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Original article

Discovery of novel indole derivatives as allosteric inhibitors of fructose-1,6-bisphosphatase

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ABSTRACT

A series of novel indole derivatives was designed and synthesized as inhibitors of fructose-1,6-bisphosphatase (FBPase). The most potent compound **14c** was identified with an IC_{50} value of 0.10 μM by testing the inhibitory activity against recombinant human FBPase. The structure-activity relationships were investigated on the substitution at 4- and 5-position of the indole scaffold. The binding interactions of the title compounds at AMP binding site of FBPase were predicted using CDOCKER algorithm.

1. Introduction

Type 2 diabetes mellitus is a metabolic disorder, which is usually characterized by insulin deficiency, insulin resistance and excess endogenous glucose production (EGP), leading to high blood glucose levels in both fasting and postprandial state of patients. At present, the majority of current anti-diabetic drugs reduce blood glucose via either augmenting insulin secretion or improving insulin resistance [1,2]. Although excess EGP is a major contributor to the hyperglycemia [3–5], Metformin is the only available drug which can effectively lower glucose levels by the indirect influence on the EGP process [6,7]. Gluconeogenesis (GNG) is a primary EGP process for liver to produce glucose, where three carbon substrates such as pyruvate, lactate and glycerol are transformed into glucose. Direct blocking GNG pathway represents a promising strategy for the development of anti-diabetics, which can be used for the treatment of both fasting and postprandial hyperglycemia. So far, not any drugs have been developed into the market by directly acting on the GNG process.

Fructose-1,6-bisphosphatase (FBPase) is a key enzyme in hepatic GNG pathway, which catalyzes the penultimate rate-limiting step, namely the conversion of fructose-1,6-bisphosphate into fructose-6-phosphate. FBPase in liver is elevated in insulin-resistant and insulin-deficient animal models of diabetes [8–10], and this fact highlights the importance of this enzyme in the control of blood glucose. In particular, the recent reports of oral FBPase inhibitors provided proof-of-concept in both T2DM animal models and patients [11–17]. Therefore, FBPase inhibitors are expected to serve as a novel class of anti-diabetic agents.

FBPase is a homotetramer which exists in active (R) and inactive (T) conformational states. It has been demonstrated that FBPase is subjected to the competitive substrate inhibition of fructose-2,6-bisphosphate and to the allosteric inhibition of AMP via the conformational change from R sate to T state or stabilization of the T state [18,19]. Up to date, a number of pharmaceutical companies and academic laboratories have been involved in exploring the allosteric inhibitors of AMP binding site in FBPase and there have been many types of chemical entities reported in the literature [11–13,20–24]. Among them, only AMP mimetic MB07803, which is a prodrug of MB07729, has been advanced into phase II clinical trial [16,17]. Therefore, there are enormous unmet needs to develop FBPase inhibitors as potential anti-diabetic agents.

In our previous work, we described 7-nitro-1*H*-indole-2-carboxylic acid derivatives [25] (**A**, Fig. 1, IC_{50}: 0.99 μM–35.6 μM) as novel allosteric inhibitors of FBPase, which were low molecular weight and devoid of a phosphonate or phosphate group. With a goal to increase the binding affinity of this series of indoles, we re-

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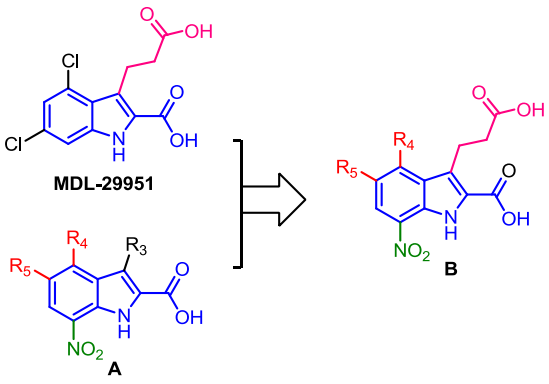


Fig. 1. Structural design of the target indole derivatives.

examined the lead structure of MDL-29951 identified by Pfizer via screening a compound library [26] and lead structure **A** discovered by our group, a series of novel indole derivatives (**B**, Fig. 1) was designed by the integration of the characteristic pharmacophore groups present in the lead structures **A** and MDL-29951. By keeping the 7-nitro-1*H*-indole-2-carboxylic acid scaffold intact, the carboxyethyl fragment was introduced as R_3 group as it was in MDL-29951. According to the SARs from series **A** [25], a wide range of hydrophobic groups were chosen as R_4 and R_5 substituents for series **B**. Herein, we described the synthesis, enzymatic activities and structure-activity relationships of these new indole derivatives as novel FBPase inhibitors.

2. Results and discussion

2.1. Chemistry

The synthesis of compounds **5a**–**g** and **6** was shown in Scheme 1 and their chemical structures were presented in Table 1. Starting from the substituted anilines **1a**–**f**, the Japp–Kingemann reactions yielded the corresponding phenylhydrazones **2a**–**f** in 15–83% yields. The subsequent Fischer esterification of phenylhydrazone **2a**–**f** delivered **c**ompounds **3a**–**f** (28–92% yields), which were converted into the key indole intermediates **4a**–**g** in 11–50% yields via Fischer indole synthesis in the presence of *p*-toluenesulfonic acid in toluene or using PPA as solvent. Under the basic reaction conditions, compounds **4a**–**g** were hydrolyzed into the corresponding carboxylic acid derivatives **5a**–**g** in high yields. Compound **6** was obtained in 64% yield directly via Fischer indole synthesis of phenylhydrazone compound **2a** under the catalysis of concentrated H_2SO_4 in toluene.

The chemical structures of target compounds (**8 and 10a**–**b**) were shown in Table 1 and their preparations were illustrated in Scheme 2. The nitro group of compound **4d** was reduced to amine **7** upon treatment with Fe/CH_3COOH in 79% yield. The followed hydrolysis of ester **7** produced the carboxylic acid **8** in good yield. The hydrolysis of esters **9a**–**b**, which were derived from the N-alkylation of compound **4d** with alkyl halides in 90–94% yields, gave rise to the formation of carboxylic acids **10a**–**b** in acceptable chemical yields.

The target compounds **12a**–**m** and **14a**–**d** were prepared according to the method as depicted in Scheme 3 and their chemical structures were shown in Table 2. Utilizing Suzuki–Miyaura reaction or Buchwald–Hartwig reaction, the intermediates **4e** and **4g** delivered the corresponding coupling products **11a**–**m** and **13a**–**d** (27–87% yields), respectively. The hydrolysis of **11a**–**m** and **13a**–**d** yielded the corresponding carboxylic acid derivatives **12a**–**m** and **14a**–**d** in good 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 [27]. All target compounds (**5a**–**g**, **6**, **8**, **10a**–**b**, **12a**–**m** and **14a**–**d**) were tested for their inhibitory activities against human liver FBPase. The corresponding results summarized in Tables 1–2 were expressed as IC_{50} values. AMP and compound MDL-29951 were used as reference molecules.

