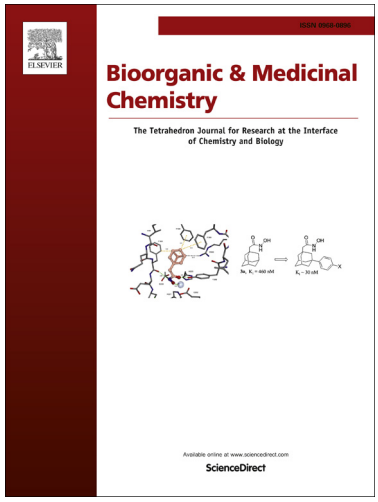

Design, synthesis and biological evaluation of 7-nitro-1*H*-indole-2-carboxylic acid derivatives as allosteric inhibitors of fructose-1,6-bisphosphatase

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Design, synthesis and biological evaluation of 7-nitro-1*H*-indole-2-carboxylic acid derivatives as allosteric inhibitors of fructose-1,6-bisphosphatase

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Abstract: A series of novel indole derivatives was synthesized as inhibitors of Fructose-1,6-bisphosphatase (FBPase). Extensive structure-activity relationships were conducted and led to a potent FBPase inhibitor **3.9** with an IC_{50} of 0.99 μM. The binding mode of this series of indoles was predicted using CDOCKER algorithm. The results of this research will shed light on the further design and optimization of novel small molecules as FBPase inhibitors.

Keywords: Fructose-1, 6-bisphosphatase inhibitor; Allosteric inhibitor; Indole; Diabetes

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease and manifests fasting hyperglycemia and exaggerated postprandial glucose levels. Excessive hepatic glucose output is a key factor leading to fasting hyperglycemia[1] and it also makes a significant contribution to postprandial hyperglycemia in type 2 diabetes patients.[2,3] Gluconeogenesis is a primary process for liver to produce glucose, where three-carbon substrates such as pyruvate, lactate and glycerol are converted into glucose. Fructose-1,6-bisphosphatase (FBPase), acting as a rate-limiting enzyme in gluconeogenesis pathway, can catalyze the hydrolysis of fructose-1,6-bisphosphate into fructose-6-phosphate and inorganic phosphate. It has been demonstrated that FBPase plays an important role in the control of blood glucose since FBPase in liver is elevated in insulin-resistant and insulin-deficient animal models of diabetes.[4,5] Furthermore, FBPase inhibitors have been verified to be capable of reducing hepatic glucose production and lower blood glucose levels in animal models of diabetes.[6-8] Therefore, Inhibition of FBPase may become a new strategy for the development of novel antidiabetic agents.

X-ray crystal structure of FBPase revealed that it is a homotetramer,[9] and each monomer consists of a substrate (fructose-1,6-bisphosphate) binding domain and an allosteric AMP binding domain. Probably due to the more druggable AMP binding site, many efforts have been devoted to the discovery of allosteric inhibitors via both

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high-throughput screening of compound libraries[10-16] and structure-guided design of AMP mimetics.[17-23] Among several classes of known FBPase inhibitors, phosphonic acid-containing thiazoles CS-917 and MB07803 have been advanced to human clinical trials.[8, 20, 21]

An indole-template FBPase inhibitor 3-(2-carboxy-ethyl)-4,6-dichloro-1*H*-indole-2-carboxylic acid[10] (MDL-29951, IC_{50} = 2.5 μM Figure 1) was identified by Pfizer via screening a compound library. In our efforts to search for FBPase inhibitors without a phosphonate or phosphate group, compound MDL-29951 was chosen as a lead structure in view of the fact that it is a small molecule with known drug-like properties. In this work, a series of novel 7-nitro-1*H*-indole-2-carboxylic acid derivatives (**A**, Figure 1) were designed by scrutinizing both the structure-activity relationships (SAR) of the known indole inhibitors and the binding features of AMP in the crystal structure of AMP complexed with FBPase[9] . The indole-2-carboxylic acid essential for the inhibitory activity was retained as a scaffold. A nitro group was tentatively incorporated on 7-position of the indole ring with an aim to construct H-bond interactions with residues Thr31 and/or Val17, which were commonly utilized by many FBPase inhibitors.[6-10,12,19,20,22] In addition, a hydrophobic surface formed by residues Met177, Leu30 and Val160 was explored by several known inhibitors.[6-10,12,19,20] Therefore, variations on 3-, 4- and/or 5-position of 7-nitro-1*H*-indole-2-carboxylic acid with a wide range of hydrophobic substituents were conducted in order to improve the hydrophobic interactions. Herein, the chemical synthesis of these new indole derivatives is described in details. The inhibitory activities against FBPase of these synthetic compounds are presented along with their SAR analysis as follows.

2. Results and discussion

2.1 Chemistry

The synthesis of compounds (**1.6a-1.6f)** was shown in Scheme 1 and their chemical structures were presented in Table 1. (2-Nitrophenyl)hydrazine hydrochloride was converted into hydrozone compound **1.2**, which was further transformed into indole **1.3** under the catalysis of PPA in 41% yield[24]. Regioselective bromination on 3-position of compound **1.3** was accomplished in 85% yield in the presence of NBS in DMF. Compounds **1.5a-1.5f** were achieved via Suzuki coupling of compound **1.4** with various boronic acids in moderate to high yields. The hydrolysis of compounds **1.5a-1.5f** yielded the corresponding carboxylic acids **1.6a-1.6f** in good yields**.**

The chemical structures of target compounds (**2.7a-2.7u, 2.8a-2.8y** and **2.10a-2.10b**) were shown in Table 2 and their preparations were illustrated in Scheme 2. 5-Chloro-4-nitroaniline was converted into (5-chloro-4-nitrophenyl)hydrazine hydrochloride **2.2** via diazotization reaction followed by reduction of diazonium salt with $SnCl_2$/HCl. The hydrazone derivative **2.3** obtained via the condensation of

compound **2.2** with ethyl pyruvate was heated at 80 □ in PPA to give rise to the key indole intermediate **2.4** in 30% yield. Utilizing Suzuki-Miyaura reaction or Buchwald-Hartwig reaction, compound **2.4** was converted into the corresponding C-C and C-N coupling products **2.5** or **2.6** (32-98% yields), respectively, which were hydrolyzed to the carboxylic acid derivatives **2.7a-2.7p** and **2.8a-2.8l** in good yields. The carboxylic acid compounds **2.10a-2.10b** were easily accessed by hydrolysis of esters **2.9a-2.9b**, which were derived from the alkylation of compound **2.5c** with alkyl halides in the yield of 48% and 20%, respectively.

The target compounds (**3.6a-3.6c, 3.9**) were synthesized according to Scheme 3 and their structures were presented in Table 3. Starting from 4-bromo-2-nitroaniline **3.1**, the indole intermediate **3.4** was prepared straightforwardly via Fischer indole synthesis. Suzuki coupling of compound **3.4** with boronic acid derivatives produced compounds **3.5a-3.5c** in moderate to high yields. Upon treatment of compound **3.5a** with NBS in DMF, compound **3.7** was afforded in 93% yield. Suzuki coupling of compound **3.7** with ethylboronic acid was conducted to generate compound **3.8** in 80% yield. The hydrolysis of compounds **3.5a-3.5c** and **3.8** provided the corresponding carboxylic acids **3.6a-3.6c** and **3.9** in reasonable yields**.**

2.2 Biological results and discussion

Recombinant human FBPase activity was assayed by employing the coupling enzymes phosphoglucose isomerase and glucose-6-phosphate dehydrogenase. The concomitant reduction of $NADP^+$ to NADPH was monitored spectrophotometrically.[25] All target compounds (**1.6a-1.6f, 2.7a-2.7p, 2.8a-2.8l, 2.10a-2.10b, 3.6a-3.6c, 3.9**) were tested for their inhibitory activities against human liver FBPase. The corresponding results summarized in Tables 1-3 were expressed as IC_{50} values. AMP and compound MDL-29951 were used as reference molecules.

Variations at 3-position of the indole scaffold were initially conducted and all of the resulted derivatives (**1.6a-1.6f**) displayed inhibitory activities against FBPase as shown in **Table 1**. The ethyl or isobutyl substituted compounds (**1.6a** and **1.6b**) showed inhibition with an IC_{50} value of 6.2 μM and 10.3 μM, respectively. When aromatic substituents were incorporated, the inhibitory activities of compounds **1.6c-1.6f** against FBPase varied with an IC_{50} value ranging from 4.5 μM to 35.6 μM. The 3-fluorophenyl substituted derivative (**1.6f,** IC_{50} **=** 4.5 μM) was the most potent compound in this series.

The SAR was extensively explored on 4-substituted indoles. As shown in Table 2, a wide range of aromatic amino groups (**2.7a-2.7p, 2.10a-2.10b**) and substituted phenyl groups (**2.8a-2.8k**) were directly installed at 4-position of the indole scaffold. In the series of diaryl amines (**2.7a-2.7p, 2.10a-2.10b**), seven compounds were found to be active against FBPase (IC_{50}, 3.4-33.9 μM). Among which, the analogs bearing 4-nitrophenylamino (**2.7l**), 3-methylphenylamino (**2.7m**) or

3,4-dimethoxyphenylamino fragment exhibited potent inhibitory activity, which was comparable to that of AMP. It was observed that the compound with a 3-methoxyphenylamino or 3-methylphenylamino substituent was more potent than that with a 4-methoxyphenylamino or 4-methylphenylamino group (compound **2.7c** *vs* **2.7d**; **2.7m** *vs* **2.7n).** In contrast, the compound bearing a 4-trifluoromethyl or 4-nitro substituent displayed stronger potency against FBPase than that with a 3-trifluoromethyl or 3-nitro group (compound **2.7g** *vs* **2.7h**; **2.7k** *vs* **2.7l).** These results suggested that the placement of a hydrophobic electron-donating group at 3-position and an electron-withdrawing group at 4-position of the phenyl ring would be favorable for potency against FBPase. In addition, no inhibitory effect was detected when phenylamino (**2.7a**), 3-chlorophenylamino (**2.7i**), 4-chlorophenylamino (**2.7j**), 3-acetamidophenylamino (**2.7o**) or 3-aminophenylamino (**2.7p**) was chosen as 4-substituent. Furthermore, in comparison with 3-methoxyphenylamino substituted derivative (**2.7c**), substitutions with methoxyl group at 2- or 4-position (**2.7b** and **2.7d**) led to complete loss of activity. Taken together, it was suggested that the substitution pattern and the electron property and hydrophobicity of the substituents on the phenylamino ring likely play a key role in the inhibitory activity of this series compounds. It was noted that alkylation of the nitrogen atom of the diaryl amine **2.7c** resulted in loss of activity completely (**2.7c** *vs* **2.10a** and **2.10b**), indicating that a hydrogen bond donor (NH) was essential or a bulky group was not tolerated in this position.

Compared to the biaryl amine derivatives, the biaryl compounds (**2.8a-2.8k**) generally produced weaker inhibition**.** In the series of the biaryl compounds**,** only two derivatives with 3-methylphenyl (**2.8g**) and 3-acetamidophenyl (**2.8j**) groups as 4-substituents showed inhibitory activity against FBPase with an IC_{50} value of 25.9 μM and 18.5 μM, respectively. Interestingly, the 4-isobutyl substituted derivative (**2.8l**) had an IC_{50} value of 9.7 μM, which was about 2-fold as potent as that of the biaryl derivatives (**2.8g** and **2.8j**).

The primary SAR investigation was conducted on 5-substituents (R_5). Three distinct groups such as isobutyl, 3-acetamidophenyl and 3-methoxyphenylamino moieties were chosen based on the SAR studies on the 3- and 4-substituents. All designed analogues (**3.6a**-**3.6c**) displayed inhibitory activity against FBPase with IC_{50} values at low micromolar level, indicating that different substituents at 5-position were tolerated. And it was also indicated that the hydrophobic binding site formed by residues Met177, Leu30 and Val160 was more easily accessible by the 5-substituents, compared to the 3- and 4-substituents. Simultaneous modification on 3- and 5-position led to indole derivative **3.9,** which is the most potent FBPase inhibitor in this work with an IC_{50} value of 0.99 μM. As compared with the corresponding monosubstituted analogs **3.6a** and **1.6a**, the inhibitory activity of compound **3.9** was 5-6 fold as active as that of compound **3.6a** and **1.6a**. Therefore, dual substitutions on 3- and 5-position were more benefical than monosubstitution to improve the binding affinity.

