9.09 (s, 2H), 8.78 (s, 1H), 8.65 (s, 1H), 8.47 (s, 1H), 7.65 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 8.8 Hz, 1H), 7.14 (s, 1H), 7.09 (d, J = 8.4 Hz, 2H), 3.40–3.10 (m, 12H), 2.84 (dd, J = 22.0, 10.8 Hz, 2H), 1.83 (d, J = 11.2 Hz, 3H), 1.38 (dd, J = 24.4, 13.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.6, 149.3, 137.4, 135.9, 133.2, 132.1, 127.8, 126.4, 122.3, 119.5, 116.9, 109.5, 103.0, 45.8, 43.6, 43.3, 43.0, 34.1, 26.5. MS (ESI) $[M+H]^+$ 418.2.

6-(4-(Piperidin-1-yl)phenyl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(58)**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H), 9.01 (s, 1H), 8.75 (s, 2H), 7.96 – 7.84 (m, 1H), 7.80 (s, 2H), 7.71 (d, J = 8.0 Hz, 1H), 7.66 (s, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.19 (s, 1H), 3.56 (s, 4H), 3.29 – 3.18 (m, 4H), 2.83 (dd, J = 22.0, 12.0 Hz, 2H), 2.17 – 1.75 (m, 7H), 1.75 – 1.58 (m, 2H), 1.41 (dd, J = 24.8, 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.2, 142.4, 141.4, 136.9, 134.3, 132.6, 128.3, 127.0, 122.3, 121.6, 119.5, 110.4, 102.8, 55.6, 43.6, 42.9, 33.9, 26.3, 23.4, 21.2. MS (ESI) $[M+H]^+$ 417.3.

N-(Piperidin-4-yl)-6-(pyridin-4-yl)-1H-indole-2-carboxamide Hydrochloride **(59)**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23 (s, 1H), 9.06 (s, 2H), 8.88–8.85 (m, 3H),, 8.34 (s, 2H), 8.01 (s, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.36 (s, 1H), 4.12 (s, 1H), 3.34 (d, J = 12.4 Hz, 2H), 3.02 (s, 2H), 2.00 (d, J = 11.6 Hz, 2H), 1.86 (dd, J = 23.6, 12.0 Hz, 2H);

^{13}C NMR (100 MHz, DMSO-d_6) δ 160.4, 156.6, 142.6, 137.1, 135.1, 129.9, 129.7, 123.8, 123.3, 119.7, 112.7, 103.9, 44.6, 42.5, 28.6. MS (ESI) $[M+H]^+$ 321.2.

N-(Piperidin-4-ylmethyl)-6-(pyridin-4-yl)-1H-indole-2-carboxamide Hydrochloride **(60).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 1H), 8.98 (brs, 1H), 8.89 (t, J = 6.4 Hz, 1H), 8.84 (d, J = 6.4 Hz, 2H), 8.73 (brs, 1H), 8.30 (d, J = 6.8 Hz, 2H), 7.98 (s, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 8.4 Hz, 1H), 7.25 (s, 1H), 3.22 (d, J = 14.4 Hz, 4H), 2.80 (dd, J = 22.4, 12.0 Hz, 2H), 1.81 (d, J = 13.2 Hz, 3H), 1.46 – 1.33 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.7, 156.1, 142.3, 136.6, 134.9, 129.6 129.2, 123.3, 122.8, 119.3, 112.3, 102.9, 43.6, 42.8, 33.8, 26.3. MS (ESI) $[M+H]^+$ 335.5.

N-(piperidin-4-ylmethyl)-6-(pyridin-3-yl)-1H-indole-2-carboxamide Hydrochloride **(61).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.09 (s, 1H), 9.17 (brs, 2H), 8.93 (brs, 3H), 8.81 (d, J = 7.2 Hz, 1H), 8.08 (s, 1H), 7.82 (d, J = 12.0 Hz, 2H), 7.52 (d, J = 8.0 Hz, 1H), 7.27 (s, 1H), 3.22 (s, 4H), 2.82 (dd, J = 23.2, 12.0 Hz, 2H), 1.84 (d, J = 12.8 Hz, 3H), 1.44 (dd, J = 24.4, 12.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.8, 142.3, 140.2, 136.7, 133.8, 129.0, 128.0, 122.6, 119.1, 111.3, 102.9, 43.6, 42.7, 33.8, 26.2. MS (ESI) $[M+H]^+$ 335.8.

6-(3,6-Dihydro-2H-pyran-4-yl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(62).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.61 (s, 1H), 8.91 (d, J = 11.6 Hz, 1H), 8.70 – 8.53 (m, 2H), 7.56 (d, J = 8.4 Hz, 1H), 7.41 (s, 1H), 7.22 (d, J = 8.4 Hz, 1H), 7.11 (s, 1H), 6.22 (s, 1H), 4.24 (d, J = 2.4 Hz, 2H), 3.84 (t, J = 5.2 Hz, 2H), 3.31 – 3.10 (m, 6H), 2.83 (dd, J = 22.8, 11.6 Hz, 2H), 1.83 (d, J = 11.2 Hz, 3H), 1.40 (q, J = 14.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.2, 136.7, 135.2, 133.7, 132.1, 126.3, 121.8, 121.3, 117.2, 107.8, 102.6, 65.2, 63.8, 43.5, 42.8, 33.8, 26.9, 26.3. MS (ESI) $[M+H]^+$ 340.4.

N-(Piperidin-4-ylmethyl)-6-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole-2-carboxamide Hydrochloride **(63).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.75 (s, 1H), 9.38 (s, 2H), 9.04 (s, 1H), 8.73 (t, J = 6.0 Hz, 2H), 7.59 (d, J = 8.4 Hz, 1H), 7.43 (s, 1H), 7.21 (d, J = 8.4 Hz, 1H), 7.14 (s, 1H), 6.15 (s, 1H), 3.74 (s, 2H), 3.30 (s, 2H), 3.21 (dd, J = 16.8, 10.8 Hz, 4H), 2.82 (dd, J = 22.4, 11.2 Hz, 2H), 2.72 (s, 2H), 1.82 (d, J = 12.4 Hz, 3H), 1.41 (dd, J = 23.2, 11.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.0, 136.5, 134.9, 134.2, 132.5, 126.7, 121.5, 117.2, 115.8, 108.3, 102.7, 43.5, 42.7, 41.5, 33.8, 26.3, 23.5. MS (ESI) $[M+H]^+$ 339.2.

N-(Piperidin-4-ylmethyl)-6-(1H-pyrazol-4-yl)-1H-indole-2-carboxamide Hydrochloride **(64).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (s, 1H), 9.16 (d, J = 9.2 Hz, 1H), 8.88 (d, J = 9.2 Hz, 1H), 8.77 (t, J = 5.6 Hz, 1H), 8.32 (s, 2H), 7.60 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 8.4 Hz, 1H), 7.15 (s, 1H), 3.33 – 3.09 (m, 4H), 2.81 (dd, J = 22.4, 11.2 Hz, 2H), 1.84 – 1.81 (m, 3H), 1.43 (dd, J = 23.2, 11.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.5, 137.4, 132.6, 130.8, 127.3, 126.4, 123.2, 122.4, 119.0, 110.0, 108.9, 103.6, 43.9, 43.2, 34.2, 26.7. MS (ESI) $[M+H]^+$ 340.1.