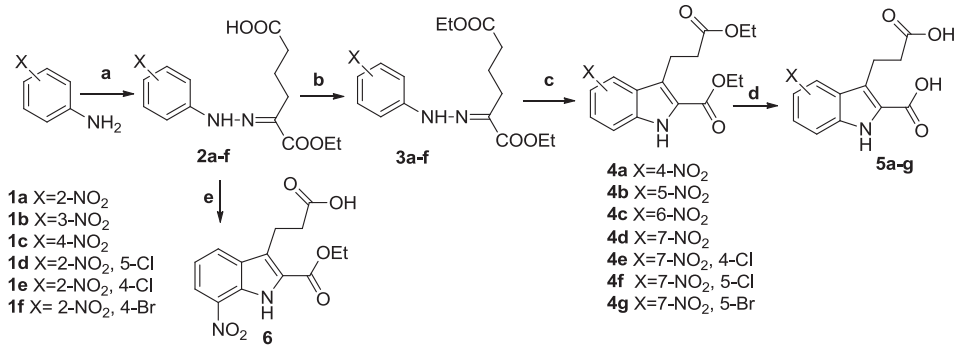
According to our design strategy, namely, installation of the carboxyethyl group at 3-position and nitro group at 7-position on the indole-2-carboxylic acid scaffold, compound **5d** was first synthesized. As shown in Table 1, compound **5d** displayed moderate inhibitory activity with an IC_{50} value of 5.17 μM. In order to further explore the contribution of 7-nitro group to the inhibition, compounds **5a**–**c** and **8** were synthesized and tested. When the nitro group was introduced at 4, 5 or 6-position of the indole ring, the inhibitory effects of the resulting compounds **5a**–**c** decreased drastically in comparison with compound **5d**. The reduction of 7-nitro group to 7-amino group (compound **8**) led to loss of activity as well. These results demonstrated that 7-nitro group played a key role in the binding interaction between FBPase and this series of indole derivatives. In addition, the esterfication of 2-carboxylic acid or *N*-alkylation of compound **5d** gave rise to compounds **6** and **10a**–**b**, which presented no inhibitory activity. These results were consistent with the known SARs of MDL-29951 derivatives [26], and it was indicated that the designed indoles very likely took the similar binding mode in the AMP binding site of FBPase as MDL-29951 did. The 2-carboxylic acid formed crucial hydrogen bonds with FBPase. The NH group on the indole ring might function as a hydrogen bond donor or a relatively bulky group was not tolerated due to the steric hindrance.

With an aim to further examine the design concept, compounds **5e**–**g** were synthesized using a chloro or bromo residue as the R_4 or R_5 substituent. Satisfyingly, compared with compound **5d**, these compouds showed stronger inhibition with IC_{50} values of 0.88–2.07 μM. In view of the above results, it was reasonably assumed that the structure **B** was a viable template to discover more potent FBPase inhibitors by variations of R_4 and R_5 substituents.

The SARs of substitution at 4-position of the indole ring with aryl, alkyl or arylamino group were summarized in Table 2. The analogs substituted with 4-aryl groups (**12a**–**h**) had IC_{50} values ranging from 1.10 to 5.27 μM. Among which, most of them showed a 2~5-fold increase in activity in comparison with compound **5d**. Substitution on the benzene ring of R_4 group in compound **12a** resulted in marginal effects on the inhibitory activity, except for compounds **12c** and **12e**. The 4-ethyl and 4-isobutyl substituted analogs (**12i** and **12j**) showed inhibition with an IC_{50} value of 2.76 μM and 0.69 μM, respectively. Introducing arylamino substituents (**12k**–**m**) at 4-position of the indole scaffold resulted in a 3~7-fold increase in potency compared with compound **5d**. It was noticed that the arylamino group was somewhat more favorable to the binding affinity than the aryl substituent, as comparing compounds **12k** (IC_{50}, 0.69 μM) and **12l** (IC_{50}, 1.31 μM) with compounds **12a** (IC_{50}, 1.40 μM) and **12e** (IC_{50}, 5.22 μM), respectively. Taken together, the SAR studies on 4-position demonstrated that a variety of hydrophobic substituents were well tolerated, and the chloro, isobutyl and phenylamino moiety were preferred.

The influence of R_5 substituents on the inhibitory activity was then explored primarily.

As summarized in Table 2, all synthesized compounds **14a**–**d** exhibited inhibitory activities with IC_{50} values in a range of



Scheme 1. Reagents and conditions: (a) (i) $NaNO_2$, HCl, H_2O, 0 °C; (ii) NaOAc, ethyl-2-oxocyclopentane-carboxylate, 15 °C; (iii) Et_3N, H_2O, reflux. (b) conc. H_2SO_4, EtOH, reflux. (c) TsOH, toluene, reflux; or PPA, 90 °C. (d) NaOH, THF, EtOH and water, rt. (e) conc. H_2SO_4, toluene, reflux.

0.10–6.43 μM. The ethyl and isobutyl substituted derivatives (**14b** and **14c**) were more potent than the analogs with a chloro, bromo, phenyl and phenylamino substituent (**5f**, **5g**, **14a** and **14d**). Compound **14c** (IC_{50}, 0.10 μM) was found to be the most potent FBPase inhibitor with the indole scaffold. It produced more than 20-fold and 50-fold enhancement in inhibition compared with MDL-29951 and compound **5d**, respectively. Therefore, it was indicated that substitution at 5-position with a small hydrophobic alkyl moiety would be favorable for binding affinity. Furthermore, it has been demonstrated that dual substitutions on 3- and 5-position could achieve more pronounced inhibitory activity as we expected [25].

Molecular docking was performed using CDOCKER program (Accelrys Discovery Studio 2.5.5) [28] to gain insights into the protein-inhibitor interactions within AMP binding site of FBPase. The coordinates of the AMP-FBPase complex (pdb code: 1FTA) [29] were employed. The representative binding pose of the synthesized indoles was illustrated by the most potent compound **14c** as shown in Fig. 2. The indole ring was situated in a hydrophobic pocket formed by residues Val17, Leu30 and Leu34. Nevertheless, in comparison with the purine ring in AMP, the indole moiety shifted slightly outward from the purine binding pocket. The 7-nitro group interacted with the hydroxyl group on the side chain of Thr31 via a hydrogen bond. The 2-carboxylate interacted with residues Thr27, Lys112 and Arg140 through a hydrogen bonding network, which was also observed between the phosphate group in AMP and FBPase. It was believed that these hydrogen bonding interactions played important roles in the binding affinity. This was in accordance with the results that esterfication of 2-carboxylic acid eliminated the inhibitory activity. The 3-carboxyethyl side chain extended into a solvent exposed surface, and that was similar to that of MDL-29951 [26]. The 5-ethyl group was located in a hydrophobic region lined with residues Leu34, Val160 and Met177. Presumably due to the better shape complementarity of ethyl group with this hydrophobic subpocket, the hydrophobic interactions made great contributions to the binding affinity of compound **14c**.