In order to get some insight for further structure-based modification of indoles as FBPase inhibitors, molecular modeling was performed using CDOCKER docking program (Accelrys Discovery Studio 2.5.5).[26] The coordinates of the AMP-FBPase complex (pdb code: 1FTA)[9] were employed. The binding modes of most of the evaluated target compounds were similar to that of AMP and exemplified by the most potent compound **3.9** as shown in Figure 2. The key hydrogen bonding interactions were observed between the carboxylate and residues Thr 27, Lys 112 and Arg 140, which were also recognized by the phosphate group in AMP. It was believed that this hydrogen bonding network made crucial contributions to the binding affinity. The indole ring was situated in a hydrophobic pocket involved in residues Leu30 and Leu34. The 7-nitro group interacted with the hydroxyl group of Thr31 via a hydrogen bond. Nevertheless, in comparison with the purine ring in AMP, the indole moiety orientated slightly outward from the purine binding pocket. Therefore, we envisioned that the indole scaffold could occupied the purine pocket more deeply if the carboxylate side chain was extended by 2 or 3-bonds length, and this could facilitate to enhance the hydrophobic contacts. The 3-ethyl and 5-isobutyl groups were positioned at a large hydrophobic site formed by residues Tyr113, Val160 and Met177. Compound **3.9** produced more potency probably because of the more hydrophobic contacts between the alkyl groups and FBPase. It should be addressed that modification on the 3-, 4- and 5-position of the indole ring simultaneously with a variety of substituents might provide more chances to improve the binding affinity.

3. Conclusion

In summary, a series of novel indole-based chemical entities were designed and synthesized as potent FBPase inhibitors. Compound **3.9** was identified as the most potent inhibitor with an IC_{50} value of 0.99 μM. The SAR and the binding mode of the titled compounds were also explored, and that will guide us to develop more potent FBPase inhibitors, which will be reported in due course.

4. Experimental section

4.1. Chemistry

4.1.1. General

Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. ^{1}H NMR (300 MHz or 400 MHz) on a Varian Mercury 300 or 400 spectrometer was recorded in DMSO-d_6, acetone-d_6 or $CDCl_3$. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). High resolution mass spectra (HRMS) were obtained on an Agilent Technologies LC/MSD TOF spectrometer. All chemicals and solvents used were of reagent grade without purified or dried before use. All the reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel G plates at 254 nm under a UV lamp.

Column chromatography separations were performed with silica gel (200-300 mesh).

4.1.2. Synthesis of 3-subsituted-7-nitro-1*H*-indole-2-carboxylic acid derivatives

4.1.2.1 Ethyl 2-(2-(2-nitrophenyl)hydrazono)propanoate (1.2)

To a solution of (2-nitrophenyl)hydrazine hydrochloride (5.0 g, 26.4 mmol) in ethanol (30 mL), ethyl pyruvate (3.7 g, 32 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 10 min, and then poured into ice-water (60 mL). After filtration, the title compound **1.2** was obtained as yellow solid (5.56 g, 82%); mp: 106-107 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.96 (brs, 1H), 8.21 (q, *J* = 8.4 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.62 (t, *J* = 8.0 Hz, 1H), 7.00 (t, *J* = 8.0 Hz, 1H), 4.36 (q, *J* = 7.2 Hz, 2H), 2.25 (s, 3H), 1.40 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{11}H_{14}N_3O_4$ [M+H$^+$]: 252.0979, found 252.0986.

4.1.2.2. Ethyl 7-nitro-1*H*-indole-2 -carboxylate (1.3)[24]

The reaction mixture of compound **1.2** (5.3 g, 21 mmol) in PPA (26.5 g) was heated at 50-60□ for 4 h and then heated at 80 □ for 3 h. The reactiong mixture was cooled to room temperature and water (30 mL) was added to the mixture to destroy the PPA. The resulting solution was extracted with EtOAc (40 mL × 2). The crude product obtained after concentration was purified with column chromatography to afford the title compound **1.3** (2.02 g, 41%) as light yellow solid; mp: 94-95 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.34 (brs, 1H), 8.30 (d, *J* = 8.0 Hz, 1H), 8.05 (d, *J* = 7.6 Hz, 1H), 7.36 (s, 1H), 7.28 (t, *J* = 7.6 Hz, 1H), 4.47 (q, *J* = 7.2 Hz, 2H), 1.45 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{11}H_{11}N_2O_4$ [M+H$^+$]: 235.0713, found 235.0722.

4.1.2.3. Ethyl 3-bromo-7-nitro-1*H*-indole-2 -carboxylate (1.4)

A solution of NBS (84 mg, 0.47 mmol) in DMF (2 mL) was added dropwise to a solution of **1.3** (100 mg, 0.43 mmol) in DMF (2 mL), which was cooled by an ice-water bath. After addition, the reaction mixture was stirred at room temperature for 1.5 hours, and then poured into ice-water (15 mL). After filtration, the title compound **1.4** was afforded as off-white solid (115 mg, 85%); mp: 135-137 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.41 (brs, 1H), 8.37 (d, *J* = 8.1 Hz, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 7.37 (t, *J* = 7.8 Hz, 1H), 4.52 (q, *J* = 7.2 Hz, 2H), 1.49 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{11}H_{10}BrN_2O_4$ [M+H$^+$]: 312.9819, found 312.9815.

4.1.2.4. Synthesis of ethyl 3-substituted-7-nitro-1*H*-indole-2-carboxylate (1.5a-1.5f)

4.1.2.4.1. Ethyl 3-ethyl-7-nitro-1*H*-indole-2 -carboxylate (1.5a)

To a solution of **1.4** (200 mg, 0.64 mmol) in toluene (15 mL), Pd $(OAc)_2$ (7 mg, 0.032 mmol), Josiphos ligand (20 mg, 0.032 mmol), K_3PO_4 (679 mg, 3.2 mmol, 1mL H_2O) and ethylboronic acid (142 mg, 1.92 mmol) were sequentially added. The reaction mixture was heated at 80 ℃ for 4 hours under argon atmosphere, and then concentrated under reduced pressure. The residue was dissolved in EtOAc (40 mL), and washed with brine (20 mL×3) and water (20 mL×3). The crude product obtained after concentration was purified with column chromatography to afford the product **1.5a** as off-white solid (165 mg, 99%); mp: 94-96 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.13 (brs, 1H), 8.29 (d, *J* = 8.0 Hz, 1H), 8.05 (d, *J* = 8.0 Hz, 1H), 7.25 (t, *J* = 8.0 Hz, 1H), 4.47 (q, *J* = 7.2 Hz, 2H), 3.16 (q, *J* = 7.2 Hz, 2H), 1.46 (t, *J* = 6.8 Hz, 3H), 1.30 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{13}H_{15}N_2O_4$ [M+H$^+$]: 263.1026, found 263.1024.

4.1.2.4.2. Ethyl 3-isobutyl-7-nitro-1*H*-indole-2-carboxylate (1.5b) bjb-1301

Following the procedure of 4.1.2.4.1, starting from compound **1.4** (200 mg, 0.64 mmol), the title compound **1.5b** was obtained as yellow solid (177 mg, 85%); mp: 95-96 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.19 (brs, 1H), 8.29 (d, J = 8.1 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.24 (t, J = 8.1 Hz, 1H), 4.46 (q, J = 7.2 Hz, 2H), 3.01 (d, J = 7.5 Hz, 2H), 1.98-2.07 (m, 1H), 1.46 (t, J = 7.2 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{19}N_2O_4$ [M+H$^+$]: 291.1339, found 291.1335.

4.1.2.4.3. Ethyl 7-nitro-3-phenyl-1*H*-indole-2 -carboxylate (1.5c)

To a solution of **1.4** (200 mg, 0.64 mmol) in 1,4-dioxane (15 mL), $PdCl_2$(dppf) (47 mg, 0.064 mmol), Na_2CO_3 (203 mg, 1.92 mmol, 2 mL H_2O) and phenylboronic acid (234 mg, 1.92 mmol) were sequentially added. The reaction mixture was heated at 100 ℃ for 2 hours under argon atmosphere, and then concentrated under reduced pressure. The residue was dissolved in EtOAc (40 mL), and washed with brine (20 mL×3) and water (20 mL×3). The crude product obtained after concentration was purified with column chromatography to afford the desired product **1.5c** as yellow solid (250 mg, 84%); mp: 118-120 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.42 (brs, 1H), 8.34 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.42-7.54 (m, 5H), 7.26 (t, J = 8.0 Hz, 1H), 4.35 (q, J = 7.2 Hz, 2H), 1.27 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{15}N_2O_4$ [M+H$^+$]: 311.1026, found 311.1019.

4.1.2.4.4. Ethyl 3-methoxyphenyl-7-nitro-1*H*-indole-2 -carboxylate (1.5d)

Following the procedure of 4.1.2.4.3, starting from compound **1.4** (200 mg, 0.64 mmol), the title compound **1.5d** was afforded as light yellow solid (185 mg, 85%); mp: 114-115 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.41 (s, 1H), 8.33 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.26 (t, J = 8.0 Hz, 1H), 7.10 (t, J = 7.6 Hz, 1H), 7.07 (s, 1H), 6.99 (d, J = 8.0 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H), 3.86 (s, 3H), 1.29 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{17}N_2O_5$ [M+H$^+$]: 341.1132, found 341.1126.

4.1.2.4.5. Ethyl 3-acetamidophenyl-7-nitro-1*H*-indole-2-carboxylate (1.5e)

Following the procedure of 4.1.2.4.3, starting from compound **1.4** (170 mg, 0.54 mmol), the title compound **1.5e** was afforded as yellow solid (150 mg, 58%), mp: 150-151 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.41 (s, 1H), 8.33 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.74 (s, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.43 (t, J = 8.0 Hz, 1H), 7.24-7.31 (m, 2H), 4.36 (q, J = 7.2 Hz, 2H), 2.19 (s, 3H), 1.29 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{19}H_{18}N_3O_5$ [M+H$^+$]: 368.1236, found 368.1241.

4.1.2.4.6. Ethyl 3-fluorophenyl-7-nitro-1*H*-indole-2 -carboxylate (1.5f)

Following the procedure of 4.1.2.4.3, starting from compound **1.4** (220 mg, 0.70 mmol), the title compound **1.5f** was obtained as light yellow solid (210 mg, 91%); mp: 124-126 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.45 (s, 1H), 8.35 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.10 (dd, J_1 = 14.0 Hz, J_2 = 7.6 Hz, 1H), 7.24-7.31 (m, 3H), 7.15 (t, J = 8.4 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H), 1.29 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{14}FN_2O_4$ [M+H$^+$]: 329.0932, found 329.0926.

4.1.2.5. Synthesis of 3-substituted-7-nitro-1*H*-indole-2-carboxylic acid (1.6a-1.6f)

4.1.2.5.1. 3-ethyl-7-nitro-1*H*-indole-2-carboxylic acid (1.6a)

To a solution of **1.5a** (100 mg, 0.38 mmol) in THF (4 mL) and ethanol (2 mL), a solution of NaOH (76 mg, 1.9 mmol) in water (2 mL) was added dropwise. The

resulting mixture was stirred at room temperature for overnight, and then concentrated under reduced pressure. The residue was dissolved in water (10 mL), which was acidified to pH=3-4 with diluted HCl. After filtration, the title compound **1.6a** was obtained as light yellow solid (60 mg, 67%); mp: 231-233 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.58 (brs, 1H), 10.58 (s, 1H), 8.28 (d, J = 8.0 Hz, 2H), 7.34 (t, J = 8.0 Hz, 1H), 3.12 (q, J = 7.2 Hz, 2H), 1.22 (t, J = 7.6 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{11}H_{11}N_2O_4$ [M+H$^+$]: 235.0713, found 235.0710.

4.1.2.5.2. 3-isobutyl-7-nitro-1*H*-indole-2-carboxylic acid (1.6b)

Following the procedure of 4.1.2.5.1, starting from compound **1.5b** (120 mg, 0.41 mmol), the title compound **1.6b** was obtained as light yellow solid (60 mg, 56%); mp: 185-187 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.54 (s, 1H), 10.60 (s, 1H), 8.27 (d, J = 8.8 Hz, 1H), 8.24 (d, J = 8.4 Hz, 1H), 7.33 (t, J = 8.0 Hz, 1H), 2.99 (d, J = 6.8 Hz, 2H), 1.93-1.99 (m, 1H), 0.89 (d, J = 6.4 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{13}H_{15}N_2O_4$ [M+H$^+$]: 263.1026, found 263.1023.

4.1.2.5.3. 7-nitro-3-phenyl-1*H*-indole-2-carboxylic acid (1.6c)

Following the procedure of 4.1.2.5.1, starting from compound **1.5b** (127 mg, 0.41 mmol), the title compound **1.6c** was obtained as off-white solid (110 mg, 95%); mp: 244-245 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.49 (brs, 1H), 11.03 (s, 1H), 8.33 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.41-7.54 (m, 5H), 7.37 (t, J = 8.0 Hz, 1H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{11}N_2O_4$ [M+H$^+$]: 283.0713, found 283.0708.

4.1.2.5.4. 3-methoxyphenyl-7-nitro-1*H*-indole-2-carboxylic acid (1.6d)

Following the procedure of 4.1.2.5.1, starting from compound **1.5d** (120 mg, 0.35 mmol), the title compound **1.6d** was obtained as light yellow solid (110 mg, 100%); mp: 219-221 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.04 (s, 1H), 8.33 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.41 (t, J = 8.4 Hz, 1H), 7.37 (t, J = 8.0 Hz, 1H), 7.09 (d, J = 7.2 Hz, 1H), 7.08 (s, 1H), 7.00 (d, J = 8.8 Hz, 1H), 3.80 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{13}N_2O_5$ [M+H$^+$]: 313.0819, found 313.0813.