N-(Piperidin-4-ylmethyl)-6-(thiophen-3-yl)-1H-indole-2-carboxamide Hydrochloride **(65).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.66 (s, 1H), 8.85 (s, 1H), 8.66 (t, J = 5.6 Hz, 1H), 8.55 (s, 1H), 7.82 – 7.73 (m, 1H), 7.70 – 7.59 (m, 3H), 7.54 – 7.48 (m, 1H), 7.46 – 7.37 (m, 1H), 7.14 (d, J = 1.2 Hz, 1H), 3.28 – 3.21 (m, 4H), 2.84 (dd, J = 21.6, 11.2 Hz, 2H), 1.85 –

1.82 (m, 3H), 1.40 (dd, *J* = 23.6, 11.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 161.1, 142.5, 136.9, 132.3, 130.7, 127.0, 126.33, 126.30, 121.9, 120.0, 119.1, 109.3, 102.7, 43.5, 42.8, 33.8, 26.3. MS (ESI) $[M+H]^+$ 340.1.

6-(Furan-3-yl)-N-(piperidin-4-ylmethyl)-1H-indole-2-carboxamide Hydrochloride **(66).** ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.64 (s, 1H), 8.90 (d, *J* = 10.4 Hz, 1H), 8.69 – 8.62 (m, 1H), 8.63 – 8.54 (m, 1H), 8.13 (s, 1H), 7.74 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.55 (s, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.13 (s, 1H), 6.90 (s, 1H), 3.28 – 3.19 (m, 4H), 2.83 (dd, *J* = 22.4, 10.8 Hz, 2H), 1.84 – 1.81 (m, 3H), 1.40 (dd, *J* = 23.6, 11.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 161.1, 144.2, 138.8, 136.9, 132.1, 127.2, 126.7, 126.2, 121.9, 118.6, 108.9, 108.7, 102.8, 43.5, 42.8, 33.8, 26.3. MS (ESI) $[M+H]^+$ 324.2.

6. General Procedure for the synthesis of compounds 9 and 70–73.—To a solution of 6-bromo-1H-pyrrolo[2,3-b]pyridine **87** (591.1 g, 3 mmol) in DCM (10 mL) was added $AlCl_3$ (2 g, 15 mmol) at room temperature. After the resulting mixture was stirred at room temperature for 10 min, 2,2,2-trichloroacetyl chloride (818.2 mg, 4.5 mmol) was added. The mixture was stirred for 12 h. Then it was poured into ice water. A lot of white solid precipitated. It was filtered to get the intermediate **88**, which was used directly for the next step.

The above solid **88** was added to 20% NaOH solution. The resulting mixture was stirred at room temperature for 16 h. After the starting material was consumed, the mixture was quenched with 1 M HCl solution. A lot of white solid precipitated from the solvent. It was filtered to get the intermediate, which was used directly for the next step. The preparation of the final compounds followed the same procedure as described for compound **1**.

6-(4-(tert-butyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide Hydrochloride **(9).** ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.16 (s, 1H), 8.94 (s, 1H), 8.66 (d, *J* = 8.0 Hz, 1H), 8.47 (d, *J* = 8.0 Hz, 1H), 8.24–8.21 (m, 2H), 8.00 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 3.23 (d, *J* = 12.0 Hz, 2H), 3.16 (t, *J* = 5.6 Hz, 2H), 2.80 (q, *J* = 12.0 Hz, 2H), 1.81 (d, *J* = 12.0 Hz, 3H), 1.43 – 1.37 (m, 2H), 1.30 (s, 9H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 164.5, 151.4, 150.8, 148.8, 136.9, 130.6, 128.9, 126.7, 125.9, 117.9, 114.2, 109.9, 43.7, 43.3, 34.8, 34.4, 31.5, 26.8. MS (ESI) $[M+H]^+$ 391.5.

N-(piperidin-4-ylmethyl)-6-(4-(trifluoromethyl)phenyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide Hydrochloride **(70).** ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.29 (s, 1H), 8.90 (s, 1H), 8.62 (s, 1H), 8.53 (d, *J* = 8.0 Hz, 1H), 8.37 – 8.19 (m, 3H), 7.88–7.82 (m, 3H), 3.24 (d, *J* = 12.0 Hz, 2H), 3.21 – 3.11 (m, 2H), 2.81 (q, *J* = 12.0 Hz, 2H), 1.82 (d, *J* = 12.0 Hz, 3H), 1.38 (q, *J* = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 163.9, 148.54, 148.50, 143.2, 130.3, 129.5, 129.0, 128.6, 128.3, 128.0, 127.6, 127.1, 125.8, 125.75, 125.70, 125.62, 125.59, 123.1, 118.4, 114.5, 109.6, 43.2, 42.9, 34.0, 26.4. MS (ESI) $[M+H]^+$ 403.2.

N-(piperidin-4-ylmethyl)-6-(pyridin-4-yl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide Hydrochloride **(71).** ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.57 (s, 1H), 8.93 (d, *J* = 8.0 Hz, 3H), 8.63 (d, *J* = 8.0 Hz, 4H), 8.48 (d, *J* = 4.0 Hz, 1H), 8.38 (t, *J* = 5.6 Hz, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 3.23 (d, *J* = 12.0 Hz, 2H), 3.20 – 3.12 (m, 3H), 2.80 (q, *J* = 12.0 Hz, 2H),

1.81 (d, J = 12.0 Hz, 3H), 1.38 (q, J = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.6, 153.5, 148.6, 144.2, 143.1, 131.8, 130.4, 122.8, 120.7, 116.1, 109.9, 43.3, 42.9, 33.9, 26.4. MS (ESI) $[M+H]^+$ 336.4.

6-(4-(piperidin-1-ylmethyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide Hydrochloride **(72).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23 (s, 1H), 10.86 (s, 1H), 9.09 (d, J = 12.0 Hz, 1H), 8.80 (d, J = 8.0 Hz, 1H), 8.50 (d, J = 8.0 Hz, 1H), 8.32–8.29 (m, 2H), 8.14 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 12.0 Hz, 1H), 7.72 (d, J = 8.0 Hz, 2H), 4.27 (d, J = 4.0 Hz, 2H), 3.53 (s, 1H), 3.28–3.14 (m, 6H), 2.87 – 2.74 (m, 4H), 1.82 – 1.64 (m, 8H), 1.44 – 1.30 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0, 149.5, 148.4, 140.2, 131.9, 130.2, 129.8, 129.1, 126.6, 118.0, 114.2, 109.6, 66.4, 58.5, 54.9, 43.2, 42.8, 40.2, 34.0, 26.3, 22.1, 21.5. MS (ESI) $[M+H]^+$ 432.3.