3. Conclusion

In summary, by integrating the key structure features of MDL-29951 and indole-based inhibitors identified previously in our group, we further developed a series of new indole derivatives as FBPase inhibitors with improved potency. The preliminary SARs were explored and led to a potent compound **14c** with an IC_{50} value of 0.10 μM. This represents a novel lead structure with low molecular weight. Therefore, it will provide more chances to further develop druggable FBPase inhibitors.

4. Experimental section

4.1. Chemistry

4.1.1. General

Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. ^1H 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).

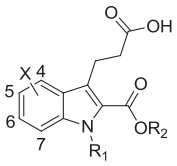
4.1.2. General procedure for synthesis of phenylhydrazones (*2a–f*)

Taking **2a** as an example: A solution of 2-nitroaniline (6.91 g, 50 mmol) in concentrated hydrochloric acid (12.5 mL) and water (100 mL) was cooled to 0 °C. A solution of $NaNO_2$ (3.45 g, 50 mmol) in water (20 mL) was then added to the above solution slowly so as to the reaction temperature was maintained below 0 °C. After the addition was complete, a solution of sodium acetate (22.56 g, 275 mmol) in water (61 mL) was prepared and added. Then ethyl 2-oxocyclopentanecarboxylate (9.37 g, 60 mmol) was added and stirred vigorously for 15 min at 0 °C and 60 min at room temperature. The mixture was extracted with ethyl acetate (70 mL × 2). The combined organic layers were concentrated in vacuo and the crude material was added to a solution of boiling Et_3N (2 mL) in water (150 mL). The solution was refluxed for 30 min and cooled to room temperature to precipitate. The yellow solid was obtained and recrystallized from ethyl acetate to give compound **2a** (8.28 g, 51%).

*4.1.2.1. 6-Ethoxy-5-(2-(2-nitrophenyl)hydrazono)-6-oxohexanoic acid (**2a**).* Starting from 2-nitroaniline (6.91 g, 50 mmol), compound **2a** was afforded as yellow solid (8.28 g, 51%); mp: 157–158 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 13.89 (s, 1H), 8.20 (d, $J_1 = 1.5$ Hz, $J_2 = 8.4$ Hz, 1H), 7.99 (d, $J = 8.7$ Hz, 1H), 7.56 (m, 1H), 6.94 (m, 1H), 4.39 (q, $J = 6.9$ Hz, 2H), 2.68 (t, $J = 7.2$ Hz, 2H), 2.48 (t, $J = 7.5$ Hz, 2H), 1.98–2.08 (m, 2H), 1.40 (t, $J = 6.9$ Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{14}H_{18}N_3O_6$ $[M+H]^+$: 324.1190, found 324.1184.

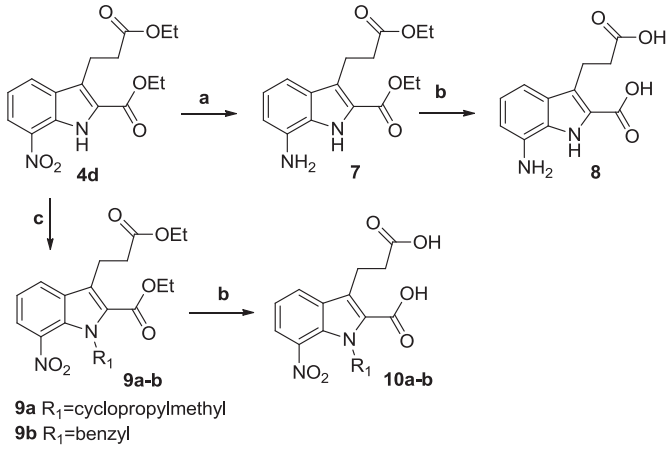
*4.1.2.2. 6-Ethoxy-5-(2-(3-nitrophenyl)hydrazono)-6-oxohexanoic acid (**2b**).* Starting from 3-nitroaniline (5.52 g, 40 mmol), compound **2b** was afforded as yellow solid (8.04 g, 59%). mp:

Table 1
The chemical structures and inhibitory activities against FBPase of compounds **5a–g, 6, 8, 10a–b**.[a]



Compd	R_1	R_2	X	IC_{50} (μM)[b]
5a	H	H	4-NO_2	ND[c]
5b	H	H	5-NO_2	>50
5c	H	H	6-NO_2	ND[c]
5d	H	H	7-NO_2	5.17
5e	H	H	4-Cl, 7-NO_2	0.88
5f	H	H	5-Cl, 7-NO_2	2.07
5g	H	H	5-Br, 7-NO_2	1.29
6	H	ethyl	7-NO_2	>50
8	H	H	7-NH_2	>50
10a	cyclo -propylmethyl	H	7-NO_2	>50
10b	benzyl	H	7-NO_2	>50

[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 FBPase activity by 50%.
[c] The inhibition of compound **5a** and **5c** were 57.5% and 79.5%, respectively, at 50 μM concentration.

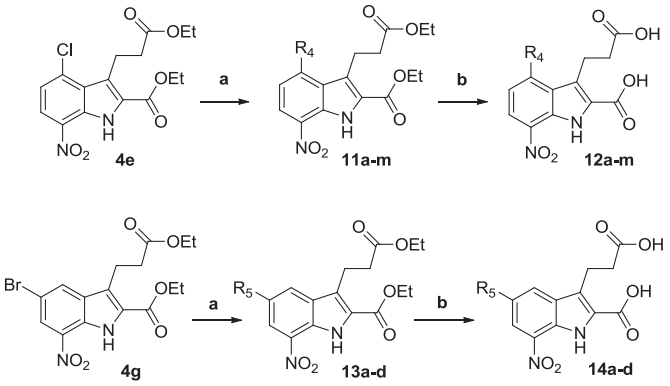


Scheme 2. Reagents and conditions: (a) Fe/CH_3COOH. (b) NaOH, THF, EtOH and water, rt. (c) Cs_2CO_3, RX, DMF, 60 °C.