4.1.2.5.5. 3-acetamidophenyl-7-nitro-1*H*-indole-2-carboxylic acid (1.6e)

Following the procedure of 4.1.2.5.1, starting from compound **1.5e** (80 mg, 0.22 mmol), the title compound **1.6e** was obtained as light yellow solid (80 mg, 100%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.06 (s, 1H), 10.05 (s, 1H), 8.33 (d, J = 8.0 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.74 (s, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.36-7.42 (m, 2H), 7.19 (d, J = 8.0 Hz, 1H), 2.06 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{14}N_3O_5$ [M+H$^+$]: 340.0928, found 340.0922.

4.1.2.5.6. 3-fluorophenyl-7-nitro-1*H*-indole-2-carboxylic acid (1.6f)

Following the procedure of 4.1.2.5.1, starting from compound **1.5f** (120 mg, 0.37 mmol), the titile compound **1.6f** was obtained as light yellow solid (90 mg, 82%), mp: 195-196 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.59 (s, 1H), 11.11 (s, 1H), 8.33 (d, J = 8.0 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.52 (dd, J_1 = 14.8 Hz, J_2 = 8.0 Hz, 1H), 7.35-7.39 (m, 3H), 7.26 (t, J = 8.4 Hz, 1H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{10}FN_2O_4$ [M+H$^+$]: 301.0619, found 301.1403.

4.1.3. Synthesis of 4-subsituted-7-nitro-1*H*-indole-2-carboxylic acid derivatives

4.1.3.1. (5-chloro-2-nitrophenyl)hydrazine hydrochloride (2.2)

A solution of 5-chloro-2-nitroaniline (5.0 g, 2.9 mmol) in concentrated HCl (57.2 g,

580 mmol) was heated at 40 □ for 2 h. The reaction mixture was cooled to -10 □, and then a solution of $NaNO_2$ (2.1 g, 30.5 mmol) in water (20 mL) was added dropwise. After stirring for 2 h, the mixture was cooled to -30 □ and added to a solution of $SnCl_2$ (19.6 g, 87 mmol) in concentrated HCl (30 mL) precooled to -30 □. The reaction mixture was stirred at -20 □ for 1, and then warmed to room temperature and filtered. The title compound **2.2** was obtained as light yellow solid (7.0 g, 100%); mp: 178-180 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 9.39 (brs, 1H), 8.15 (d, J = 8.8 Hz, 1H), 7.45 (s, 1H), 7.06 (d, J = 8.4 Hz, 1H), 3.83 (brs, 3H); HRMS (ESI): *m/z*, calcd for $C_6H_7ClN_3O_2$ [M+H$^+$]: 188.0221, found 188.0220.

4.1.3.2. Ethyl 2-(2-(5-chloro-2-nitrophenyl)hydrazono)propanoate (2.3)

To a solution of **2.2** (1.0 g, 4.46 mmol) in ethanol (20 mL), ethyl pyruvate (0.54 g, 4.69 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 5 min, and then poured into ice-water (60 mL). After filtration, the title compound **2.3** was obtained as yellow solid (469 mg, 37%); mp: 107-109 □; ^{1}H NMR (acetone-d_6) δ (ppm): 10.82 (br, 1H), 8.24 (d, J = 8.8 Hz, 1H), 7.95 (s, 1H), 7.10 (dd, J_1 = 9.2 Hz, J_2 = 1.6 Hz, 1H), 4.32 (q, J = 7.2 Hz, 2H), 2.26 (s, 3H), 1.35 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{11}H_{13}ClN_3O_4$ [M+H$^+$]: 286.0589, found 286.0582.

4.1.3.3. Ethyl 4-chloro-7-nitro-1*H*-indole-2-carboxylate (2.4)

The reaction mixture of compound **2.3** (2.84 g, 9.94 mmol) in PPA (57 g) was heated at 80℃ for 12 h, and then cooled to room temperature. Water (30 mL) was added to the mixture to destroy the PPA, and the residue was extracted with EtOAc (40 mL×2). The crude product obtained after concentration was purified with column chromatography to afford **2.4** (810 mg, 30%) as light yellow solid; mp: 170-171 □; ^{1}H NMR (acetone-d_6) δ (ppm): 11.01 (brs, 1H), 8.31 (d, J = 8.7 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.40 (s, 1H), 4.45 (q, J = 7.2 Hz, 2H), 1.42 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{11}H_{10}ClN_2O_4$ [M+H+]: 269.0324, found 269.0318.

4.1.3.4. Synthesis of ethyl 4-substituted-7-nitro-1*H*-indole-2-carboxylate

4.1.3.4.1. Ethyl 7-nitro-4-phenylamino-1*H*-indole-2-carboxylate (2.5a)

To a solution of **2.4** (50 mg, 0.19 mmol) in 1,4-dioxane (10 mL), $Pd_2(dba)_3$ (17 mg, 0.019 mmol), Xantphos (22 mg, 0.038 mmol) and t-BuONa (55 mg, 0.57 mmol) and aniline (53 mg, 0.57 mmol) were added in order. The reaction mixture was refluxed for 3 h under argon atmosphere, and then concentrated under reduced pressure. The residue was dissolved in EtOAc (20 mL), and washed with brine (10 mL×3) and water (10 mL×3). The crude product obtained after concentration was purified with column chromatography to afford product **2.6a** as yellow solid (42 mg, 65%); mp: 211-212 □; ^{1}H NMR (acetone-d_6) δ (ppm): 10.59 (brs, 1H), 8.85 (brs, 1H), 8.17 (d, J = 9.0 Hz, 1H), 7.69 (s, 1H), 7.46-7.51 (m, 4H), 7.22-7.27 (m, 1H), 6.84-6.88 (m, 1H), 4.41 (q, J = 7.5 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{16}N_3O_4$ [M+H$^+$]: 326.1135, found 326.1126.

4.1.3.4.2. Ethyl 4-(2-methoxyphenylamino)-7-nitro-1*H*-indole-2-carboxylate (2.5b)

To a solution of **2.4** (250 mg, 0.93 mmol) in toluene (15 mL), $Pd_2(dba)_3$ (85 mg, 0.093 mmol), Xantphos (108 mg, 0.19 mmol) and Na_2CO_3 (296 mg, 2.79 mmol, 2 mL H_2O) and 2-methoxyaniline (344 mg, 2.79 mmol) were added in order. The reaction mixture was refluxed for 4 h under argon atmosphere, and then concentrated under

reduced pressure. The residue was dissolved in EtOAc (30 mL), and washed with brine (20 mL×3) and water (20 mL×3). The crude product obtained after concentration was purified with column chromatography to afford product **2.6b** as yellow solid (270 mg, 82%); mp: 137-139 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.46 (brs, 1H), 8.20 (d, *J* = 9.2 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 1.2 Hz, 1H), 7.16 (t, *J* = 8.0 Hz, 2H), 7.00-7.04 (m, 3H), 6.92 (d, *J* = 8.8 Hz, 1H), 4.46 (q, *J* = 7.2 Hz, 2H), 3.92 (s, 3H), 1.45 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{18}N_3O_5$ [M+H$^+$]: 356.1241, found 356.1231.

4.1.3.4.3. Ethyl 4-(3-methoxyphenylamino)-7-nitro-1*H*-indole-2-carboxylate (2.5c)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (220 mg, 0.82 mmol), the title compound **2.5c** was obtained as yellow solid (285 mg, 98%); mp: 188-190 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.94 (s, 1H), 9.64 (s, 1H), 8.13 (d, *J* = 9.3 Hz, 1H), 7.84 (s, 1H), 7.35 (t, *J* = 7.8 Hz, 1H), 6.96 (d, *J* = 7.8 Hz, 1H), 6.92-6.93 (m, 1H), 6.77-6.82 (m, 2H), 4.37 (q, *J* = 7.5 Hz, 2H), 3.78 (s, 3H), 1.36 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{18}N_3O_5$ [M+H$^+$]: 356.1241, found 356.1231.

4.1.3.4.4. Ethyl 4-(4-methoxyphenylamino)-7-nitro-1*H*-indole-2-carboxylate (2.5d)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (350 mg, 1.30 mmol), the title compound **2.5d** was obtained as yellow solid (389 mg, 84%); mp: 209-211 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.47 (brs, 1H), 8.14 (d, *J* = 9.3 Hz, 1H), 7.33 (d, *J* = 2.4 Hz, 1H), 7.25 (d, *J* = 8.7 Hz, 2H), 6.98 (d, *J* = 9.0 Hz, 1H), 6.72 (brs, 1H), 6.50 (d, *J* = 9.0 Hz, 1H), 4.43 (q, *J* = 7.2 Hz, 2H), 3.86 (s, 1H), 1.43 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{18}N_3O_5$ [M+H$^+$]: 356.1231, found 356.1241.

4.1.3.4.5. Ethyl 4-(3,4-dimethoxyphenylamino)-7-nitro-1*H*-indole-2-carboxylate (2.5e)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (300 mg, 1.12 mmol), the title compound **2.5e** was obtained as orange-red solid (420 mg, 98%); mp: 205-206 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.46 (brs, 1H), 8.15 (d, *J* = 9.0 Hz, 1H), 7.35 (d, *J* = 1.8 Hz, 1H), 6.78-6.95 (m, 4H), 6.55 (d, *J* = 8.7 Hz, 1H), 4.43 (q, *J* = 7.2 Hz, 2H), 3.93 (s, 1H), 3.88 (s, 3H), 1.42 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{19}H_{20}N_3O_6$ [M+H$^+$]: 386.1347, found 386.1333.

4.1.3.4.6. Ethyl 4-(3,4,5-trimethoxyphenylamino)-7-nitro-1*H*-indole-2-carboxy -late (2.5f)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (200 mg, 0.74 mmol), the title compound **2.5f** was obtained as orange-red solid (292 mg, 94%); mp: 179-181 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.46 (brs, 1H), 8.18 (d, *J* = 9.2 Hz, 1H), 7.35 (d, *J* = 1.2 Hz, 1H), 6.75 (brs, 1H), 6.70 (d, *J* = 9.2 Hz, 1H), 6.56 (s, 2H), 4.44 (q, *J* = 7.2 Hz, 2H), 3.89 (s, 1H), 3.86 (s, 6H), 1.43 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{20}H_{22}N_3O_7$ [M+H$^+$]: 416.1452, found 416.1439.

4.1.3.4.7. Ethyl 4-(3-(trifluoromethyl)phenylamino)-7-nitro-1*H*-indole-2-carboxy -late (2.5g)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (250 mg, 0.93 mmol), the title compound **2.5g** was obtained as light yellow solid (360 mg, 98%); mp >250 □; ^{1}H NMR (acetone-d_6) δ (ppm): 10.62 (s, 1H), 8.96 (s, 1H), 8.21 (d, *J* =

9.2 Hz, 1H), 7.67-7.80 (m, 4H), 7.52 (d, *J* = 7.6 Hz, 1H), 6.99-7.01 (m, 1H), 4.41 (q, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{15}F_3N_3O_4$ [M+H$^+$]: 394.1009, found 394.0997.

4.1.3.4.8. Ethyl 4-(4-(trifluoromethyl)phenylamino)-7-nitro-1*H*-indole-2-carboxy -late (2.5h)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (200 mg, 0.74 mmol), the title compound **2.5h** was obtained as yellow solid (250 mg, 86%); mp: 239-241 □; ^{1}H NMR (acetone-d_6) δ (ppm): 10.67 (brs, 1H), 9.05 (s, 1H), 8.23 (d, *J* = 8.8 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.69 (s, 1H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.12-7.15 (m, 1H), 4.41 (q, *J* = 7.2 Hz, 2H), 1.39 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{15}F_3N_3O_4$ [M+H$^+$]: 394.1009, found 394.0997.

4.1.3.4.9. Ethyl 4-(3-chlorophenylamino)-7-nitro-1*H*-indole-2-carboxylate (2.5i)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (200 mg, 0.74 mmol), the title compound **2.5i** was obtained as light yellow solid (257 mg, 96%); mp: 220-221 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.01 (s, 1H), 9.67 (s, 1H), 8.16 (d, *J* = 8.8 Hz, 1H), 7.81 (s, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.42 (s, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 7.6 Hz, 1H), 6.84 (d, *J* = 8.8 Hz, 1H), 4.37 (q, *J* = 7.5 Hz, 2H), 1.36 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{15}ClN_3O_4$ [M+H$^+$]: 360.0746, found 360.0736.

4.1.3.4.10. Ethyl 4-(4-chlorophenylamino)-7-nitro-1*H*-indole-2-carboxylate (2.5j)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (200 mg, 0.74 mmol), the title compound **2.5j** was obtained as yellow solid (265 mg, 99%); mp: 244-245 □; ^{1}H NMR (acetone-d_6) δ (ppm): 10.63 (brs, 1H), 8.87 (s, 1H), 8.19 (d, *J* = 9.2 Hz, 1H), 7.69 (s, 1H), 7.48 (m, 4H), 6.89-6.91 (m, 1H), 4.41 (q, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 7.2 Hz, 3H) ; HRMS (ESI): *m/z*, calcd for $C_{17}H_{15}ClN_3O_4$ [M+H$^+$]: 360.0746, found 360.0736.