6-(4-(morpholinomethyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide Hydrochloride **(73).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.21 (s, 1H), 11.45 (s, 1H), 8.94 (d, J = 12.0 Hz, 1H), 8.65 (d, J = 12.0 Hz, 1H), 8.50 (d, J = 8.0 Hz, 1H), 8.26 (s, 2H), 8.16 (d, J = 8.0 Hz, 2H), 7.82 (d, J = 8.0 Hz, 1H), 7.72 (d, J = 8.0 Hz, 2H), 4.36 (d, J = 4.0 Hz, 2H), 3.92 (d, J = 12.0 Hz, 2H), 3.81 (t, J = 12.0 Hz, 2H), 3.25 – 3.07 (m, 8H), 2.80 (q, J = 12.0 Hz, 2H), 1.81 (d, J = 12.0 Hz, 3H), 1.38 (q, J = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0, 149.5, 148.4, 140.4, 132.0, 130.2, 129.2, 129.0, 126.7, 118.0, 114.2, 109.6, 63.1, 58.6, 50.6, 43.2, 42.8, 34.0, 26.4. MS (ESI) $[M+H]^+$ 434.3.

7. Synthesis of compound 10—4-Bromobenzene-1,2-diamine **90** (187 mg, 1 mmol), (4-(*tert*-butyl)phenyl)boronic acid (214 mg, 1.2 mmol), tetrakis(triphenylphosphine)palladium (58 mg, 0.05 mmol), and sodium carbonate (210 mg, 2 mmol) in DMF/H_2O (10/2 mL) were placed in a sealed tube. The mixture was degassed and heated to 90 °C for 12 h. The reaction was then cooled and quenched with brine (5 mL). The product was extracted with ethyl acetate (3 × 15 mL) and the combined organic layers were washed with water and brine and dried over Na_2SO_4. The volatiles were removed *in vacuo* to give a yellow solid, which was purified by column chromatography (silica gel, hexanes: ethyl acetate from 1:1 to 1:2) to afford the corresponding coupling product.

To a solution of the above coupling product (0.8 mmol) in AcOH (5 mL) were added Methyl 2,2,2-trichloroacetimidate (176.4 mg, 0.96 mmol). The mixture was stirred for 16 h before it was quenched with $NaHCO_3$ aq. The precipitate was filtered and washed with water to afford a yellow solid, which was used directly for next step.

To the above intermediate **91** (0.7 mmol) was added 2 N NaOH (5 mL). The resulting mixture was stirred at room temperature for 12 h before it was quenched with 1M HCl aq. The precipitate was filtered and washed with water to afford a yellow solid, which was used directly for next step.

To a solution of the above intermediate (0.6 mmol) in DCM (5 mL) were added HOBt (81.1 mg, 0.6 mmol) and EDCl (230 mg, 1.2 mmol). The resulting mixture was stirred at room temperature for 0.5 h. Then 4-aminomethyl-1-Boc-piperidine (193 mg, 0.9 mmol) and TEA (0.42 mL, 3 mmol) were added to the mixture. The mixture was stirred for 12 h before it was

quenched with H_2O. The mixture was extracted with DCM (3 × 30 mL) and the combined organic layers were washed with water and brine and dried over Na_2SO_4. The volatiles were removed in vacuo to afford a crude oil, which was purified by column chromatography (silica gel, hexanes: ethyl acetate = 2:1) to afford a white solid, which was used directly for next step.

To a solution of this intermediate in DCM (4 mL) was added dropwise HCl (0.4 mL, 4 N in *p*-dioxane) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 4 h. The volatiles were removed *in vacuo* to afford the final product as a hydrochloric salt.

5-(4-(tert-butyl)phenyl)-N-(piperidin-4-ylmethyl)-1H-benzo[d]imidazole-2 carboxamide Hydrochloride **(10).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (d, J = 4.0 Hz, 1H), 8.88 (s, 1H), 8.60 (s, 1H), 7.79 (s, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.59 (d, J = 8.0 Hz, 3H), 7.47 (d, J = 8.4 Hz, 2H), 3.32 – 3.13 (m, 4H), 2.91 – 2.72 (m, 2H), 2.06 (s, 2H), 1.95 – 1.69 (m, 3H), 1.40 (t, J = 11.6 Hz, 2H), 1.30 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.8, 150.1, 146.2, 138.2, 136.8, 127.1, 126.2, 123.6, 44.0, 43.2, 34.7, 33.9, 31.6, 31.1, 26.6. MS (ESI) $[M+H]^+$ 391.6.

8. Synthesis of compound 11, 12, 13 and 68, 69.—To a solution of the corresponding carboxylic acid **92** (4.0 mmol, 1 equiv.) and amine (4.4 mmol, 1.1 equiv.) in DMF (20 mL) were added HATU (4.8 mmol, 1.2 equiv.) and DIPEA (6 mmol, 1.5 equiv.). The reaction was stirred overnight at room temperature. After completion, the mixture was diluted with water (30 mL) and then was extracted with DCM (40 mL). The organic phase was washed with brine three times. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel to give amides **93**.

Compound **93** (0.2 mmol), aryl boronic acid or aryl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.24 mmol), tetrakis(triphenylphosphine)palladium (11.6 mg, 0.01 mmol), and sodium carbonate (42 mg, 0.4 mmol) in *p*-dioxane/H_2O (5/1, v/v, 6 mL) were placed in a sealed tube. The mixture was degassed and heated to 80 °C for overnight. The reaction was then cooled and quenched with brine (10 mL). The mixture was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with water and brine and dried over Na_2SO_4. The solvent were removed *in vacuum* to give a crude oil, which was purified by column chromatography (silica gel, hexanes: ethyl acetate from 4:1 to 1:1) to afford the corresponding coupling product. Then the Boc-protected intermediate was treated with 0.5 mL HCl (4*N* in dioxane) in DCM (5 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 6 h. The solvent were removed in vacuum, which was triturated in diether ether and solidified to give the final product as a hydrochloric salt.

5-(4-(Tert-butyl)phenyl)-N-(piperidin-4-yl)benzo[b]thiophene-2-carboxamide Hydrochloride (**11).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.87 (d, J = 7.6 Hz, 1H), 8.85 – 8.68 (m, 2H), 8.23 (s, 1H), 8.14 (s, 1H), 8.07 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 8.0 Hz, 2H), 7.50 (d, J = 8.0 Hz, 2H), 4.10 – 4.00 (m, 1H), 3.35 – 3.30 (m, 2H), 3.10 – 3.00 (m, 2H), 2.05 – 1.95 (m, 2H), 1.87 – 1.74 (m, 2H), 1.31 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ

161.1, 150.0, 140.5, 139.9, 139.1, 137.3, 137.1, 126.7, 125.8, 125.39, 125.35, 123.3, 122.7, 44.6, 42.3, 34.3, 31.2, 28.2; MS (ESI) $[M+H]^+$ 393.1.