164–165 °C; ^{1}H NMR (300 MHz, acetone-d_6): δ 10.21 (s, 1H), 8.07–8.08 (m, 1H), 7.73–7.76 (m, 1H), 7.64–7.67 (m, 1H), 7.55 (t, J = 8.4 Hz, 1H), 4.26 (q, J = 7.2 Hz, 2H), 2.68 (t, J = 8.1 Hz, 2H), 2.51 (t, J = 6.6 Hz, 2H), 1.73–1.82 (m, 2H), 1.32 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{14}H_{18}N_3O_6$ $[M+H]^+$: 324.1190, found 324.1183.

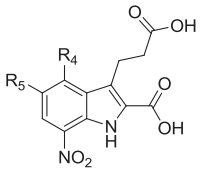
*4.1.2.3. 6-Ethoxy-5-(2-(4-nitrophenyl)hydrazono)-6-oxohexanoic acid **(2c)**.* Starting from 4-nitroaniline (3.46 g, 25 mmol), compound **2c** was afforded as yellow solid (6.70 g, 83%). mp: 164–165 °C; ^{1}H NMR (300 MHz, acetone-d_6): δ 10.44 (s, 1H), 8.20 (d, J = 9.0 Hz, 2H), 7.40 (d, J = 9.0 Hz, 2H), 4.26 (q, J = 7.2 Hz, 2H), 2.70 (t, J = 7.5 Hz, 2H), 2.52 (t, J = 6.6 Hz, 2H), 1.72–1.82 (m, 2H), 1.32 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{14}H_{18}N_3O_6$ $[M+H]^+$: 324.1190, found 324.1185.

*4.1.2.4. 6-Ethoxy-5-(2-(5-chloro-2-nitrophenyl)hydrazono)-6-oxohexanoic acid **(2d)**.* Starting from 5-chloro-2-nitroaniline



Scheme 3. Reagents and conditions: (a) $ArB(OH)_2$ or $ArNH_2$, $Pd_2(dba)_3$, Xantphos, Na_2CO_3(aq), toluene, 80 °C; or Alkylboronic acid, $Pd(OAc)_2$, $tBu_3P{\cdot}HBF_4$, K_3PO_4(aq), toluene, 90 °C; or $ArNH_2$, $Pd_2(dba)_3$, Davephos, K_3PO_4(aq), toluene, 80 °C. (b) NaOH, THF, EtOH and H_2O, rt.

Table 2
The chemical structures and inhibitory activities against FBPase of compounds **12a–m, 14a–d**.[a]



Compd	R_4	R_5	IC_{50} (μM)[b]
12a	phenyl	H	1.40
12b	2-fluorophenyl	H	2.40
12c	3-fluorophenyl	H	5.27
12d	4-fluorophenyl	H	2.40
12e	3-methoxyphenyl	H	5.22
12f	4-methoxyphenyl	H	1.80
12g	3-nitrophenyl	H	1.10
12h	4-nitrophenyl	H	2.00
12i	ethyl	H	2.76
12j	isobutyl	H	0.69
12k	phenylamino	H	0.69
12l	(3-methoxyphenyl)amino	H	1.31
12m	(4-methoxyphenyl)amino	H	1.7
14a	H	phenyl	6.43
14b	H	isobutyl	0.45
14c	H	ethyl	0.10
14d	H	phenylamino	1.60

[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 FBPase activity by 50%.

(1.73 g, 10 mmol), compound **2d** was afforded as yellow solid (1.56 g, 44%). mp: 168–169 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.91 (s, 1H), 8.15 (d, J = 8.8 Hz, 1H), 7.97 (s, 1H), 6.90 (d, J = 8.8 Hz, 1H), 4.40 (q, J = 7.2 Hz, 2H), 2.69 (t, J = 7.2 Hz, 2H), 2.48 (t, J = 7.2 Hz, 2H), 2.03 (m, 2H), 1.40 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{14}H_{17}N_3O_6Cl$ $[M+H]^+$: 358.0800, found 358.0797.

*4.1.2.5. 6-Ethoxy-5-(2-(4-chloro-2-nitrophenyl)hydrazono)-6-oxohexanoic acid **(2e)**.* Starting from 4-chloro-2-nitroaniline (5.2 g, 30 mmol), compound **2e** was afforded as yellow solid (2.44 g, 23%). mp: 168–169 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.71 (s, 1H), 12.71 (brs,1H), 8.15 (d, J = 2.4 Hz, 1H), 7.91–7.95 (d, J = 9.0 Hz, 1H), 7.77 (dd, J_1 = 2.4 Hz, J_2 = 9.0 Hz, 1H), 4.31 (q, J = 7.2 Hz, 2H), 2.57 (t, J = 7.2 Hz, 2H), 2.30 (t, J = 7.2 Hz, 2H), 1.84 (m, 2H), 1.31 (t, J = 7.2 Hz,

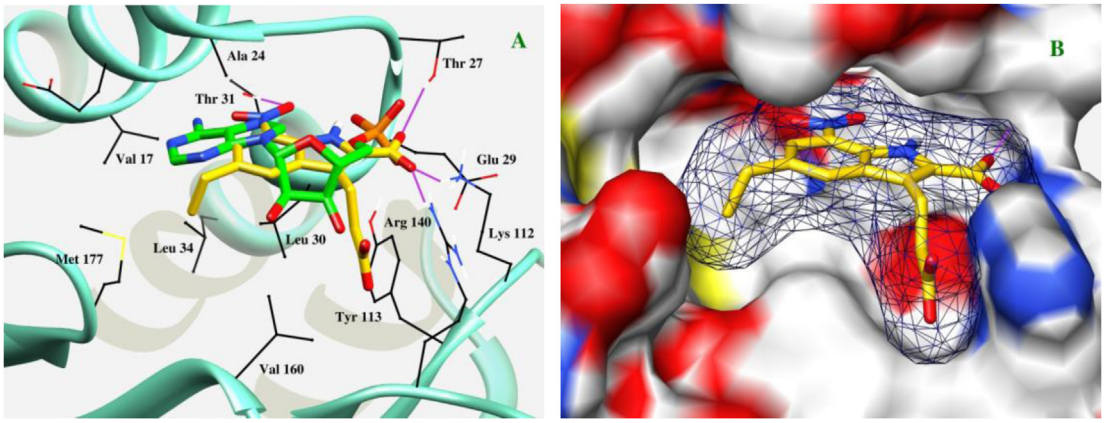


Fig. 2. CDOCKER-modeled binding mode of compound **14c** (carbon atoms colored orange). (A) The binding orientation and interactions of compound **14c** in comparison with that of AMP (carbon atoms colored green) in the cocrystal structure (1FTA in PDB) [29]; (B) The molecular shape and volume of compound **14c** in the binding site of FBPase (1FTA in PDB). H-Bonding interactions are presented with purple lines. The surface of compound **14c** was presented with blue meshes. Molecular image was generated with UCSF Chimera.[30].