4.1.3.4.11. Ethyl 4-(3-nitrophenylamino)-7-nitro-1*H*-indole-2-carboxylate (2.5k)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (220 mg, 0.82 mmol), the title compound **2.5k** was obtained as light yellow solid (265 mg, 87%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.05 (s, 1H), 9.84 (s, 1H), 8.17 (d, *J* = 9.2 Hz, 1H), 8.14 (s, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.79 (s, 1H), 7.69 (t, *J* = 8.0 Hz, 1H), 6.96 (d, *J* = 8.8 Hz, 1H), 4.37 (q, *J* = 7.2 Hz, 2H), 1.36 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{15}N_4O_6$ [M+H$^+$]: 371.0986, found 371.0981.

4.1.3.4.12. Ethyl 4-(4-nitrophenylamino)-7-nitro-1*H*-indole-2-carboxylate (2.5l)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (220 mg, 0.82 mmol), the title compound **2.5l** was obtained as yellow solid (242 mg, 84%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.16 (s, 1H), 10.00 (s, 1H), 8.24 (d, *J* = 8.8 Hz, 2H), 8.21 (d, *J* = 9.2 Hz, 1H), 7.78 (s, 1H), 7.53 (d, *J* = 9.2 Hz, 2H), 7.15 (d, *J* = 8.8 Hz, 1H), 4.37 (q, *J* = 7.2 Hz, 2H), 1.36 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{14}N_4O_6$ [M+H$^+$]: 371.0986, found 371.0973.

4.1.3.4.13. Ethyl 4-(3-methylphenylamino)-7-nitro-1*H*-indole-2-carboxylate (2.5m)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (220 mg, 0.82

mmol), the title compound **2.5m** was obtained as yellow solid (260 mg, 93%); mp: 190-191 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.89 (s, 1H), 9.62 (s, 1H), 8.13 (d, J = 9.2 Hz, 1H), 7.83 (s, 1H), 7.33 (t, J = 8.0 Hz, 1H), 7.16-7.19 (m, 2H), 7.04 (d, J = 7.6 Hz, 1H), 6.72 (d, J = 9.2 Hz, 1H), 4.37 (q, J = 7.2 Hz, 2H), 2.34 (s, 3H), 1.36 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{18}N_3O_4$ [M+H$^+$]: 340.1292, found 340.1289.

4.1.3.4.14. Ethyl 4-(4-methylphenylamino)-7-nitro-1*H*-indole-2-carboxylate (2.5n)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (250 mg, 0.93 mmol), the title compound **2.5m** was obtained as yellow solid (256 mg, 81%); mp: 232-234 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.47 (brs, 1H), 8.16 (d, J = 9.2 Hz, 1H), 7.33 (s, 1H), 7.25 (d, J = 7.2 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 6.71 (brs, 1H), 6.65 (d, J = 8.8 Hz, 2H), 4.44 (q, J = 7.2 Hz, 2H), 2.40 (s, 3H), 1.43 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{18}N_3O_4$ [M+H$^+$]: 340.1292, found 340.1281.

4.1.3.4.15. Ethyl 4-(3-(acetamidophenyl)amino)-7-nitro-1*H*-indole-2-carboxylate (2.5o)

Following the procedure of 4.1.3.4.2, starting from compound **2.4** (220 mg, 0.82 mmol), the title compound **2.5o** was obtained as brick-red solid (170 mg, 54%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.91 (s, 1H), 10.05 (s, 1H), 9.69 (s, 1H), 8.12 (d, J = 9.3 Hz, 1H), 7.85-7.86 (m, 1H), 7.79 (s, 1H), 7.29-7.35 (m, 2H), 7.04 (d, J = 6.9 Hz, 1H), 6.77 (d, J= 9.3 Hz, 1H), 4.37 (q, J = 7.2 Hz, 2H), 2.05 (s, 3H), 1.35 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{19}H_{19}N_4O_5$ [M+H$^+$]: 383.1350, found 383.1336.

4.1.3.5. Synthesis of 4-substituted-7-nitro-1*H*-indole-2-carboxylic acid (2.7a-2.7p)

4.1.3.5.1. 7-nitro-4-phenylamino-1*H*-indole-2-carboxylic acid (2.7a)

Following the procedure of 4.1.2.5.1, starting from compound **2.5a** (100 mg, 0.31 mmol), the title compound **2.7a** was obtained as orange solid (70 mg, 76%); mp: 237-240 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.37 (brs, 1H), 10.72 (s, 1H), 9.65 (s, 1H), 8.11 (d, J = 9.2 Hz, 1H), 7.74 (s, 1H), 7.46 (t, J = 7.6 Hz, 2H), 7.38 (d, J = 7.6 Hz, 1H), 7.22 (t, J = 6.8 Hz, 1H), 6.71 (q, J = 9.2 Hz, 1H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{12}N_3O_4$ [M+H$^+$]: 298.0822, found 298.0811.

4.1.3.5.2. 4-(2-methoxyphenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7b)

Following the procedure of 4.1.2.5.1, starting from compound **2.5b** (180 mg, 0.51 mmol), the title compound **2.7b** was obtained as orange solid (130 mg, 78%); mp: 245-246 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.65 (s, 1H), 9.38 (s, 1H), 8.05 (d, J = 9.2 Hz, 1H), 7.70 (s, 1H), 7.33-7.37 (m, 2H), 7.21 (d, J = 8.0 Hz, 1H), 7.06 (t, J = 7.6 Hz, 1H), 6.14 (d, J = 9.2 Hz, 1H), 3.78 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{14}N_3O_5$ [M+H$^+$]: 328.0928, found 328.0923.

4.1.3.5.3. 4-(3-methoxyphenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7c)

Following the procedure of 4.1.2.5.1, starting from compound **2.5c** (140 mg, 0.39 mmol), the title compound **2.7c** was obtained as orange solid (102 mg, 79%); mp: 217-219 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.73 (s, 1H), 9.62 (s, 1H), 8.12 (d, J = 9.2 Hz, 1H), 7.75 (s, 1H), 7.35 (t, J = 8.0 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 6.92 (s, 1H), 6.77-6.80 (m, 2H), 3.77 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{14}N_3O_5$ [M+H$^+$]: 328.0928, found 328.0921.

4.1.3.5.4. 4-(4-methoxyphenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7d)

Following the procedure of 4.1.2.5.1, starting from compound **2.5d** (350 mg, 0.99 mmol), the title compound **2.7d** was obtained as orange solid (300 mg, 99%); mp: 254-255 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.59 (s, 1H), 9.55 (s, 1H), 8.05 (d, J = 9.3 Hz, 1H), 7.61 (s, 1H), 7.31 (d, J = 8.4 Hz, 2H), 7.04 (d, J = 8.4 Hz, 2H), 6.48 (d, J = 9.3 Hz, 1H), 3.80 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{14}N_3O_5$ [M+H$^+$]: 328.0928, found 328.0921.

4.1.3.5.5. 4-(3,4-dimethoxyphenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7e)

Following the procedure of 4.1.2.5.1, starting from compound **2.5e** (320 mg, 0.83 mmol), the title compound **2.7e** was obtained as sorrel solid (291 mg, 98%); mp: 267-269 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.34 (brs, 1H), 10.69 (s, 1H), 9.61 (s, 1H), 8.09 (d, J = 9.3 Hz, 1H), 7.72 (d, J = 2.1 Hz, 1H), 7.04 (d, J = 8.7 Hz, 1H), 6.91-6.97 (m, 2H), 6.58 (d, J = 9.3 Hz, 1H), 3.79 (s, 3H), 3.77 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{16}N_3O_6$ [M+H$^+$]: 358.1034, found 358.1029.

4.1.3.5.6. 4-(3,4,5-trimethoxyphenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7f)

Following the procedure of 4.1.2.5.1, starting from compound **2.5f** (190 mg, 0.46 mmol), the title compound **2.7f** was obtained as sorrel solid (145 mg, 82%); mp: 278-280 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.73 (brs, 1H), 9.66 (s, 1H), 8.12 (d, J = 9.0 Hz, 1H), 7.76 (d, J = 2.1 Hz, 1H), 6.78 (d, J = 9.3 Hz, 1H), 6.70 (s, 2H), 3.80 (s, 6H), 3.69 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{18}N_3O_7$ [M+H$^+$]: 388.1139, found 388.1133.

4.1.3.5.7. 7-nitro-4-(3-(trifluoromethyl)phenylamino)-1*H*-indole-2-carboxylic acid (2.7g)

Following the procedure of 4.1.2.5.1, starting from compound **2.5g** (124 mg, 0.32 mmol), the title compound **2.7g** was obtained as sorrel solid (42 mg, 37%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.81 (s, 1H), 9.75 (s, 1H), 8.16 (d, J = 9.2 Hz, 1H), 7.64-7.71 (m, 4H), 7.51 (d, J = 7.2 Hz, 1H), 6.85 (d, J = 9.2 Hz, 1H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{11}F_3N_3O_4$ [M+H$^+$]: 366.0696, found 366.0694.

4.1.3.5.8. 7-nitro-4-(4-(trifluoromethyl)phenylamino)-1*H*-indole-2-carboxylic acid (2.7h)

Following the procedure of 4.1.2.5.1, starting from compound **2.5h** (140 mg, 0.36 mmol), the title compound **2.7h** was obtained as orange solid (90 mg, 69%); mp: 244-246 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.41 (brs, 1H), 10.84 (s, 1H), 9.79 (s, 1H), 8.15 (d, J = 8.8 Hz, 1H), 7.74-7.76 (m, 3H),7.55 (d, J = 8.0 Hz, 2H), 6.98 (d, J = 8.8 Hz, 1H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{11}F_3N_3O_4$ [M+H$^+$]: 366.0696, found 366.0694.

4.1.3.5.9. 4-(3-chlorophenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7i)

Following the procedure of 4.1.2.5.1, starting from compound **2.5i** (190 mg, 0.46 mmol), the title compound **2.7i** was obtained as orange solid (103 mg, 81%); mp >250 □; ^{1}H NMR (acetone-d_6) δ (ppm): 13.20 (brs, 1H), 10.78 (s, 1H), 9.64 (s, 1H), 8.14 (d, J = 9.2 Hz, 1H), 7.71 (d, J = 1.2 Hz, 1H), 7.45 (t, J = 8.0 Hz, 1H), 7.41 (s, 1H), 7.36 (d, J = 7.6 Hz, 1H), 7.23 (d, J = 7.6 Hz, 1H), 6.82 (d, J = 8.8 Hz, 1H);

HRMS (ESI): *m/z*, calcd for $C_{15}H_{11}ClN_3O_4$ [M+H$^+$]: 332.0432, found 332.0427.

4.1.3.5.10. 4-(4-chlorophenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7j)

Following the procedure of 4.1.2.5.1, starting from compound **2.5j** (190 mg, 0.46 mmol), the title compound **2.7j** was obtained as orange solid (145 mg, 82%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.78 (s, 1H), 10.77 (s, 1H), 9.65 (s, 1H), 8.12 (d, *J* = 9.2 Hz, 1H), 7.72 (s, 1H), 7.49 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 6.74 (d, *J* = 9.2 Hz, 1H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{11}ClN_3O_4$ [M+H$^+$]: 332.0432, found 332.0430.

4.1.3.5.11. 4-(3-nitrophenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7k)

Following the procedure of 4.1.2.5.1, starting from compound **2.5k** (122 mg, 0.33 mmol), the title compound **2.7k** was obtained as orange solid (105 mg, 93%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.42 (brs, 1H), 10.86 (s, 1H), 9.84 (s, 1H), 8.15-8.17 (m, 2H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.72 (s, 1H), 7.69 (t, *J* = 8.0 Hz, 1H), 6.95 (d, *J* = 9.2 Hz, 1H); HRMS (ESI): m/z, calcd for $C_{15}H_{10}N_4O_6$ [M+H$^+$]: 343.0673, found 343.0665.

4.1.3.5.12. 4-(4-nitrophenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7l)

Following the procedure of 4.1.2.5.1, starting from compound **2.5l** (140 mg, 0.38 mmol), the title compound **2.7l** was obtained as orange solid (113 mg, 88%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.97 (s, 1H), 10.03 (s, 1H), 8.25 (d, *J* = 8.8 Hz, 2H), 8.21 (d, *J* = 8.8 Hz, 1H), 7.12 (s, 1H), 7.53 (d, *J* = 8.8 Hz, 2H), 7.15 (d, *J* = 9.2 Hz, 1H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{11}N_4O_6$ [M+H$^+$]: 343.0673, found 343.0672.

4.1.3.5.13. 4-(3-methylphenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7m)

Following the procedure of 4.1.2.5.1, starting from compound **2.5m** (190 mg, 0.46 mmol), the title compound **2.7m** was obtained as yellow solid (90 mg, 80%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.43 (s, 1H), 9.49 (s, 1H), 8.03 (d, *J* = 8.8 Hz, 1H), 7.47 (s, 1H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.16-7.19 (m, 2H), 7.01 (d, *J* = 7.6 Hz, 1H), 6.69 (d, *J* = 9.2 Hz, 1H), 2.33 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{14}N_3O_4$ [M+H+]: 312.0979, found 312.0976.