6-(4-(Tert-butyl)phenyl)-N-(piperidin-4-yl)benzo[b]thiophene-2-carboxamide Hydrochloride **(12).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 – 8.75 (m, 3H), 8.28 (s, 1H), 8.21 (s, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.75 – 7.65 (m, 3H), 7.53 – 7.45 (m, 2H), 4.10 – 4.00 (m, 1H), 3.39 – 3.25 (m, 2H), 3.08 – 2.93 (m, 2H), 2.05 – 1.95 (m, 2H), 1.85 – 1.73 (m, 2H), 1.31 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.1, 150.3, 141.2, 140.0, 138.2, 136.8, 126.8, 125.9, 125.5, 124.9, 124.1, 120.3, 44.6, 42.2, 34.3, 31.1, 28.2; MS (ESI) $[M+H]^+$ 393.2.

6-(4-(Tert-butyl)phenyl)-N-(piperidin-4-yl)benzofuran-3-carboxamide Hydrochloride **(13).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (s, 1H), 8.69 – 8.50 (m, 3H), 8.09 (d, J = 8.4 Hz, 1H), 7.91 (s, 1H), 7.69 – 7.60 (m, 3H), 7.48 – 7.40 (m, 2H), 3.27 – 3.17 (m, 3H), 2.89 – 2.75 (m, 2H), 1.89 – 1.76 (m, 2H), 1.46 – 1.35 (m, 2H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.1, 155.4, 149.9, 147.8, 137.5, 136.9, 126.7, 125.7, 124.3, 122.7, 122.1, 116.9, 109.3, 43.4, 42.8, 34.3, 33.7, 31.1. MS (ESI) $[M+H]^+$ 377.4.

6-(Furan-3-yl)-N-(piperidin-4-yl)benzofuran-3-carboxamide Hydrochloride **(68).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (br, 2H), 8.67 (s, 1H), 8.49 (d, J = 7.6 Hz, 1H), 8.26 (s, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.91 (s, 1H), 7.75 (s, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.06 (s, 1H), 4.13 – 4.00 (m, 1H), 3.35 – 3.25 (m, 2H), 3.08 – 2.93 (m, 2H), 2.05 – 1.95 (m, 2H), 1.85 – 1.70 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.5, 155.3, 147.8, 144.4, 139.6, 129.4, 125.7, 124.1, 122.1, 121.7, 116.7, 108.9, 108.3, 43.8, 42.1, 28.2; MS (ESI) $[M+H]^+$ 311.1.

6-(4-Cyanophenyl)-N-(piperidin-4-yl)benzofuran-3-carboxamide Hydrochloride **(69).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (br, 2H), 8.77 (s, 1H), 8.57 (d, J = 7.2 Hz, 1H), 8.14 (d, J = 8.4 Hz, 1H), 8.08 (s, 1H), 7.99 – 7.90 (m, 4H), 7.75 (d, J = 8.4 Hz, 1H), 4.15 – 4.00 (m, 1H), 3.36 – 3.25 (m, 2H), 3.08 – 2.94 (m, 2H), 2.08 – 1.95 (m, 2H), 1.85 – 1.70 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.4, 155.3, 148.9, 144.3, 135.5, 132.9, 127.9, 125.8, 123.1, 122.5, 118.9, 116.6, 110.3, 110.0, 43.9, 42.2, 28.2; MS (ESI) $[M+H]^+$ 346.2.

Activity and inhibition assays for Flavivirus proteases.

Expression and purification of Flavivirus proteases were performed using our previous methods[32]. The biochemical assay for ZVpro was performed using the enzyme (1 nM) and benzoyl-norleucine-lysine-lysine-arginine 7-amino-4-methylcoumarine (Bz-Nle-Lys-Lys-Arg-AMC, 10 μM) as the substrate in a HEPES buffer (20mM, pH 7.3) containing 0.05% Triton X-100. To determine the IC_{50} values, triplicate samples of a compound with increasing concentrations were pre-incubated with the enzyme for 10 min before adding the substrate in 96-well plate (100 μL final volume). The fluorescence signal (Ex: 360 nm, Em: 460 nm) of each well was monitored every 30s, using a Tecan microplate reader. Data were imported into Prism (version 5.0), and IC_{50} values from 3 independent experiments with standard deviation were obtained by using a standard dose-response curve fitting in the program. The biochemical assays for DV2pro and WVpro were done similarly.

Cellular antiviral activity testing.

Antiviral activity was evaluated in human U87 glioma following our previous methods[32]. 2×10^4 U87 cells/well were seeded into 96-well plates and cultured in DMEM media with 2% FBS. 0.01 MOI (multiplicity of infection) of ZIKV was added to each well of the plates. After 1h, the supernatant was removed and cells were washed with PBS to remove unattached viral particles. Fresh medium (150 μL/well) containing various concentrations of a compound in triplicate were then added to each well. Upon incubation at 37 °C for 2 days, the supernatant of each well was used to determine ZIKV titers. Half-log serial dilution of the viral supernatant (50 μL) was added to a monolayer of Vero cells in quadruplicate in 96-well plates and cultured for 7 days. CPE/cell lysis was determined with microscope followed by MTT assay. $TCID_{50}$ was calculated based on the highest dilution in which ≥50% (i.e., ≥2 out of the 4 quadruplicate wells) of Vero cells were infected with ZIKV. Compared to controls, the ability for a compound to reduce $TCID_{50}$ can be determined. The results were from at least 2 independent experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights:

- Compound screening identified indole-containing compounds **1** and **2** to be novel inhibitors of Zika virus NS2B-NS3 protease.
- Medicinal chemistry optimization gave a significantly more potent inhibitor **66**.
- Compound **66** exhibited a strong antiviral activity against cellular replication of Zika virus.