3H). HRMS (ESI): *m/z*, calcd. for $C_{14}H_{17}N_3O_6Cl$ $[M+H]^+$: 358.0800, found 358.0793.

*4.1.2.6. 6-Ethoxy-5-(2-(4-bromo-2-nitrophenyl)hydrazono)-6-oxohexanoic acid **(2f)**.* Starting from 4-bromo-2-nitroaniline (6.5 g, 30 mmol), compound **2f** was afforded as yellow solid (1.8 g, 15%). mp: 159–161 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H), 4.40 (q, J = 7.2 Hz, 2H), 2.68 (t, J = 8.0 Hz, 2H), 2.47 (t, J = 8.0 Hz, 2H), 2.02 (m, 2H), 1.40 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{14}H_{17}N_3O_6Br$ $[M+H]^+$: 402.0295, found 402.0289.

*4.1.3. General procedure for synthesis of diethyl derivatives **(3a-f)***

Taking **3a** as an example: To a solution of **2a** (1.00 g, 3.10 mmol) in ethanol (20 mL), concentrated H_2SO_4 (0.31 mL) was added dropwise. The reaction mixture was heated to reflux for 4 h, and then poured into the ice-water. The mixture was filtered to afford the crude product, which was dried and recrystallized from ethyl acetate to give the desired product **3a** (856 mg, 79%) as orange solid.

*4.1.3.1. Diethyl 2-(2-(2-nitrophenyl)hydrazono)hexanedioate **(3a)**.* Starting from compound **2a** (1.00 g, 3.1 mmol), compound **3a** was afforded as orange solid (856 mg, 79%). mp: 77–78 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.89 (s, 1H), 8.20 (d, J = 8.1 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.57 (t, J = 8.1 Hz, 1H), 6.96 (t, J = 7.8 Hz, 1H), 4.40 (q, J = 7.2 Hz, 2H), 4.14 (q, J = 7.2 Hz, 2H), 2.66 (t, J = 7.5 Hz, 2H), 2.42 (t, J = 7.2 Hz, 2H), 2.02 (quint, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H), 1.25 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{22}N_3O_6$ $[M+H]^+$: 352.1503, found 352.1498.

*4.1.3.2. Diethyl 2-(2-(3-nitrophenyl)hydrazono)hexanedioate **(3b)**.* Starting from compound **2b** (3.69 g, 11.42 mmol), compound **3b** was afforded as yellow solid (1.13 g, 28%). mp: 82–83 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.12 (s, 1H), 7.86 (s, 1H), 7.61 (m, 1H), 7.25 (m, 2H), 4.15 (q, J = 7.2 Hz, 2H), 3.97 (q, J = 6.9 Hz, 2H), 2.43 (t, J = 7.2 Hz, 2H), 2.25 (t, J = 7.2 Hz, 2H), 1.82 (quint, J = 7.2 Hz, 2H), 1.22 (t, J = 7.2 Hz, 3H), 1.09 (t, J = 6.9 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{22}N_3O_6$ $[M+H]^+$: 352.1503, found 352.1497.

*4.1.3.3. Diethyl 2-(2-(4-nitrophenyl)hydrazono)hexanedioate **(3c)**.* Starting from compound **2c** (2.00 g, 6.19 mmol), compound **3c** was afforded as yellow solid (1.94 g, 90%). mp: 80–82 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.36 (s, 1H), 8.21 (d, J = 9.0 Hz, 2H), 7.40 (d, J = 9.0 Hz, 2H), 4.27–4.37 (m, 4H), 2.65 (t, J = 7.5 Hz, 2H), 2.50 (m, 2H), 1.77 (m, 2H), 1.33–1.42 (m, 6H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{22}N_3O_6$ $[M+H]^+$: 352.1503, found 352.1498.

*4.1.3.4. Diethyl 2-(2-(5-chloro-2-nitrophenyl)hydrazono)hexanedioate **(3d)**.* Starting from compound **2d** (1.56 g, 4.36 mmol), compound **3d** was afforded as yellow solid (1.24 g, 74%). mp: 80–81 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.91 (s, 1H), 8.15 (d, J = 8.8 Hz, 1H), 7.97 (s, 1H), 6.90 (d, J = 8.8 Hz, 1H), 4.40 (q, J = 7.2 Hz, 2H), 4.15 (q, J = 7.2 Hz, 2H), 2.67 (t, J = 7.2 Hz, 2H), 2.42 (t, J = 7.2 Hz, 2H), 2.01 (quint, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H), 1.26 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{21}O_6N_3Cl$ $[M+H]^+$: 386.1113, found 386.1116.

*4.1.3.5. Diethyl 2-(2-(4-chloro-2-nitrophenyl)hydrazono)hexanedioate **(3e)**.* Starting from compound **2e** (2.44 g, 6.82 mmol), compound **3e** was afforded as yellow solid (2.41 g, 92%). mp: 79–80 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.81 (s, 1H), 8.19 (d, J = 2.4 Hz, 1H), 7.96 (d, J = 9.0 Hz, 1H), 7.77 (dd, J_1 = 2.4 Hz, J_2 = 9.0 Hz, 1H), 4.39 (q, J = 7.2 Hz, 2H), 4.13 (q, J = 7.2 Hz, 2H), 2.65 (t, J = 7.2 Hz, 2H), 2.40 (t, J = 7.2 Hz, 2H), 2.00 (quint, J = 7.2 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H), 1.25 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{21}O_6N_3Cl$ $[M+H]^+$: 386.1113, found 386.1108.

*4.1.3.6. Diethyl 2-(2-(4-bromo-2-nitrophenyl)hydrazono)hexanedioate **(3f)**.* Starting from compound **2f** (1.70 g, 3.95 mmol), compound **3f** was afforded as light yellow solid (1.57 g, 92%). mp: 80–82 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.91 (s, 1H), 8.34 (d, J = 2.1 Hz, 1H), 7.90 (d, J = 8.7 Hz, 1H), 7.61–7.65 (dd, J_1 = 2.1 Hz, J_2 = 8.7 Hz, 1H), 4.39 (q, J = 6.9 Hz, 2H), 4.41 (q, J = 7.2 Hz, 2H), 2.65 (t, J = 7.2 Hz, 2H), 2.41 (t, J = 7.2 Hz, 2H), 2.01 (quint, J = 7.2 Hz, 2H), 1.40 (t, J = 6.9 Hz, 3H), 1.25 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{21}O_6N_3Br$ $[M+H]^+$: 430.0608, found 430.0609.