4.1.3.5.14. 4-(4-methylphenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7n)

Following the procedure of 4.1.2.5.1, starting from compound **2.5n** (190 mg, 0.46 mmol), the title compound **2.7n** was obtained as yellow solid (160 mg, 97%); mp: 257-259 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.70 (s, 1H), 9.61 (s, 1H), 8.09 (d, *J* = 9.2 Hz, 1H), 7.74 (s, 1H), 7.27 (s, 4H), 6.62 (d, *J* = 9.2 Hz, 1H), 2.34 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{14}N_3O_4$ [M+H$^+$]: 312.0979, found 312.0973.

4.1.3.5.15. 4-(3-acetamidophenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7o)

Following the procedure of 4.1.2.5.1, starting from compound **2.5o** (190 mg, 0.46 mmol), the title compound **2.7o** was obtained as sorrel solid (100 mg, 98%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.72 (s, 1H), 10.08 (s, 1H), 9.69 (s ,1H), 8.11 (d, *J* = 9.2 Hz, 1H), 7.78 (m, 2H), 7.33-7.36 (m, 2H), 7.04 (d, *J* = 5.6 Hz, 1H), 6.75 (d, *J* = 8.8 Hz, 1H), 2.05 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{15}N_4O_5$ [M+H$^+$]: 355.1037, found 355.1033.

4.1.3.5.16. 4-(3-aminophenylamino)-7-nitro-1*H*-indole-2-carboxylic acid (2.7p)

To a solution of **2.7o** (120 mg, 0.339 mmol) in acetic acid (4 mL), concentrated HCl (2 mL) was added. The resulting mixture was refluxed for 12 hours. After filtration, the residue was dissolved in NaOH aqueous (2 mL, 2M), and extacted with EtOAc. The aqueous layer was acidified to PH=3-4 with diluted HCl. After filtration, the title compound **2.7p** was obtained as dark solid (60 mg, 57%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.81 (s, 1H), 9.78 (s, 1H), 8.14 (d, J = 9.0 Hz, 1H), 7.79 (d, J = 2.1 Hz, 1H), 7.45 (t, J = 7.8 Hz, 1H), 7.24 (m, 2H), 7.01 (d, J = 7.5 Hz, 1H), 6.84 (d, J = 9.0 Hz, 1H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{13}N_4O_4$ [M+H$^+$]: 313.0931, found 313.0927.

4.1.3.6. Synthesis of ethyl 4-substituted-7-nitro-1*H*-indole-2-carboxylate (2.6a-2.6j, 2.6l)

4.1.3.6.1. Ethyl 7-nitro-4-phenyl-1*H*-indole-2-carboxylate (2.6a)

To a solution of **2.4** (50 mg, 0.19 mmol) in DMF (5 mL), $Pd(OAc)_2$ (8.4 mg, 0.037 mmol), dppf (41 mg, 0.074 mmol), Et_3N (95 mg, 0.94 mmol) and phenylboronic acid (68 mg, 0.56 mmol) were added in order. The reaction mixture was heated to 80 □ for 20 h under argon atmosphere, and concentrated under reduced pressure. The residue was dissolved in EtOAc (20 mL), and washed with brine (10 mL×3) and water (10 mL×3). The crude product obtained after concentration was purified with column chromatography to afford product **2.6a** as light yellow solid (90 mg, 60%); mp: 180-181 □; ^{1}H NMR (acetone-d_6) δ (ppm): 10.86 (brs, 1H), 8.41 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 7.6 Hz, 2H), 7.54-7.64 (m, 3H), 7.47 (d, J = 8.4 Hz, 1H), 7.44 (s, 1H), 4.43 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{15}N_2O_4$ [M+H$^+$]: 311.1026, found 311.1017.

4.1.3.6.2. Ethyl 4-(3-methoxyphenyl)-7-nitro-1*H*-indole-2-carboxylate (2.6b)

To a solution of **2.4** (250 mg, 0.93 mmol) in toluene (15 mL), $Pd_2(dba)_3$ (85 mg, 0.093 mmol), Xantphos (108 mg, 0.186 mmol) and Na_2CO_3 (296 mg, 2.79 mmol, 2 mL H_2O) and 3-methoxyphenylboronic acid (424 mg, 2.79 mmol) were sequentially added. The reaction mixture was refluxed for 3 h under argon atmosphere, and concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL), and washed with brine (20 mL×3) and water (20 mL×3). The crude product obtained after concentration was purified with column chromatography to afford product **2.6b** as light yellow solid (268 mg, 85%); mp: 182-183 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.47 (s, 1H), 8.35 (d, J = 8.4 Hz, 1H), 7.45-7.47 (m, 2H), 7.31 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 9.2 Hz, 1H), 7.18 (s, 1H), 7.04 (d, J = 8.4 Hz, 1H), 4.46 (q, J = 7.2 Hz, 2H), 3.89 (s, 3H), 1.43 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{17}N_2O_5$ [M+H$^+$]: 341.1132, found 341.1119.

4.1.3.6.3. Ethyl 4-(3-chlorophenyl)-7-nitro-1*H*-indole-2-carboxylate (2.6c)

Following the procedure of 4.1.3.6.2, starting from compound **2.4** (220 mg, 0.82 mmol), the title compound **2.6c** was obtained as light yellow solid (190 mg, 67%), mp: 148-150 □; ^{1}H NMR (acetone-d_6) δ (ppm): 10.90 (brs, 1H), 8.40 (d, J = 8.4 Hz, 1H), 7.75 (s, 1H), 7.72 (d, J = 7.6 Hz, 1H), 7.64 (t, J = 8.0 Hz, 1H), 7.58 (d, J = 8.0 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.41 (s, 1H), 4.43 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{14}ClN_2O_4$ [M+H$^+$]: 345.0637, found 345.0624.

4.1.3.6.4. Ethyl 4-(4-chlorophenyl)-7-nitro-1*H*-indole-2-carboxylate (2.6d)

Following the procedure of 4.1.3.6.2, starting from compound **2.4** (220 mg, 0.82 mmol), the title compound **2.6d** was obtained as light yellow solid light green solid (200 mg, 71%); mp: 186-187 ℃; ^{1}H NMR (acetone-d_6) δ (ppm): 8.40 (d, J = 8.4 Hz, 1H), 7.78-7.81 (m, 2H), 7.63-7.66 (m, 2H), 7.43-7.49 (m, 2H), 4.43 (q, J = 7.2 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{14}ClN_2O_4$ [M+H$^+$]: 345.0637, found 345.0625.

4.1.3.6.5. Ethyl 4-(3-cyanophenyl)-7-nitro-1*H*-indole-2-carboxylate (2.6e)

Following the procedure of 4.1.3.6.2, starting from compound **2.4** (250 mg, 0.93 mmol), the title compound **2.6e** was obtained as light yellow solid (200 mg, 71%); mp: 195-197 ℃; ^{1}H NMR (acetone-d_6) δ (ppm): 10.95 (brs, 1H), 8.44 (d, J = 8.4 Hz, 1H), 8.17 (s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.96 (d, J = 7.6 Hz, 1H), 7.85 (t, J = 8.0 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.48 (s, 1H), 4.44 (q, J = 7.2 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{14}N_3O_4$ [M+H$^+$]: 336.0979, found 336.0968.

4.1.3.6.6. Ethyl 4-(4-cyanophenyl)-7-nitro-1*H*-indole-2-carboxylate (2.6f)

Following the procedure of 4.1.3.6.2, starting from compound **2.4** (250 mg, 0.93 mmol), the title compound **2.6f** was obtained as light yellow solid light yellow solid (248 mg, 80%); mp: 213-215 ℃; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.52 (brs, 1H), 8.39 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 8.7 Hz, 2H), 7.78 (d, J = 8.1 Hz, 2H),7.37-7.38 (m, 1H), 7.32 (d, J = 8.1 Hz, 1H), 4.47 (q, J = 6.9 Hz, 2H), 1.44 (t, J = 6.9 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{14}N_3O_4$ [M+H$^+$]: 336.0979, found 336.0968.

4.1.3.6.7. Ethyl 4-(3-methylphenyl)-7-nitro-1*H*-indole-2-carboxylate (2.6g)

Following the procedure of 4.1.3.6.2, starting from compound **2.4** (220 mg, 0.82 mmol), the title compound **2.6g** was obtained as light yellow solid (176 mg, 66%); mp: 91-92 ℃; ^{1}H NMR (acetone-d_6) δ (ppm): 10.83 (brs, 1H), 8.39 (d, J = 8.0 Hz, 1H), 7.43-7.57 (m, 5H), 7.37 (d, J = 7.2 Hz, 1H), 4.43 (q, J = 7.2 Hz, 2H), 2.47 (s, 3H), 1.39 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{17}N_2O_4$ [M+H$^+$]: 325.1183, found 325.1172.

4.1.3.6.8. Ethyl 7-nitro-4-(3-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (2.6h)

Following the procedure of 4.1.3.6.2, starting from compound **2.4** (220 mg, 0.82 mmol), the title compound **2.6h** was obtained as light yellow solid (256 mg, 83%); mp: 137-139 ℃; ^{1}H NMR (acetone-d_6) δ (ppm): 11.02 (brs, 1H), 8.51 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 6.8 Hz, 1H), 7.13 (s, 1H), 7.96-7.98 (m, 2H), 7.63 (d, J = 8.4 Hz, 1H), 7.48 (s, 1H), 4.52 (q, J = 7.2 Hz, 2H), 1.47 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{14}F_3N_2O_4$ [M+H$^+$]: 379.0900, found 379.0886.

4.1.3.6.9. Ethyl 7-nitro-4-(3-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (2.6i)

Following the procedure of 4.1.3.6.2, starting from compound **2.4** (220 mg, 0.82 mmol), the title compound **2.6i** was obtained as light yellow solid (225 mg, 73%); mp: 178-179 ℃; ^{1}H NMR (acetone-d_6) δ (ppm): 10.96 (brs, 1H), 8.43 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 8.4 Hz, 2H), 7.96 (d, J = 8.0 Hz, 2H), 7.54 (d, J = 8.4 Hz, 1H), 7.44 (s, 1H), 4.43 (q, J = 7.2 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{14}F_3N_2O_4$ [M+H$^+$]: 379.0900, found 379.0890.

4.1.3.6.10. Ethyl 4-(3-acetamidophenyl)-7-nitro-1*H*-indole-2-carboxylate (2.6j)

Following the procedure of 4.1.3.6.2, starting from compound **2.4** (300 mg, 1.12 mmol), the title compound **2.6j** was obtained as light yellow solid (248 mg, 60%); mp: 183-184 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.45 (s, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 7.84 (s, 1H), 7.63 (d, *J* = 7.6 Hz, 1H), 7.47-7.51 (m, 3H), 7.39 (d, *J*= 7.6 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 4.45 (q, *J* = 7.2 Hz, 2H), 2.23 (s, 3H), 1.43 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{19}H_{18}N_3O_5$ [M+H$^+$]: 368.1241, found 368.1228.

4.1.3.6.11. Ethyl 4-isobutyl-7-nitro-1*H*-indole-2-carboxylate (2.6l)

To a solution of compound **2.4** (250 mg, 0.93 mmol) in toluene (12 mL), $Pd(OAc)_2$ (21 mg, 0.093 mmol), t-$Bu_3P{\cdot}HBF_4$ (54 mg, 0.186 mmol) and K_3PO_4 (987 mg, 4.65 mmol, 1 mL H_2O) and isobutylboronic acid (284 mg, 2.79 mmol) were sequentially added. The reaction mixture was heated to 90□ for 2 h under argon atmosphere, and concentrated under reduced pressure. The residue was dissolved in EtOAc (20 mL), and washed with brine (20 mL×3) and water (20 mL×3). The crude product obtained after concentration was purified with column chromatography to afford product **2.6n** as off-white solid (216 mg, 80%); mp: 108-109 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.36 (s, 1H), 8.23 (d, *J* = 8.0 Hz, 1H), 7.38 (s, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 4.47 (q, *J* = 7.2 Hz, 2H), 2.86 (d, *J* = 7.2 Hz, 2H), 2.04-2.15 (m, 1H), 1.46 (t, *J* = 7.2 Hz, 2H), 0.97 (d, *J* = 7.2 Hz, 6H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{19}N_2O_4$ [M+H$^+$]: 291.1339, found 291.1331.

4.1.3.7. Synthesis of 4-substituted-7-nitro-1*H*-indole-2-carboxylic acid (2.8a-2.8l)

4.1.3.7.1. 7-nitro-4-phenyl-1*H*-indole-2-carboxylic acid (2.8a)

Following the procedure of 4.1.2.5.1, starting from compound **2.6a** (90 mg, 0.29 mmol), the title compound **2.8a** was obtained as light yellow solid (55 mg, 68%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.61 (brs, 1H), 11.26 (s, 1H), 8.34 (d, *J* = 8.4 Hz, 1H), 7.72 (d, *J* = 6.9 Hz, 2H), 7.53-7.62 (m, 3H), 7.42 (d, *J* = 8.4 Hz, 1H), 7.31 (s, 1H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{11}N_2O_4$ [M+H$^+$]: 283.0713, found 283.0710.