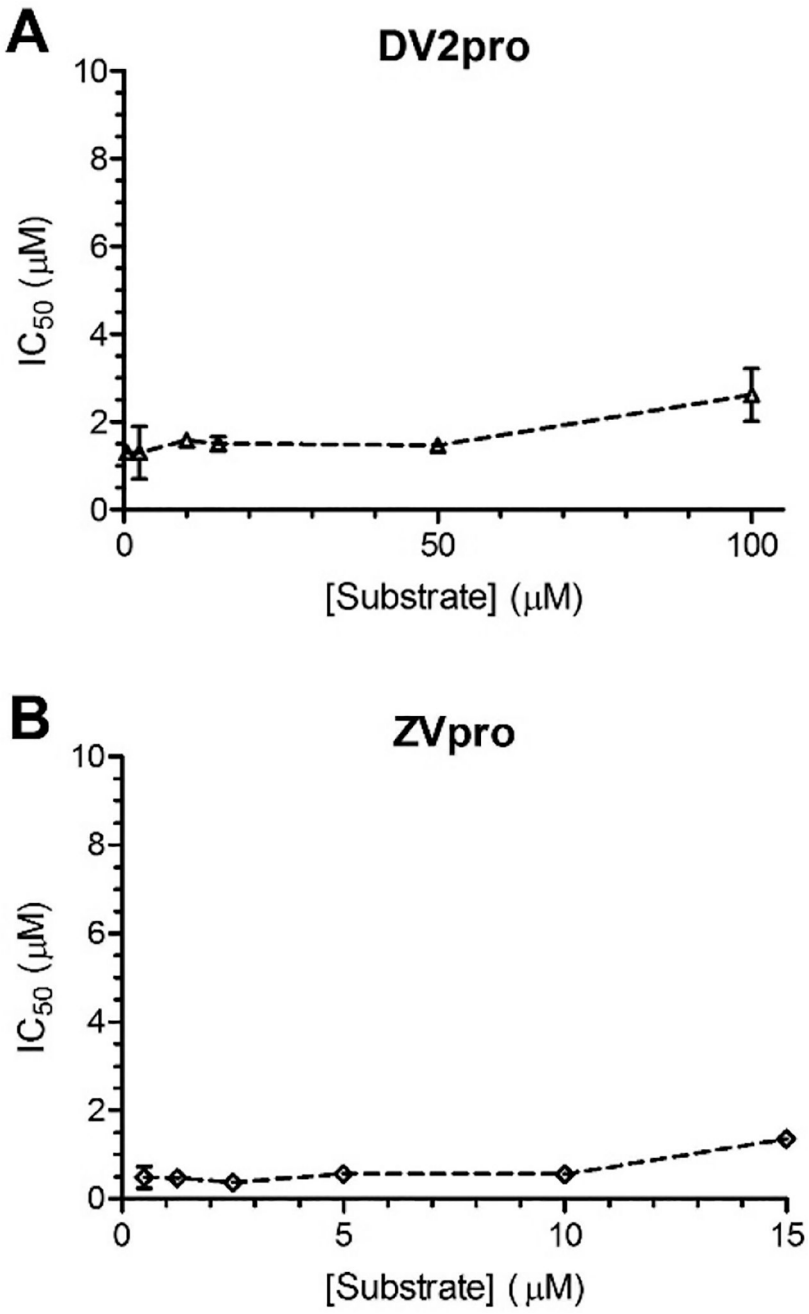
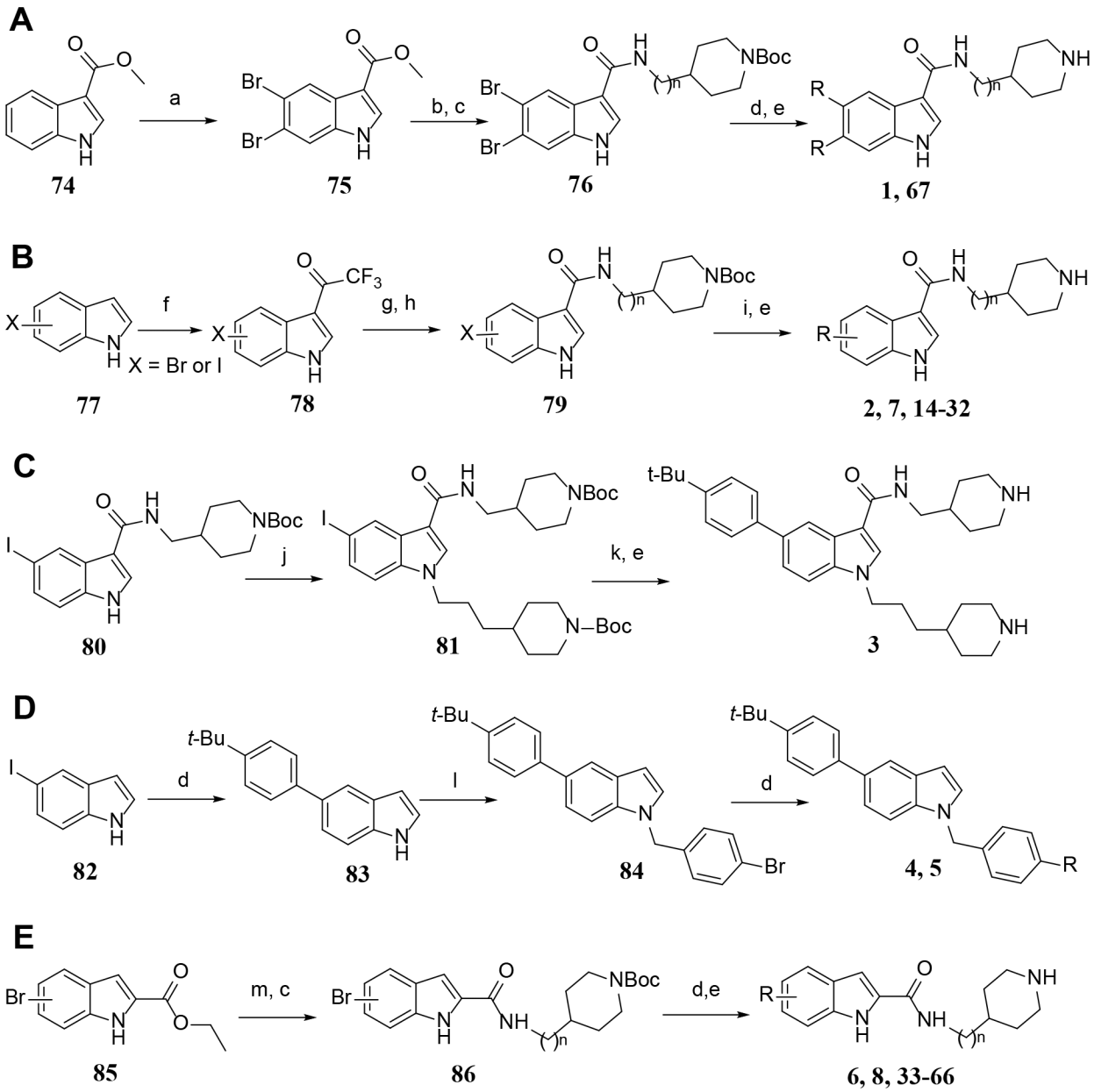


Figure 1.

Enzyme kinetics studies of (**A**) DV2pro and (**B**) ZVpro with inhibitor **66** at the increasing concentrations of the substrate from 0.5 to 100 μM (~0.05–11×K_m for DV2pro and 0.03× to 1×K_m for ZVpro). The IC_{50} values against DV2pro do not linearly increase according to the Cheng-Prusoff equation ($IC_{50} = K_i + K_i/K_m \times [S]$), suggesting compound **66** is not a competitive inhibitor of DV2pro and more likely adopts a non-competitive mode of inhibition.