*4.1.4. General procedure for synthesis of indole-2-carboxylate derivatives **(4a–d)** and **(4e–g)***

Taking **4a** and **4c** as an example by which compounds **4a–d** were prepared in toluene: To a solution of **3b** (0.95 g, 2.71 mmol) in toluene (20 mL), *p*-toluenesulfonic acid (0.78 g, 4.07 mmol) was added. The resulting mixture was heated to reflux for 12 h and then concentrated under vacuum. The residue was dissolved in EtOAc (40 mL), washed with saturated $NaHCO_3$ aqueous solution (30 mL × 2) and dried over anhydrous $MgSO_4$. The crude product obtained after concentration was purified by column

chromatography to afford isomers **4a** (272 mg, 30%) and **4c** (260 mg, 29%) as yellow solid, respectively.

Taking **4e** as an example by which compounds **4e**–**g** were prepared in PPA: The reaction mixture of compound **3d** (1.0 g, 2.60 mmol) in PPA (2 g) was heated at 90–100 °C for 4 h. The reaction 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 (20 mL × 2). The crude product obtained after concentration was purified by column chromatography to afford the title compound **4e** (252 mg, 26%) as light yellow solid.

*4.1.4.1. Ethyl 3-(3-ethoxy-3-oxopropyl)-4-nitro-1H-indole-2-carboxylate (4a) and Ethyl 3-(3-ethoxy-3-oxopropyl)-6-nitro-1H-indole-2-carboxylate **(4c)**.* Starting from compound **3b** (0.95 g, 2.71 mmol), compounds **4a** (272 mg, 30%) and **4c** (260 mg, 29%) were afforded as yellow solid, respectively.

(**4a**) mp: 115–117 °C; ^{1}H NMR (300 MHz, $CDCl_3$): 9.39 (s, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.35 (m, 1H), 4.47 (q, J = 7.2 Hz, 2H), 4.12 (q, J = 6.9 Hz, 2H), 3.45 (t, J = 7.5 Hz, 2H), 2.70 (t, J = 8.1 Hz, 2H), 1.45 (t, J = 7.2 Hz, 3H), 1.23 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{19}N_2O_6$ $[M+H]^+$: 335.1238, found 335.1231.

(**4c**) mp: 134–135 °C; ^{1}H NMR (300 MHz, $CDCl_3$): 9.29 (s, 1H), 8.33 (s, 1H), 8.02 (d, J = 9.0 Hz, 1H), 7.83 (d, J = 9.3 Hz, 1H), 4.47 (q, J = 6.9 Hz, 2H), 4.09 (q, J = 6.9 Hz, 2H), 3.42 (t, J = 7.5 Hz, 2H), 2.70 (t, J = 7.5 Hz, 2H), 1.46 (t, J = 6.9 Hz, 3H), 1.19 (t, J = 6.9 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{19}N_2O_6$ $[M+H]^+$: 335.1238, found 335.1231.

*4.1.4.2. Ethyl 3-(3-ethoxy-3-oxopropyl)-5-nitro-1H-indole-2-carboxylate **(4b)**.* Starting from compound **3c** (1.95 g, 5.54 mmol), compound **4b** was afforded as yellow solid (402 mg, 22%). mp: 163.5–164.0 °C; ^{1}H NMR (300 MHz, acetone-d_6): δ 11.34 (brs, 1H), 8.78 (d, J = 1.8 Hz, 1H), 8.16 (dd, J_1 = 1.8 Hz, J_2 = 9.0 Hz, 1H), 7.65 (d, J = 9.0 Hz, 1H), 4.42 (q, J = 6.9 Hz, 2H), 4.03 (q, J = 6.9 Hz, 2H), 3.48 (t, J = 7.5 Hz, 2H), 2.72 (t, J = 7.5 Hz, 2H), 1.40 (t, J = 6.9 Hz, 3H), 1.14 (t, J = 6.9 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{19}N_2O_6$ $[M+H]^+$: 335.1238, found 335.1234.

*4.1.4.3. Ethyl 3-(3-ethoxy-3-oxopropyl)-7-nitro-1H-indole-2-carboxylate **(4d)**.* Starting from compound **3a** (0.79 g, 2.25 mmol), compound **4d** was afforded as light yellow solid (372 mg, 50%). mp: 76–78 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.21 (s, 1H), 8.30 (d, J = 8.1 Hz, 1H), 8.14 (d, J = 8.1 Hz, 1H), 7.27 (t, J = 8.1 Hz, 1H), 4.48 (q, J = 7.2 Hz, 2H), 4.08 (q, J = 7.2 Hz, 2H), 3.44 (t, J = 7.5 Hz, 2H), 2.71 (t, J = 7.5 Hz, 2H), 1.47 (t, J = 7.2 Hz, 3H), 1.19 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{19}N_2O_6$ $[M+H]^+$: 335.1237, found 335.1233.

*4.1.4.4. Ethyl 4-chloro-3-(3-ethoxy-3-oxopropyl)-7-nitro-1H-indole-2-carboxylate **(4e)**.* Starting from compound **3d** (1.00 g, 2.60 mmol), compound **4e** was afforded as light yellow solid (252 mg, 26%). mp: 135–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.37 (s, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.22 (d, J = 8.4 Hz, 1H), 4.48 (q, J = 7.2 Hz, 2H), 4.15 (q, J = 7.2 Hz, 2H), 3.73 (t, J = 8.4 Hz, 2H), 2.68 (t, J = 8.4 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H), 1.25 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{18}O_6N_2Cl$ $[M+H]^+$: 369.0848, found 369.0850.

*4.1.4.5. Ethyl 5-chloro-3-(3-ethoxy-3-oxopropyl)-7-nitro-1H-indole-2-carboxylate **(4f)**.* Starting from compound **3e** (2.25 g, 5.8 mmol), compound **4f** was afforded as off-white solid (706 mg, 11%). mp: 120–121 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.17 (s, 1H), 8.27 (s, 1H), 8.10 (s, 1H), 4.48 (q, J = 7.2 Hz, 2H), 4.09 (q, J = 7.2 Hz, 2H), 3.39 (t, J = 7.2 Hz, 2H), 2.69 (t, J = 7.2 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H), 1.21 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{18}O_6N_2Cl$ $[M+H]^+$: 369.0848, found 369.0844.

*4.1.4.6. Ethyl 5-bromo-3-(3-ethoxy-3-oxopropyl)-7-nitro-1H-indole-2-carboxylate **(4g)**.* Starting from compound **3f** (1.47 g, 3.4 mmol), compound **4g** was afforded as off-white solid (690 mg, 49%). mp: 120–121 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.18 (s, 1H), 8.40 (s, 1H), 8.24 (s, 1H), 4.48 (q, J = 6.9 Hz, 2H), 4.10 (q, J = 6.9 Hz, 2H), 3.39 (t, J = 7.2 Hz, 2H), 2.69 (t, J = 7.2 Hz, 2H), 1.46 (t, J = 6.9 Hz, 3H), 1.22 (t, J = 6.9 Hz, 3H). HRMS (ESI): *m/z*, calcd. for $C_{16}H_{18}O_6N_2Br$ $[M+H]^+$: 413.0343, found 413.0338.