4.1.3.7.2. 4-(3-methoxyphenyl)-7-nitro-1*H*-indole-2-carboxylic acid (2.8b)

Following the procedure of 4.1.2.5.1, starting from compound **2.6b** (128 mg, 0.38 mmol), the title compound **2.8b** was obtained as light yellow solid (106 mg, 91%); mp: 248-249 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.59 (brs, 1H), 11.27 (s, 1H), 8.33 (d, *J* = 8.4 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.31 (s, 1H), 7.30 (d, *J* = 7.6 Hz, 1H), 7.22 (s, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 3.80 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{13}N_2O_5$ [M+H$^+$]: 313.0819, found 313.0817.

4.1.3.7.3. 4-(3-chlorophenyl)-7-nitro-1*H*-indole-2-carboxylic acid (2.8c)

Following the procedure of 4.1.2.5.1, starting from compound **2.6c** (130 mg, 0.38 mmol), the title compound **2.8c** was obtained as light yellow solid (50 mg, 42%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.70 (brs, 1H), 11.25 (s, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 7.75 (s, 1H), 7.70 (d, *J* = 6.4 Hz, 1H), 7.61-7.64 (m, 2H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.24 (s, 1H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{10}ClN_2O_4$ [M+H$^+$]: 317.0324, found 317.0316.

4.1.3.7.4. 4-(4-chlorophenyl)-7-nitro-1*H*-indole-2-carboxylic acid (2.8d)

Following the procedure of 4.1.2.5.1, starting from compound **2.6d** (100 mg, 0.29

mmol), the title compound **2.8d** was obtained as light yellow solid (25 mg, 27%); mp >250 □; ^{1}HNMR (DMSO-d_6) δ (ppm): 13.62 (brs, 1H), 11.32 (s, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.32 (s, 1H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{10}ClN_2O_4$ [M+H$^+$]: 317.0324, found 317.0323.

4.1.3.7.5. 4-(3-cyanophenyl)-7-nitro-1*H*-indole-2-carboxylic acid (2.8e)

Following the procedure of 4.1.2.5.1, starting from compound **2.6e** (110 mg, 0.33 mmol), the title compound **2.8e** was obtained as white solid (96 mg, 95%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.32 (s, 1H), 8.33 (d, *J* = 8.4 Hz, 1H), 8.17 (s, 1H), 8.05 (d, *J* = 7.6 Hz, 1H), 7.98 (d, *J* = 7.2 Hz, 1H), 7.78 (t, *J* = 7.6 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.32 (s, 1H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{10}N_3O_4$ [M+H$^+$]: 308.0666, found 308.0652.

4.1.3.7.6. 4-(4-cyanophenyl)-7-nitro-1*H*-indole-2-carboxylic acid (2.8f)

Following the procedure of 4.1.2.5.1, starting from compound **2.6f** (202 mg, 0.60 mmol), the title compound **2.8f** was obtained as light yellow solid (116 mg, 63%); mp >250 □;^{1}H NMR (DMSO-d_6) δ (ppm): 13.64 (brs, 1H), 11.38 (s, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 2H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.30 (s, 1H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{11}N_3O_4$ [M+H$^+$]: 308.0666, found 308.0657.

4.1.3.7.7. 4-(3-methylphenyl)-7-nitro-1*H*-indole-2-carboxylic acid (2.8g)

Following the procedure of 4.1.2.5.1, starting from compound **2.6g** (116 mg, 0.36 mmol), the title compound **2.8g** was obtained as light yellow solid (80 mg, 75%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.61 (brs, 1H), 11.26 (s, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 7.47-7.54 (m, 3H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 7.2 Hz, 1H), 7.32 (s, 1H), 2.44 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{13}N_2O_4$ [M+H$^+$]: 297.0870, found 297.0867.

4.1.3.7.8. 4-(3-(trifluromethyl)phenyl)-7-nitro-1*H*-indole-2-carboxylic acid (2.8h)

Following the procedure of 4.1.2.5.1, starting from compound **2.6h** (141 mg, 0.37 mmol), the title compound **2.8h** was obtained as light yellow solid (68 mg, 52%); mp: 217-219 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 11.29 (brs, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 8.05 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.83 (t, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.23 (s, 1H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{10}F_3N_2O_4$ [M+H$^+$]: 351.0587, found 351.0580.

4.1.3.7.9. 4-(4-(trifluoromethyl)phenyl)-7-nitro-1*H*-indole-2-carboxylic acid (2.8i)

Following the procedure of 4.1.2.5.1, starting from compound **2.6i** (140 mg, 0.37 mmol), the title compound **2.8i** was obtained as light yellow solid (75 mg, 58%); mp: 221-223 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.63 (brs, 1H), 11.39 (s, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 7.95 (m, 4H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.34 (s, 1H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{10}F_3N_2O_4$ [M+H$^+$]: 351.0587, found 351.0587.

4.1.3.7.10. 4-(3-acetamidophenyl)-7-nitro-1*H*-indole-2-carboxylic acid (2.8j)

Following the procedure of 4.1.2.5.1, starting from compound **2.6j** (90 mg, 0.25 mmol), the title compound **2.8j** was obtained as light yellow solid (65 mg, 78%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.60 (brs, 1H), 11.27 (s, 1H), 10.17 (s, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 8.02 (s, 1H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.50 (t, *J* = 7.8

Hz, 1H), 7.36-7.42 (m, 3H), 2.08 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{14}N_3O_5$ [M+H$^+$]: 340.0928, found 340.0921.

4.1.3.7.11. 4-(3-aminophenyl)-7-nitro-1*H*-indole-2-carboxylic acid (2.8k)

To a solution of **2.8j** (125 mg, 0.37 mmol) in methanol (3 mL), concentrated HCl (3 mL, 6 N) was added. The resulting mixture was refluxed for 3 h. After filtration, the residue was dissolved in NaOH aqueous (2 mL, 2M), and extracted with EtOAc. The aqueous layer was acidified to pH=3-4 with diluted HCl. After filtration, the title compound **2.8k** was obtained as light yellow solid (38 mg, 35%); mp >250 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.30 (brs, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 7.23 (d, *J* = 8.8 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 6.95 (s, 1H), 6.91 (s, 1H), 6.83 (d, *J* = 7.6 Hz, 1H), 6.68 (d, *J* = 7.6 Hz, 1H), 5.33 (s, 2H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{12}N_3O_4$ [M+H$^+$]: 298.0822, found 298.0819.

4.1.3.7.12. 4-isobutyl-7-nitro-1*H*-indole-2-carboxylic acid (2.8l)

Following the procedure of 4.1.2.5.1, starting from compound **2.6l** (156 mg, 0.54 mmol), the title compound **2.8l** was obtained as white solid (116 mg, 82%); mp: 195-197 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.53 (s, 1H), 11.02 (s, 1H), 8.21 (d, *J* = 8.4 Hz, 1H), 7.49 (s, 1H), 7.15 (d, *J* = 8.0 Hz, 1H), 2.88 (d, *J* = 7.2 Hz, 2H), 1.96-2.06 (m, 1H), 0.91 (d, *J* = 6.4 Hz, 6H); HRMS (ESI): *m/z*, calcd for $C_{13}H_{15}N_2O_4$ [M+H$^+$]: 263.1026, found 263.1024.

4.1.3.8. Synthesis of ethyl 4-substituted-7-nitro-1*H*-indole-2-carboxylate (2.9a-2.9b)

4.1.3.8.1. Ethyl 4-((3-methoxyphenyl)(methyl)amino)-7-nitro-1*H*-indole-2-carboxylate (2.9a)

To a solution of **2.6c** (200 mg, 0.56 mmol) in DMF (10 mL), Cs_2CO_3 (363 mg, 1.12 mmol) and iodomethane (79 mg, 0.56 mmol) was added. The reaction mixture was stirred at room temperature for 3 h, and then concentrated under reduced pressure. The residue was dissolved in EtOAc (20 mL), washed with water (20 m ×3), dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using petroleum ether/ethyl acetate as eluent to provide compound **2.9a** as yellow solid (100 mg, 48%); mp: 138-139 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.44 (brs, 1H), 8.22 (d, *J* = 9.3 Hz, 1H), 7.36 (t, *J* = 8.1 Hz, 1H), 6.89-6.92 (dd,?? 1H), 6.83 (d, *J* = 7.2 Hz, 1H), 6.77 (s, 1H), 6.50 (d, *J* = 9.0 Hz, 1H), 6.25-6.26 (m, 1H), 4.34 (q, *J* = 7.2 Hz, 2H), 3.80 (s, 3H), 3.60 (s, 3H), 1.35 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{19}H_{20}N_3O_5$ [M+H$^+$]: 370.1398, found 370.1386.

4.1.3.8.2. Ethyl 4-((3-methoxyphenyl)(propyl)amino)-7-nitro-1*H*-indole-2-carboxylate (2.9b)

Following the procedure of 4.1.3.8.1, starting from compound **2.6c** (250 mg, 0.70 mmol), the title compound **2.8l** was obtained as light yellow solid (55 mg, 20%), mp: 115-117 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.43 (brs, 1H), 8.20 (d, *J* = 9.0 Hz, 1H), 7.36 (t, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 1H), 6.82 (d, *J* = 7.8 Hz, 1H), 6.76 (s, 1H), 6.50 (d, *J* = 9.3 Hz, 1H), 6.05 (d, *J* = 2.1 Hz, 1H), 4.32 (q, *J* = 7.2 Hz, 2H), 3.87 (t, *J* = 8.1 Hz, 2H), 3.81 (s, 3H), 1.76-1.89 (m, 2H), 1.34 (t, *J* = 7.2 Hz, 3H), 1.00 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{21}H_{24}N_3O_5$ [M+H$^+$]: 398.1711, found 398.1697.

4.1.3.9. Synthesis of 4-substituted-7-nitro-1H-indole-2-carboxylic (2.10a-2.10b)

4.1.3.9.1 4-((3-methoxyphenyl)(methyl)amino)-7-nitro-1*H*-indole-2-carboxylic acid (2.10a)

Following the procedure of 4.1.2.5.1, starting from compound **2.9a** (100 mg, 0.27 mmol), the title compound **2.10a** was obtained as orange solid (30 mg, 33%); mp: 223-225 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.24 (brs, 1H), 10.64 (s, 1H), 8.20 (d, *J* = 9.2 Hz, 1H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.00 (d, *J* = 8.0 Hz, 1H), 6.95 (s, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.69 (d, *J* = 9.2 Hz, 1H), 5.94 (s, 1H), 3.76 (s, 3H), 3.55 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{16}N_3O_5$ [M+H$^+$]: 342.1085, found 342.1083.

4.1.3.9.2. 4-((3-methoxyphenyl)(propyl)amino)-7-nitro-1*H*-indole-2-carboxylic acid (2.10b)

Following the procedure of 4.1.2.5.1, starting from compound **2.9b** (50 mg, 0.13 mmol), the title compound **2.10b** was obtained as yellow solid (40 mg, 86%); mp: 191-192 □; ^{1}HNMR (DMSO-d_6) δ (ppm): 13.23 (brs, 1H), 10.60 (brs, 1H), 8.18 (d, *J* = 9.3 Hz, 1H), 7.42 (t, *J* = 7.8 Hz, 1H), 7.01 (d, *J* = 8.1 Hz, 1H), 6.93 (s, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 6.75 (d, *J* = 9.3 Hz, 1H), 5.73 (d, *J* = 1.5 Hz, 1H), 3.89 (t, *J* = 7.8 Hz, 2H), 3.77 (s, 3H), 1.67-1.75 (m, 2H), 0.94 (t, *J* = 7.5 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{19}H_{20}N_3O_5$ [M+H$^+$]: 370.1398, found 370.1398.

4.1.4. Synthesis of 5-subsituted-7-nitro-1*H*-indole-2-carboxylic acid derivatives

4.1.4.1. Ethyl 2-(2-(4-bromo-2-nitrophenyl)hydrazono)propanoate (3.3) bjb-1540

A solution of 4-bromo-2-nitroaniline (20 g, 92 mmol) in concentrated HCl (100 mL) was heated to 40 □ for 2 h. The mixture was then cooled to -10 □, and a solution of $NaNO_2$ (6.98 g, 101.2 mmol) in water (50 mL) was added `dropwise. After 2 h, the mixture was cooled to -30 □ and added to a solution of $SnCl_2$ (62 g, 276 mmol) in concentrated HCl (100 mL) precooled to -30 □. The reaction mixture was stirred at -20 □ for 1 h, then warmed to room temperature and filtered. The title compound **3.2** was obtained as light yellow solid (24 g, 97%);

To a solution of compound **3.2** (24 g, 89.3 mmol) in ethanol (60 mL) ethyl pyruvate (11.4 g, 98.3 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 10 min. After filtration, the title compound **3.3** was obtained as yellow solid (27 g, 92%); mp: 124-125 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 13.91 (brs, 1H), 8.34 (s, 1H), 7.92 (d, *J* = 9.2 Hz, 1H), 7.62 (d, *J* = 9.2 Hz, 1H), 4.39 (q, *J* = 7.2 Hz, 2H), 2.25 (s, 3H), 1.40 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{11}H_{13}BrN_3O_4$ [M+H$^+$]: 330.0084, found 330.0074.