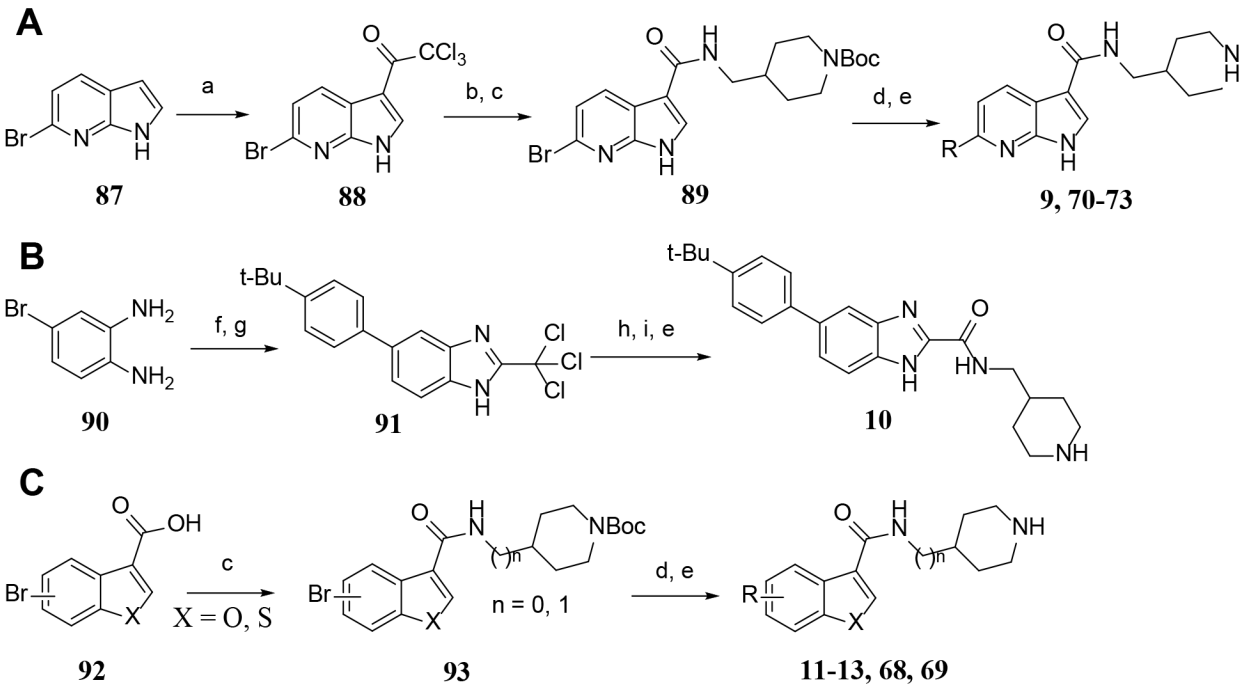


Scheme 1.

General methods for synthesis of indole compounds **1**−**8**, and **14-67**.[a]

[a]*Reagents and conditions:* (a) Br_2, AcOH, rt, 72 h; (b) NaOH, MeOH-H_2O, 50 °C, 12 h; (c) HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate), diisopropylethylamine, DMF, 12 h, 4-amino-1-Boc-piperidine, or 4-aminomethyl-1-Boc-piperidine; (d) Aryl boronic acid or Aryl 4,4,5,5-tetramethyl-1,3,2-dioxaborolane, $Pd(PPh_3)_4$, Na_2CO_3, *p*-dioxane-H_2O, 100 °C; (e) HCl (4 N in *p*-dioxane), CH_2Cl_2, 0 °C; (f) TFAA, 0 °C to room temperature, 2 h; (g) NaOH (20% aq.), 80 °C, 2 days; (h) 4-aminomethyl-1-Boc-piperidine, *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide (EDC), 1-Hydroxybenzotriazole (HOBt), triethylamine, CH_2Cl_2; (i) Aryl-boronic acid or Aryl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, Na_2CO_3, toluene-DMF-

EtOH, 80 °C, N_2; (j) NaH, 1-Boc-4-(3-bromopropyl)piperidine, DMF, 14 h; (k) (4-(*tert*-butyl)phenyl)boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene-DMF-EtOH-H_2O, 80 °C, 14 h; (l) 4-bromobenzyl bromide, K_2CO_3, DMF, 16 h; (m) NaOH, THF-H_2O, 50 °C, 5 h.



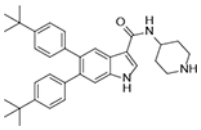
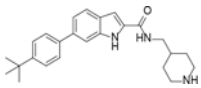
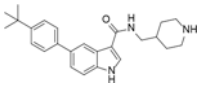
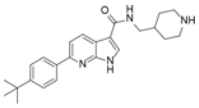
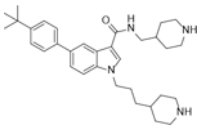
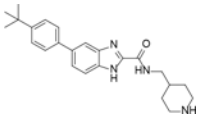
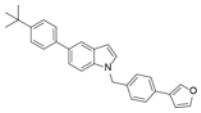
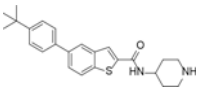
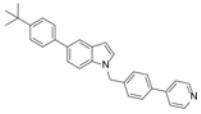
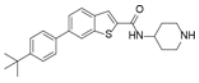
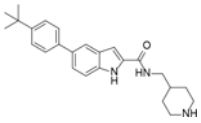
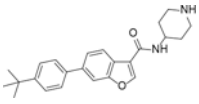
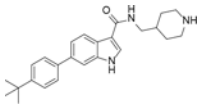
Scheme 2.

General methods for synthesis of compounds **9**-**13**, and **68**-**73**.[a]

[a]*Reagents and conditions:* (a) $AlCl_3$, CH_2Cl_2, 10 min; then, 2,2,2-trichloroacetyl chloride, 12 h; (b) NaOH (20% aq.), 16 h; (c) 4-aminomethyl-1-Boc-piperidine, HATU, diisopropylethylamine, DMF, 12 h; (d) Aryl-boronic acid or Aryl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, $Pd(PPh_3)_4$, Na_2CO_3, *p*-dioxane-H_2O, 90 °C; (e) HCl (4 N in *p*-dioxane), CH_2Cl_2, 0 °C; (f) 4-*tert*-butylphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DMF, 90 °C, 12 h; (g) Methyl 2,2,2-trichloroacetimidate, AcOH, 16 h; (h) NaOH (2 N), 12 h; (i) 4-aminomethyl-1-Boc-piperidine, EDC, HOBt, triethylamine, CH_2Cl_2.

Table 1.