*4.1.5. General procedure for synthesis of indole-2-carboxylic acid derivatives **(5a-g)***

Taking **5a** as an example: A solution of NaOH (140 mg, 3.50 mmol) in water (2 mL) was added dropwise to a solution of compound **4a** (236 mg, 0.70 mmol) in THF (4 mL) and ethanol (2 mL). The reaction mixture was stirred at room temperature for overnight and concentrated under vacuum. The residue was dissolved in water (10 mL), which was acidified to pH = 3–4 with diluted HCl. After filtration, the title compound **5a** was obtained as yellow solid (752 mg, 90%).

*4.1.5.1. 3-(2-Carboxyethyl)-4-nitro-1H-indole-2-carboxylic acid **(5a)**.* Starting from compound **4a** (236 mg, 0.71 mmol), compound **5a** was afforded as light yellow solid (163 mg, 83%). mp: >300 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 12.76 (brs, 2H), 12.37 (s, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 7.5 Hz, 1H), 7.36–7.41 (m, 1H), 3.25 (t, J = 8.1 Hz, 2H), 2.43 (t, J = 8.1 Hz, 2H). HRMS (ESI): *m/z*, calcd. for $C_{12}H_{11}N_2O_6$ $[M+H]^+$: 279.0607, found 279.0612.

*4.1.5.2. 3-(2-Carboxyethyl)-5-nitro-1H-indole-2-carboxylic acid **(5b)**.* Starting from compound **4b** (200 mg, 0.60 mmol), compound **5b** was afforded as light yellow solid (145 mg, 87%). mp: 265–267 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 12.23 (s, 1H), 8.74 (s, 1H), 8.09–8.12 (m, 1H), 7.54 (d, J = 9.2 Hz, 1H), 3.30–3.32 (m, 2H), 2.56 (t, J = 7.6 Hz, 2H). HRMS (ESI): *m/z*, calcd. for $C_{12}H_{11}N_2O_6$ $[M+H]^+$: 279.0608, found 279.0612.

*4.1.5.3. 3-(2-Carboxyethyl)-6-nitro-1H-indole-2-carboxylic acid **(5c)**.* Starting from compound **4c** (285 mg, 0.85 mmol), compound **5c** was afforded as light yellow solid (173 mg, 73%). mp: 262–264 °C; ^{1}H NMR (300 MHz, acetone-d_6): δ 11.08 (s, 1H), 8.22 (s, 1H), 7.72–7.80 (m, 2H), 3.23 (t, J = 7.8 Hz, 2H), 2.49 (t, J = 7.8 Hz, 2H). HRMS (ESI): *m/z*, calcd. for $C_{12}H_{11}N_2O_6$ $[M+H]^+$: 279.0612, found 279.0609.

*4.1.5.4. 3-(2-Carboxyethyl)-7-nitro-1H-indole-2-carboxylic acid **(5d)**.* Starting from compound **4d** (1.00 g, 3.00 mmol), compound **5d** was afforded as yellow solid (752 mg, 90%). mp: 254–256 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.86 (brs, 2H), 10.60 (s, 1H), 8.25 (d, J = 8.0 Hz, 1H), 8.23 (d, J = 8.0 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H), 3.28 (t, J = 7.6 Hz, 2H), 2.55 (t, J = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.71, 162.16, 132.89, 131.18, 129.59, 127.96, 126.92, 122.96, 122.11, 119.55, 34.82, 19.55. HRMS (ESI): *m/z*, calcd. for $C_{12}H_{11}N_2O_6$ $[M+H]^+$: 279.0612, found 279.0609.

*4.1.5.5. 3-(2-Carboxyethyl)-4-chloro-7-nitro-1H-indole-2-carboxylic acid **(5e)**.* Starting from compound **4e** (120 mg, 0.33 mmol), compound **5e** was afforded as light yellow solid (90 mg, 44%). mp: >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.80 (brs, 1H), 12.23 (brs, 1H), 10.94 (s, 1H), 8.23 (d, J = 8.4 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 3.56 (t, J = 8.4 Hz, 2H), 2.53 (t, J = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.34, 161.82, 135.06, 132.12, 129.19, 128.27, 126.57, 122.78, 122.17, 121.10, 35.67, 19.76. HRMS (ESI): *m/z*, calcd. for $C_{12}H_{10}N_2O_6Cl$ $[M+H]^+$: 313.0222, found 313.0221.

*4.1.5.6. 3-(2-Carboxyethyl)-5-chloro-7-nitro-1H-indole-2-carboxylic acid **(5f)**.* Starting from compound **4f** (130 mg, 0.35 mmol), compound **5f** was afforded as off-white solid (105 mg, 96%). mp: >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.93 (s, 1H), 8.40 (s, 1H), 8.23 (s, 1H), 3.28 (t, J = 7.6 Hz, 2H), 2.56 (t, J = 7.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.71, 161.90, 133.05, 132.18, 128.53, 128.50, 126.59, 123.38, 122.45, 121.32, 34.69, 19.33. HRMS (ESI): *m/z,* calcd. for $C_{12}H_{10}N_2O_6Cl$ $[M+H]^+$: 313.0222, found 313.0215.

*4.1.5.7. 3-(2-Carboxyethyl)-5-bromo-7-nitro-1H-indole-2-carboxylic acid **(5g)**.* Starting from compound **4g** (130 mg, 0.31 mmol), compound **5g** was afforded as off-white solid (102 mg, 91%). mp: >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.93 (s, 1H), 8.51 (s, 1H), 8.30 (s, 1H), 3.27 (t, J = 7.6 Hz, 2H), 2.54 (t, J = 7.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.71, 161.90, 133.36, 132.73, 131.45, 128.29, 126.85, 123.79, 122.35, 110.41, 34.69, 19.33. HRMS (ESI): *m/z,* calcd. for $C_{12}H_{10}N_2O_6Br$ $[M+H]^+$: 356.9710, found 356.9717.

*4.1.6. 3-(2-(Ethoxycarbonyl)-7-nitro-1H-indol-3-yl)propanoic acid **(6)***

To a solution of **2a** (10.57 g, 33 mmol) in toluene (165 mL), concentrated H_2SO_4 (0.31 mL) was dropwise added. The reaction mixture was heated to reflux for 4 h, and then poured into the ice-water. The residue was filtered, dried and recrystallized from ethyl acetate to give product **6** (6.46 g, 64%) as yellow solid. mp: 186–187 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.22 (s, 1H), 8.30 (d, J = 7.8 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H), 7.24–7.29 (m, 1H), 4.48 (q, J = 7.2 Hz, 2H), 3.44 (t, J = 7.5 Hz, 2H), 2.76 (t, J = 7.5 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{14}H_{15}N_2O_6$ $[M+H]^+$: 307.0925, found 307.0918.