4.1.4.2. Ethyl 5-bromo-7-nitro-1*H*-indole-2 -carboxylate (3.4)

The reaction mixture of compound **3.3** (27 g, 81.8 mmol) in PPA (270 g) was heated at 90-100□ for 4 h, and then cooled to room temperature. Water (400 mL) was added to the mixture to destroy the PPA, and the residue was extracted with EtOAc (100 mL×4). The crude product obtained after concentration was purified with column chromatography to afford title compound **3.4** (11.7 g, 44%) as light yellow solid; mp: 135-137 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.31 (brs, 1H), 8.40 (s, 1H), 8.17 (s, 1H), 7.30 (s, 1H), 4.47 (q, *J* = 7.2 Hz, 2H), 1.45 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{11}H_{10}BrN_2O_4$ [M+H$^+$]: 312.9818, found 312.9807.

4.1.4.3. Synthesis of ethyl 5-substituted-7-nitro-1*H*-indole-2-carboxylate

(3.5a-3.5c)

4.1.4.3.1. Ethyl 5-isobutyl-7-nitro-1*H*-indole-2 -carboxylate (3.5a)

To a solution of compound **3.4** (750 mg, 2.40 mmol) in toluene (28 mL), $Pd(OAc)_2$ (54 mg, 0.24 mmol), t-$Bu_3P \cdot HBF_4$ (139 mg, 0.48 mmol) and K_3PO_4 (2.04 g, 9.6 mmol, 5.5 mL H_2O) and isobutylboronic acid (489 mg, 4.8 mmol) were sequentially added. The reaction mixture was heated at 90 ℃ for 5 h under argon atmosphere, and then concentrated under reduced pressure. The residue was dissolved in EtOAc (60 mL), and washed with brine (20 mL×3) and water (20 mL×3). The crude product obtained after concentration was purified with column chromatography to afford title product **3.5a** as light yellow solid (614 mg, 88%); mp: 99-100 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.23 (brs, 1H), 8.11 (s, 1H), 7.81 (s, 1H), 7.29 (s, 1H), 4.46 (q, *J* = 7.2 Hz, 2H), 2.65 (d, *J* = 7.2 Hz, 2H), 1.91-1.98 (m, 1H), 1.44 (t, *J* = 7.2 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 6H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{19}N_2O_4$ [M+H$^+$]: 291.1339, found 291.1330.

4.1.4.3.2. Ethyl 5-(3-acetamidophenyl)-7-nitro-1*H*-indole-2-carboxylate (3.5b)

Following the procedure of 4.1.3.4.2, starting from compound **3.4** (250 mg, 0.64 mmol), the title compound **3.5b** was obtained as yellow solid (238 mg, 81%); mp: 124-125 ℃; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.30 (s, 1H), 8.50 (s, 1H), 8.23 (s, 1H), 7.89 (s, 1H), 7.37-7.49 (m, 5H), 4.48 (q, *J* = 7.2 Hz, 2H), 2.23 (s, 3H), 1.46 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{19}H_{18}N_3O_5$ [M+H$^+$]: 368.1241, found 368.1223.

4.1.4.3.3. Ethyl 5-((3-methoxyphenyl)amino)-7-nitro-1*H*-indole-2-carboxylate (3.5c)

To a solution of compound **3.4** (300 mg, 0.96 mmol) in toluene (19 mL), $Pd_2(dba)_3$ (88 mg, 0.096 mmol), Davephos (76 mg, 0.19 mmol) and K_3PO_4 (611 mg, 2.88 mmol, 2.5 mL H_2O) and 3-methoxyaniline (489 mg, 4.8 mmol) were sequentially added. The reaction mixture was heated at 90□ for 10 h under argon atmosphere, and then concentrated under reduced pressure. The residue was dissolved in EtOAc (40 mL), and washed with brine (20 mL×3) and water (20 mL×3). The crude product obtained after concentration was purified with column chromatography to afford title product **3.5c** as sorrel solid (242 mg, 71%); mp: 99-100 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.18 (brs, 1H), 8.11 (d, *J* = 2.1 Hz, 1H), 7.79 (d, *J* = 1.5 Hz, 1H), 7.18-7.24 (m, 2H), 6.51-6.63 (m, 3H), 4.46 (q, *J* = 7.2 Hz, 2H), 3.79 (s, 3H), 1.44 (t, *J* = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{18}N_3O_5$ [M+H$^+$]: 356.1241, found 356.1228.

4.1.4.4. Synthesis of 5-substituted-7-nitro-1*H*-indole-2-carboxylic acid (3.6a-3.6c)

4.1.4.4.1. 5-isobutyl-7-nitro-1*H*-indole-2-carboxylic acid (3.6a)

Following the procedure of 4.1.2.5.1, starting from compound **3.5a** (166 mg, 0.57 mmol), the title compound **3.6a** was obtained as light yellow solid (155 mg, 100%); mp >300 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.25 (brs, 1H), 7.93 (s, 1H), 7.89 (s, 1H), 6.86 (s, 1H), 2.63 (d, *J* = 7.2 Hz, 2H),1.86-1.94 (m, 1H), 0.89 (d, *J* = 6.9 Hz, 6H); HRMS (ESI): *m/z*, calcd for $C_{13}H_{15}N_2O_4$ [M+H$^+$]: 263.1026, found 263.1026.

4.1.4.4.2. 5-(3-acetamidophenyl)-7-nitro-1*H*-indole-2-carboxylic acid (3.6b)

Following the procedure of 4.1.2.5.1, starting from compound **3.5b** (142 mg, 0.39

mmol), the title compound **3.6b** was obtained as yellow solid (130 mg, 100%); mp: 282-284 ℃; ^{1}H NMR (DMSO-d_6) δ (ppm): 13.60 (brs, 1H), 11.25 (s, 1H), 10.09 (s, 1H), 8.45 (s, 1H), 8.40 (d, J = 1.5 Hz, 1H), 7.98 (s, 1H), 7.60-7.63 (m, 1H), 7.39-7.45 (m, 3H), 2.06 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{14}N_3O_5$ [M+H$^+$]: 340.0928, found 340.0920.

4.1.4.4.3. 5-((3-methoxyphenyl)amino)-7-nitro-1*H*-indole-2-carboxylic acid (3.6c)

Following the procedure of 4.1.2.5.1, starting from compound **3.6c** (130 mg, 0.37 mmol), the title compound **3.6c** was obtained as yellow solid (120 mg, 100%); mp >300 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.86 (s, 1H), 8.42 (s, 1H), 8.00 (d, J = 0.9 Hz, 1H), 7.91 (d, J = 1.5 Hz, 1H), 7.27 (d, J = 1.8 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H), 6.66 (d, J = 7.8 Hz, 1H), 6.61 (s, 1H), 6.45 (dd, J_1 = 7.8 Hz, J_2 = 1.5 Hz, 1H), 3.72 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{14}N_3O_5$ [M+H$^+$]: 328.0928, found 328.0923.

4.1.4.5. Ethyl 3-bromo-5-isobutyl-7-nitro-1*H*-indole-2-carboxylate (3.7)

To a solution of **3.5a** (250 mg, 0.86 mmol) in DMF (4 mL), a solution of NBS (168 mg, 0.95 mmol) in DMF (2 mL) was added dropwise under the ice-water bath. The reaction mixture was stirred at room temperature for 2 h, and then poured into ice-water (35 mL). After filtration, the title compound **1.4** was afforded as light yellow solid (295 mg, 93%); mp: 84-86 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.29 (brs, 1H), 8.17 (s, 1H), 7.80 (s, 1H), 4.50 (q, J = 7.2 Hz, 2H), 2.69 (d, J = 7.2 Hz, 2H), 1.96-2.01 (m, 1H), 1.48 (t, J = 7.2 Hz, 3H), 0.96 (d, J = 6.8 Hz, 6H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{18}BrN_2O_4$ [M+H$^+$]: 369.0445, found 369.0441.

4.1.4.6. Ethyl 3-ethyl-5-isobutyl-7-nitro-1*H*-indole-2-carboxylate (3.8)

To a solution of **3.7** (250 mg, 0.68 mmol) in toluene (18 mL), Pd $(OAc)_2$ (7.6 mg, 0.034 mmol), Josiphos (44 mg, 0.068 mmol) and K_3PO_4 (722 mg, 3.4 mmol, 2.5 mL H_2O) and ethylboronic acid (151 mg, 2.04 mmol) were sequentially added. The reaction mixture was heated at 80 □ for 36 h under argon atmosphere, and then concentrated under reduced pressure. The residue was dissolved in EtOAc (40 mL), and washed with brine (20 mL×3) and water (20 mL×3). The crude product obtained after concentration was purified with column chromatography to afford product **3.8** as light yellow solid (171 mg, 80%); mp: 72-73 □; ^{1}H NMR ($CDCl_3$) δ (ppm): 10.03 (brs, 1H), 8.11 (s, 1H), 7.79 (s, 1H), 4.46 (q, J = 7.2 Hz, 2H), 3.14 (q, J = 7.2 Hz, 2H), 2.66 (d, J = 7.2 Hz, 2H), 1.91-1.98 (m, 1H), 1.45 (t, J = 7.2 Hz, 3H), 1.29 (t, J = 7.2 Hz, 3H), 0.95 (d, J = 6.4 Hz, 6H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{23}N_2O_4$ [M+H$^+$]: 319.1652, found 319.16510.

4.1.4.7. 3-ethyl-5-isobutyl-7-nitro-1H-indole-2-carboxylic acid (3.9)

Following the procedure of 4.1.2.5.1, starting from compound **3.8** (120 mg, 0.38 mmol), the title compound **3.9** was obtained as light yellow solid (97 mg, 89%); mp: 168-170 □; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.47 (s, 1H), 8.08 (s, 1H), 8.05 (s, 1H), 3.10 (q, J = 6.9 Hz, 2H), 2.68 (d, J = 6.9 Hz, 2H), 1.90-1.94 (m, 1H), 1.21 (t, J = 7.2 Hz, 3H), 0.90 (d, J = 6.6 Hz, 6H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{19}N_2O_4$ [M+H$^+$]:

291.1339, found 291.1337.

4.2 Biological evaluation

4.2.1 Isolation and purification of human liver FBPase.

An expression plasmid (pcDNA3.1-hFBP) for human liver FPBase was constructed by inserting the human liver FBPase gene from plasmid EX-C0133-B31 (GeneCopoeia) into the vector pcDNA3.1. The FBPase gene from EX-C0133-B31 had a C-terminal 6xHis tag for use in purification.

For expression of the human liver FBPase, chemically competent BL21(DE3) Rosetta cells (Novagen, Inc.) were transformed with pcDNA3.1-hFBP. A 5 mL overnight culture grown in LB media with 150 μg/mL of ampicillin was back-diluted 1000-fold into 2 L of 2YT media with 0.4% glycerol and 150 μg/mL ampicillin. Bacterial growth at 37°C was monitored using the A560 and the doubling-time was calculated. When the growth reached an A560 of 0.4, 0.1 mM IPTG was added to induce the cell, following which the cells were allowed to grow for three additional doubling times. The cells were then harvested by centrifugation (4400 x g), resuspended and lysed by sonication. The lysate was clarified by centrifugation at 31,000 x g for 15 min. The protein was purified through a Chelating Sepharose Fast Flow column (GE Healthcare), which had been charged with 0.3 M $NiSO_4$. The human liver FBPase was eluted with buffer containing 50 mM sodium citrate and 50 mM NaCl, pH 4.0. The protein-containing fractions were pooled and dialyzed against 50 mM Tris-acetate buffer, 150 mM NaCl, pH 7.5 at 4 °C overnight.

Protein purity was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Protein concentration was determined using the BioRad version of the Bradford dye-binding assay with bovine serum albumin as the standard.[28]

4.2.2 Measurement of FBPase activity

FBPase activity was measured spectrophotometrically by employing the coupling enzymes phosphoglucose isomerase and glucose-6-phosphate dehydrogenase. The reduction of $NADP^+$ to NADPH was monitored directly at 340 nm.[29] Specifically, buffer (100 mM Tris, 2 mM MgCl2, 0.1 mM EDTA, pH 7.5), 10 μM of inhibitor and 0.72 unit of FBPase were mixed in a cuvette and equilibrated at 37°C. 0.2 mM of $NADP^+$, 0.01 units of phosphoglucose isomerase, 0.01 units of glucose-6-phosphate dehydrogenase and FBP were then added to initiate the reaction. Reactions were performed in duplicate. Inhibition curves were obtained for each compound by plotting the relative activity versus inhibitor concentration.