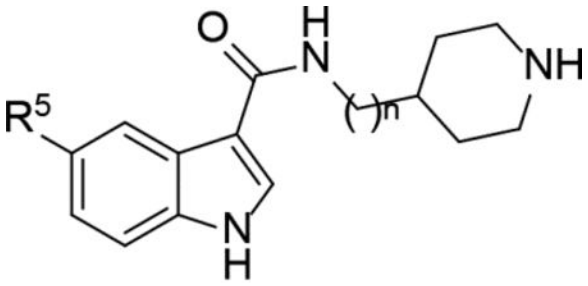
Structures and ZVpro inhibitory activities of compounds **1**−**13** with a *para*-*tert*-butylphenyl group.[a]

Cpd #	Structure	IC_{50} (μM)	Cpd #	Structure	IC_{50} (μM)
1		4.5	**8**		1.3
2		11.0	**9**		9.1
3		14.6	**10**		3.1
4		>50	**11**		3.2
5		>50	**12**		2.1
6		7.0	**13**		5.1
7		5.0			

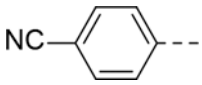
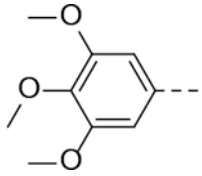
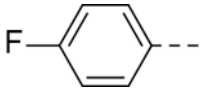
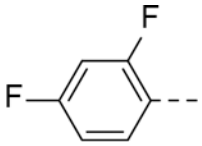
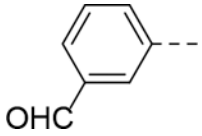
[a]Standard errors of all IC_{50} values are less than 30%.

Table 2.

Structures and ZVpro inhibitory activities of 5-substituted indole-3-carboxamide compounds **14**-**18**.[a]



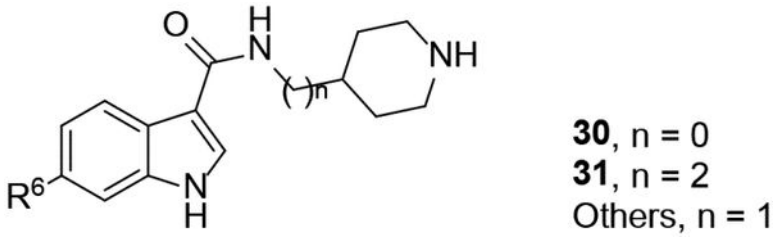
For **14** and **15**, n = 0
16, **17**, and **18**, n = 1

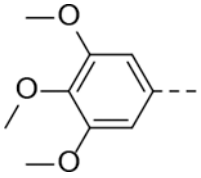
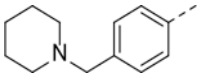
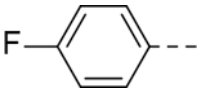
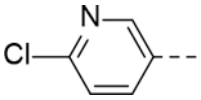
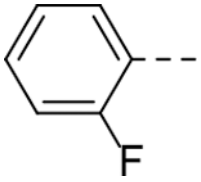
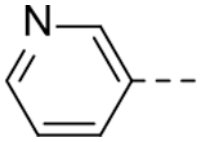
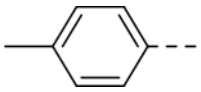
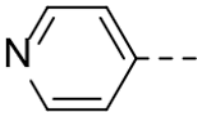
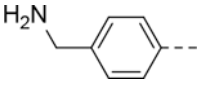
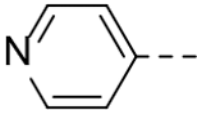
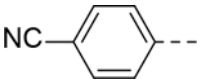
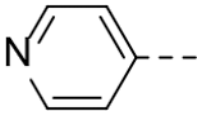
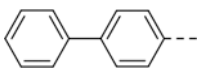
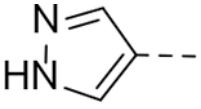
Cpd #	R^5	IC_{50} (μM)
14		>50
15		>50
16		>50
17		>50
18		>50

[a]Standard errors of all IC_{50} values are less than 30%.

Table 3.

Structures and ZVpro inhibitory activities of 6-substituted indole-3-carboxamide compounds **19**-**32**.[a]

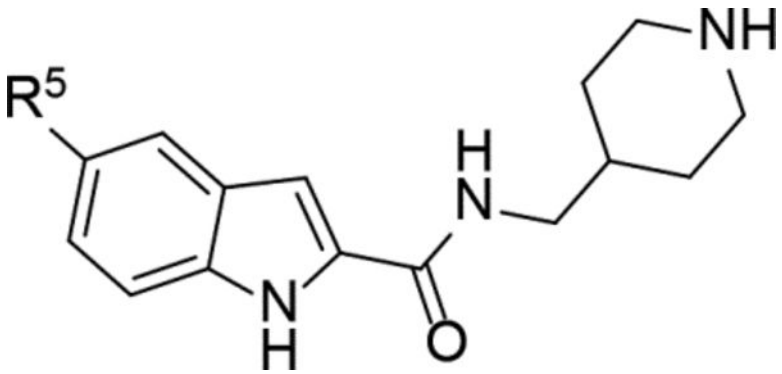


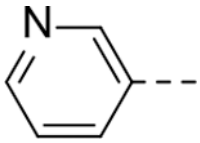
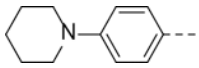
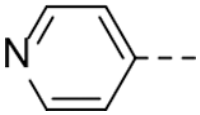
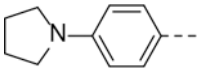
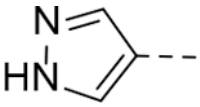
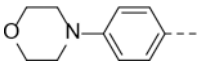
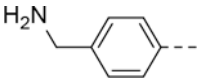
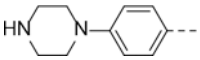
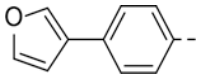
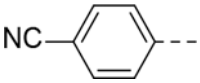
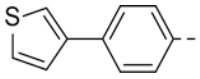
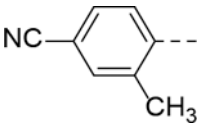
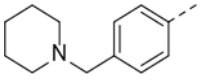
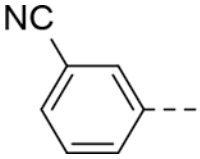
Cpd #	R[6]	IC_{50} (μM)	Cpd #	R[6]	IC_{50} (μM)
19		>50	**26**		3.1
20		>50	**27**		42
21		>50	**28**		1.1
22		48.9	**29**		0.39
23		>50	**30**		1.0
24		21.8	**31**		0.63
25		4.2	**32**		5.7

[a]Standard errors of all IC_{50} values are less than 30%.

Table 4.