4.3 Molecular docking

All molecular computation studies were performed on a Dell 2.83 GHz Core 2 running Windows XP. The X-ray crystal structure of FBPase complexed with AMP was retrieved from protein data bank (PDB code: 1FTA).[9] The CDOCKER protocol in Discovery Studio 2.5.5 (Accelrys Software Inc., San Diego, CA) was used in this study to investigate the binding mode of compound **3.9** in the crystal structure FBPase.[26] CDOCKER uses molecular dynamics (MD) with CHARMm force field

scheme to dock ligands into a binding site of targeted protein. The water molecules in protein were removed and the protein was prepared by adding hydrogen and correcting the incomplete residues using Clean Protein tool of DS, then the protein was refined with CHARMm. The binding site was constructed within 7.6 Å with AMP set as the center. Compound **3.9** was built and minimized using Prepare Ligands tool of DS and refined with CHARMm force field. Docking of compound **3.9** into FBPase with CDOCKER was done using the default parameters except that Pose Cluster Radius was defined as 0.5 Å for increasing the diversity of the docked poses. The pose with the top –CDOCKER_INTERACTION_ENERGY was chosen for analyzing the binding features of compound **3.9** and FBPase.

Acknowledgments

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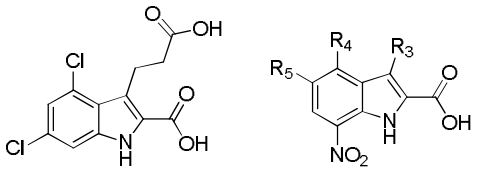


MDL-29951 **A**

Figure 1. Chemical structures of MDL-29951 and designed compounds (A).

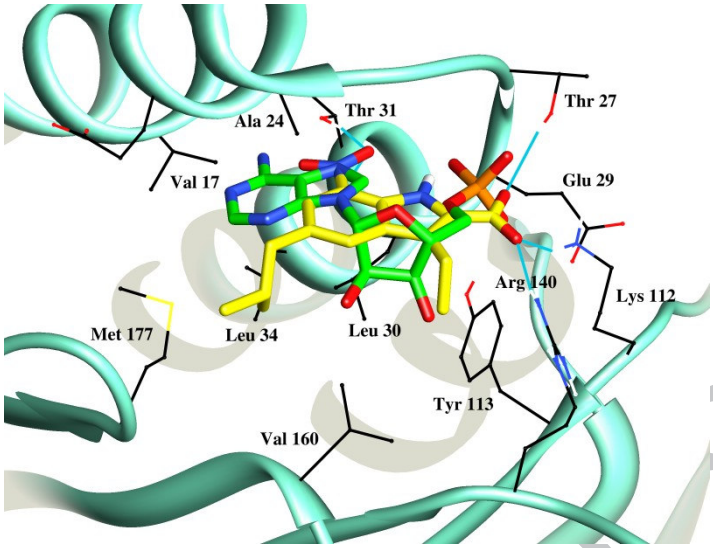
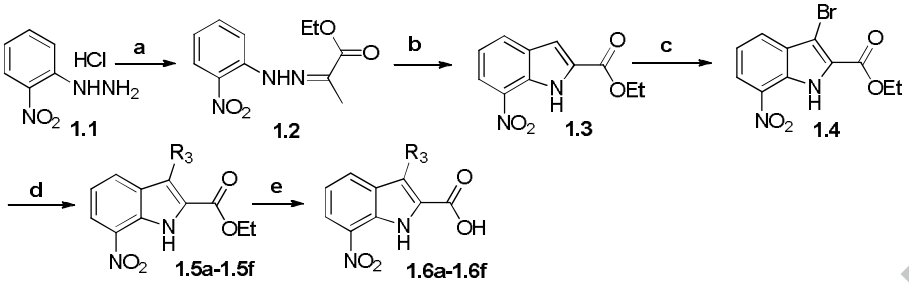
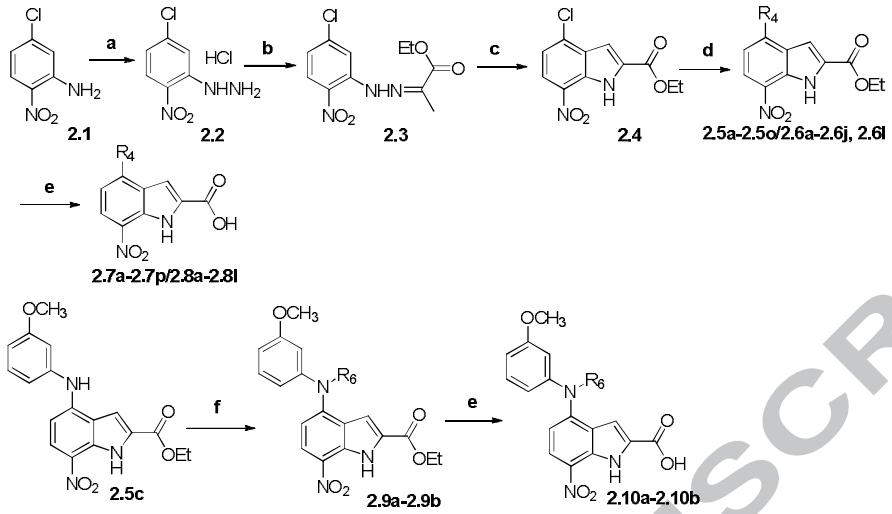


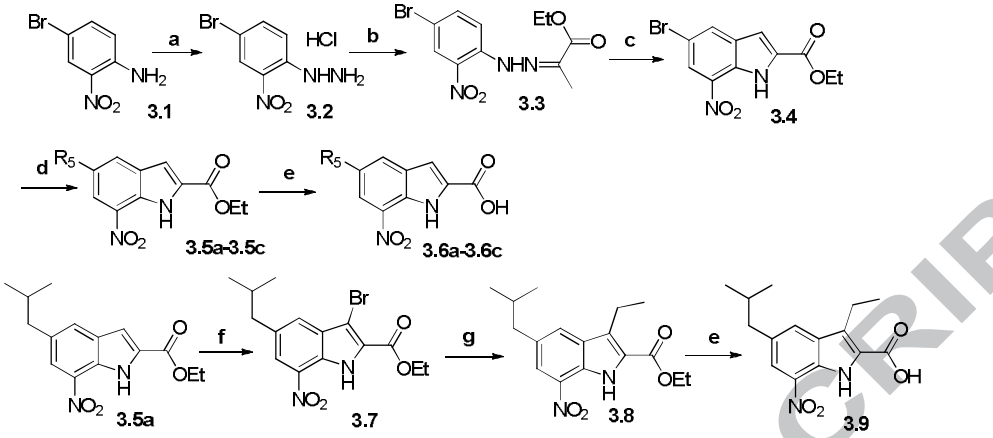
Figure 2. CDOCKER-modeled binding mode of compound **3.9** (carbon atoms colored orange) in comparison with the crystal structure (1FTA in PDB)[9] of AMP (carbon atoms colored green). H-Bonding interactions are presented with light blue line. The chemical structure of reference molecule is presented. Molecular image was generated with UCSF Chimera.[27]



Scheme 1. Reagents and conditions: (a) ethyl pyruvate, EtOH, r.t. (b) PPA, 80 □. (c) NBS, DMF. (d) $R_3B(OH)_2$, $Pd(OAc)_2$, Josiphos, K_3PO_4(aq), toluene, 80 □; or $R_3B(OH)_2$, $PdCl_2$(dppf), Na_2CO_3(aq), 1,4-dioxane, 100 □. (e) NaOH, THF, EtOH and H_2O, r.t.



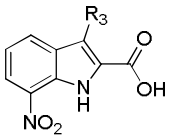
Scheme 2. Reagents and conditions: (a) (i) $NaNO_2$, HCl, -10 □; (ii) $SnCl_2$, HCl, -30 □. (b) ethyl pyruvate, EtOH, r.t. (c) PPA, 80 □. (d) $R_4B(OH)_2$ or $ArNH_2$, $Pd_2(dba)_3$, Xantphos, 1M Na_2CO_3(aq), toluene, 80-110□; or i-$BuB(OH)_2$, $Pd(OAc)_2$, t-$Bu_3P{\cdot}HBF_4$, K_3PO_4(aq), toluene, 90 □. (e) NaOH, THF, EtOH and H_2O, r.t. (f) R_6X, Cs_2CO_3, DMF, 80 □.



Scheme 3. Reagents and conditions: (a) (i) $NaNO_2$, HCl, -10 □; (ii) $SnCl_2$, HCl, -30 □. (b) ethyl pyruvate, EtOH, r.t. (c) PPA, 80 □. (d) $ArB(OH)_2$, $Pd_2(dba)_3$, Xantphos, 1M Na_2CO_3(aq), toluene, 80□; or $ArNH_2$, $Pd_2(dba)_3$, DavePhos, K_3PO_4(aq), toluene, 80□; or $iBuB(OH)_2$, $Pd(OAc)_2$, $tBu_3P{\cdot}HBF_4$, K_3PO_4(aq), toluene, 90 □. (e) NaOH, THF, EtOH and H_2O, r.t. (f) NBS, DMF. (g) $EtB(OH)_2$, $Pd(OAc)_2$, Josiphos, K_3PO_4(aq), toluene, 80℃.

Table 1

The chemical structures and inhibitory activities against F16BPase of compounds **1.6a-1.6f**.[a]



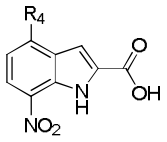
Compound	R_3	IC_{50} (μM)[b]
1.6a	ethyl	6.2
1.6b	isobutyl	10.3
1.6c	phenyl	12.7
1.6d	3-methoxyphenyl	35.6
1.6e	3-acetamidophenyl	7.4
1.6f	3-fluorophenyl	4.5

[a]AMP and compound MDL-29951 were used as reference molecules. IC_{50} for AMP was 3.2 μM. IC_{50} for compound MDL-29951 was 2.0-2.8 μM.

[b]Compound dose (μM) required to inhibit F16BPase activity by 50%.

Table 2

The chemical structures and inhibitory activities against FBPase of compounds **2.7a–2.7p, 2.8a-2.8l, 2.10a-2.10b**.[a]



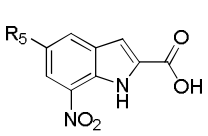
Compd	R_4	IC_{50} (μM)[b]
2.7a	phenylamino	>50
2.7b	2-methoxyphenylamino	>50
2.7c	3-methoxyphenylamino	25.6
2.7d	4-methoxyphenylamino	>50
2.7e	3,4-dimethoxyphenylamino	7.7
2.7f	3,4,5-trimethoxyphenylamino	>50
2.7g	3-(trifluoromethyl)phenylamino	29.1
2.7h	4-(trifluoromethyl)phenylamino	12.2
2.7i	3-chlorophenylamino	>50
2.7j	4-chlorophenylamino	>50
2.7k	3-nitrophenylamino	>50
2.7l	4-nitrophenylamino	3.4
2.7m	3-methylphenylamino	4.3
2.7n	4-methylphenylamino	33.9
2.7o	3-acetamidophenylamino	>50
2.7p	3-aminophenylamino	>50
2.10a	(3-methoxyphenyl)(methyl)amino	>50
2.10b	(3-methoxyphenyl)(propyl)amino	>50
2.8a	phenyl	>50
2.8b	3-methoxyphenyl	>50
2.8c	3-chlorophenyl	>50
2.8d	4-chlorophenyl	>50
2.8e	3-cyanophenyl	>50
2.8f	4-cyanophenyl	>50
2.8g	3-methylphenyl	25.9
2.8h	3-(trifluoromethyl)phenyl	>50
2.8i	4-(trifluoromethyl)phenyl	>50
2.8j	3-acetamidophenyl	18.5
2.8k	3-aminophenyl	>50
2.8l	isobutyl	9.7

[a]AMP and compound MDL-29951 were used as reference molecules. IC_{50} for AMP was 3.2 μM. IC_{50} for compound MDL-29951 was 2.0-2.8 μM.

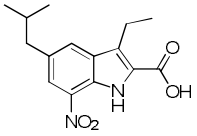
[b]Compound dose (μM) required to inhibit F16BPase activity by 50%.

Table 3

The chemical structures and inhibitory activities on FBPase of compounds **3.6a-3.6c, 3.9**.[a]



3.6a-3.6c



3.9

Compd	R_3	IC_{50} (μM)[b]
3.6a	isobutyl	5.40
3.6b	3-acetamidophenyl	3.70
3.6c	3-methoxyphenylamino	4.06
3.9	\	0.99

[a]AMP and compound MDL-29951 were used as reference molecules. IC_{50} for AMP was 3.2 μM. IC_{50} for compound MDL-29951 was 2.0-2.8 μM.

[b]Compound dose (μM) required to inhibit F16BPase activity by 50%.

Graphical Abstract:

Design, synthesis and biological evaluation of 7-nitro-1*H*-indole-2-carboxylic acid derivatives as allosteric inhibitors of fructose-1,6-bisphosphatase

Jianbo Bie, Shuainan Liu, Jie Zhou, Bailing Xu, Zhufang Shen

A series of novel indole derivatives was synthesized as inhibitors of Fructose-1,6-bisphosphatase (FBPase). Extensive structure-activity relationships were conducted and led to a potent FBPase inhibitor **3.9** with an IC_{50} of 0.99 μM. The binding mode of this series indoles was predicted using CDOCKER algorithm.