Structures and ZVpro inhibitory activities of 5-substituted indole-2-carboxamide compounds **33**-**46**.[a]

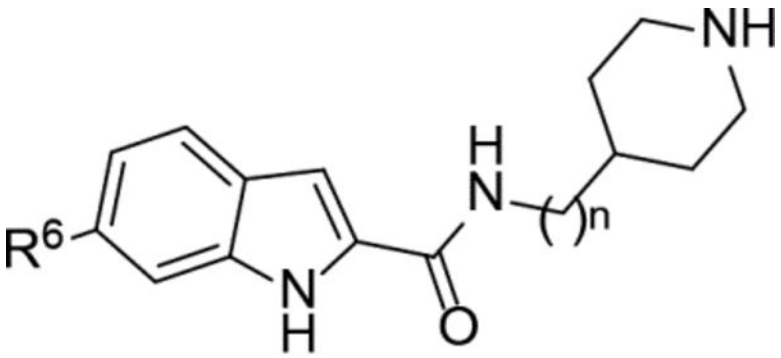


Cpd #	R^5	IC_{50} (μM)	Cpd #	R^5	IC_{50} (μM)
33		>50	**40**		11.4
34		37.0	**41**		3.4
35		6.0	**42**		33.0
36		9.5	**43**		8.8
37		0.87	**44**		9.2
38		1.1	**45**		40.2
39		4.7	**46**		30.5

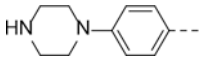
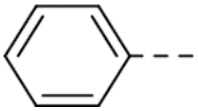
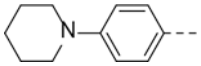
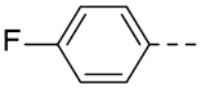
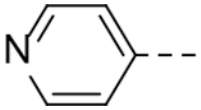
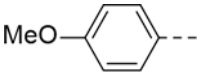
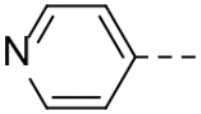
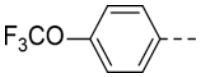
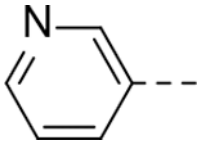
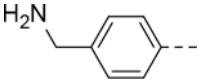
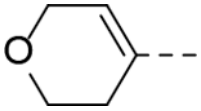
[a]Standard errors of all IC_{50} values are less than 30%.

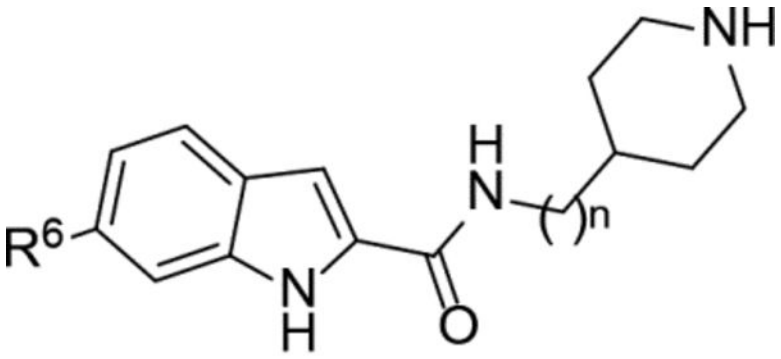
Table 5.

Structures and ZVpro inhibitory activities of 6-substituted indole-2-carboxamide compounds **47**-**66**.[a]

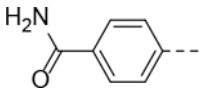
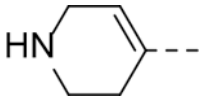
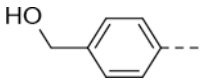
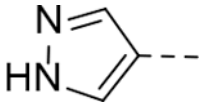
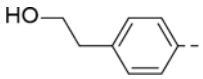
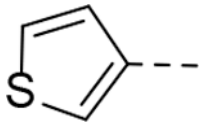
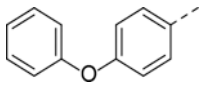
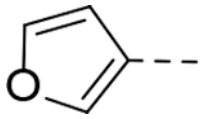


59, n = 0
Others, n = 1

Cpd #	R[6]	IC_{50} (μM)	Cpd #	R[6]	IC_{50} (μM)
47	H	24.6	**57**		5.6
48		9.4	**58**		7.9
49		14.4	**59**		9.8
50		1.0	**60**		19.7
51		3.2	**61**		27.2
52		3.2	**62**		2.2



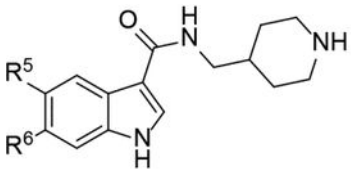
59, n = 0
Others, n = 1

Cpd #	R^6	IC_{50} (μM)	Cpd #	R^6	IC_{50} (μM)
53		7.1	**63**		21.0
54		3.1	**64**		0.99
55		45.6	**65**		6.4
56		1.6	**66**		0.32

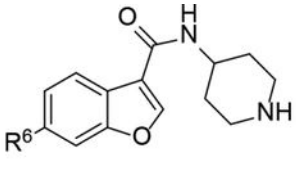
[a]Standard errors of all IC_{50} values are less than 30%.

Table 6.

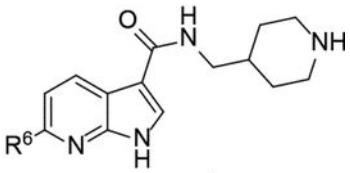
Structures and ZVpro inhibitory activities of compounds **67**–**73**.[a]



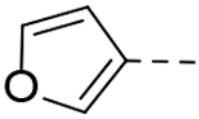
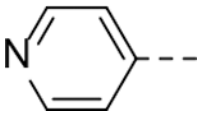
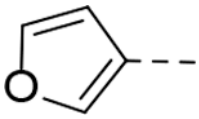
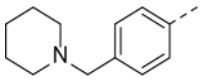
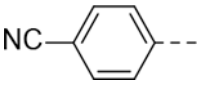
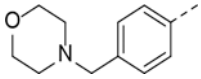
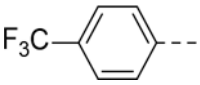
67, $R^5 = R_6$



68, **69**



70, **71**, **72** and **73**

Cpd #	R^6	IC_{50} (μM)	Cpd #	R^6	IC_{50} (μM)
67		0.37	**71**		1.9
68		2.86	**72**		0.45
69		>50	**73**		0.60
70		18.1			

[a]Standard errors of all IC_{50} values are less than 30%.

Table 7.

Inhibitory activity IC_{50} (μM) against Flavivirus proteases ZVpro, DV2pro and WVpro.[a]

	ZVpro	DV2pro	WVpro
66	0.32	1.6	5.7
67	0.37	3.1	3.7
72	0.45	9.2	8.6
64	0.99	10.3	3.5
30	1.0	10.0	10.5
50	1.0	10.6	20.1
8	1.3	15.0	21.8
56	1.6	6.5	6.6
26	3.1	49.7	32.0
51	3.2	10.7	16.0
7	5.0	30.4	35.1
32	5.7	48.3	42.0
40	11.4	40.2	31.0
24	21.8	12.0	50.5
45	40.2	>50	>50
22	48.9	>50	>50

[a]Standard errors of all IC_{50} values are less than 30%.

Table 8.

Antiviral EC_{68} (μM) against ZIKV-FLR in U87 cells.

	ZVpro IC_{50} (μM)	ZIKV-FLR EC_{68} (μM)	U87 Cytotoxicity CC_{50} (μM)
66	0.32	1.0	>10
67	0.37	3.0	>10
72	0.45	10.0	>10
73	0.60	>10	>10
31	0.63	10.0	>10
64	0.99	>10	>10
30	1.0	10.0	>10
56	1.6	2.5	>10
26	3.1	10.0	>10
54	3.1	>10	>10
51	3.2	>10	>10
25	4.2	>10	>10