*4.1.7. Ethyl 7-amino-3-(3-ethoxy-3-oxopropyl)-1H-indole-2-carboxylate **(7)***

To a solution of **4d** (500 mg, 1.50 mmol) in acetic acid (30 mL), Fe powder (672 mg, 12.00 mmol) was added. The reaction mixture was heated to 40 °C for 30 min, diluted with EtOAc (20 mL), filtered to remove Fe residue and concentrated under vacuum. The residue was dissolved in EtOAc (30 mL × 3), washed with saturated $NaHCO_3$ aqueous solution (10 mL × 3) and water (10 mL × 3) and dried over anhydrous $MgSO_4$. The crude product obtained after concentration was purified by silica gel column chromatography to afford **7** as light yellow solid (361 mg, 79%). mp: 99–100 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.34 (s, 1H), 7.22 (d, J = 8.1 Hz, 1H), 6.98–7.03 (m, 1H), 6.68 (d, J = 7.5 Hz, 1H), 4.45 (q, J = 7.2 Hz, 2H), 4.14 (q, J = 7.2 Hz, 2H), 3.97 (brs, 2H), 3.41 (t, J = 8.1 Hz, 2H), 2.68 (t, J = 8.1 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H),1.24 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{16}H_{21}N_2O_4$ $[M+H]^+$: 305.1496, found 305.1491.

*4.1.8. 7-Amino-3-(2-carboxyethyl)-1H-indole-2-carboxylic acid **(8)***

Following the procedure of **4.1.5**, starting from compound **7** (604 mg, 1.98 mmol), compound **8** was afforded as off-white solid (441 mg, 90%). mp: >300 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.03 (s, 2H), 6.84 (d, J = 8.1 Hz, 1H), 6.74–6.79 (m, 1H), 6.38 (d, J = 7.2 Hz, 1H), 3.19 (t, J = 7.8 Hz, 2H), 2.46 (t, J = 7.8 Hz, 2H). HRMS (ESI): *m/z,* calcd. for $C_{12}H_{13}N_2O_4$ $[M+H]^+$: 249.0870, found 249.0865.

*4.1.9. General procedure for synthesis of N-alkyl indole-2-carboxylate derivatives **(9a-b)***

Taking **9a** as an example: To a solution of **4d** (250 mg, 0.75 mmol) in DMF (8 mL), Cs_2CO_3 (719 mg, 2.25 mmol), a catalytic amount of KI and cyclopropylmethyl bromide (253 mg, 1.88 mmol) was sequentially added. The reaction mixture was heated to 60 °C for 2 h, and then concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL), washed with water (10 mL × 3), dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography to provide compound **9a** as yellow oil (283 mg, 97%).

*4.1.9.1. Ethyl 1-(cyclopropyl)methyl-3-(3-ethoxy-3-oxopropyl)-7-nitro-1H-indole -2-carboxylate **(9a)**.* Starting from compound **4d** (250 mg, 0.75 mmol), compound **9a** was afforded as yellow oil (283 mg, 97%). ^{1}H NMR (300 MHz, acetone-d_6): δ 8.19 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 7.32–7.38 (m, 1H), 4.43–4.50 (m, 4H), 4.05 (q, J = 7.2 Hz, 2H), 3.41 (t, J = 7.5 Hz, 2H), 2.67 (t, J = 7.5 Hz, 2H), 1.44 (t, J = 7.2 Hz, 3H), 1.14 (t, J = 7.2 Hz, 3H), 0.72–0.81 (m, 1H), 0.28–0.34 (m, 2H), 0.08–0.13 (m, 2H). HRMS (ESI): *m/z,* calcd. for $C_{20}H_{25}N_2O_6$ $[M+H]^+$: 389.1699, found 389.1707.

*4.1.9.2. Ethyl 1-benzyl-3-(3-ethoxy-3-oxopropyl)-7-nitro-1H-indole-2-carboxylate **(9b)**.* Starting from compound **4d** (260 mg, 0.78 mmol) and benzyl bromide (334 mg, 1.95 mmol), compound **9b** was afforded as yellow oil (305 mg, 92%). ^{1}H NMR (300 MHz, acetone-d_6): δ 8.21 (dd, J_1 = 7.8 Hz, J_2 = 0.9 Hz, 1H), 7.83 (d, J_1 = 7.8 Hz, J_2 = 0.9 Hz, 1H), 7.32 (t, J = 7.8 Hz, 1H), 7.14–7.16 (m, 3H), 6.69–6.72 (m, 2H), 5.84 (s, 2H), 4.44 (q, J = 7.2 Hz, 2H), 4.05 (q, J = 7.2 Hz, 2H), 3.45 (t, J = 7.5 Hz, 2H), 2.71 (t, J = 7.5 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H), 1.15 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{23}H_{25}N_2O_6$ $[M+H]^+$: 425.1707, found 425.1697.

*4.1.10. Synthesis of N-alkyl indole-2-carboxylic acid derivatives **(10a–b)***

*4.1.10.1. 1-(Cyclopropyl)methyl-3-(2-carboxyethyl)-7-nitro-1h-indole-2-carboxy -lic acid **(10a)**.* Following the procedure of **4.1.5**, and starting from compound **9a** (160 mg, 0.41 mmol), compound **10a** was afforded as white solid (112 mg, 82%). mp: 216–218 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 8.23 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 8.1 Hz, 1H), 7.35 (t, J = 8.1 Hz, 1H), 4.50 (d, J = 6.9 Hz, 2H), 3.45 (t, J = 7.5 Hz, 2H), 2.72 (t, J = 7.5 Hz, 2H), 0.74–0.82 (m, 1H), 0.28–0.34 (m, 2H), 0.09–0.14 (m, 2H). HRMS (ESI): *m/z,* calcd. for $C_{16}H_{17}N_2O_6$ $[M+H]^+$: 333.1081, found 333.1075.

*4.1.10.2. 1-Benzyl-3-(2-carboxyethyl)-7-nitro-1H-indole-2-carboxylic acid **(10b)**.* Following the procedure of **4.1.5**, and starting from compound **9b** (170 mg, 0.40 mmol), compound **10b** was afforded as white solid (130 mg, 88%). mp: >250 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 8.25 (d, J = 7.8 Hz, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.32 (t, J = 8.1 Hz, 1H), 7.13–7.15 (m, 3H), 6.70–6.73 (m, 2H), 5.90 (s, 2H), 3.50 (t, J = 7.5 Hz, 2H), 2.76 (t, J = 7.5 Hz, 2H). HRMS (ESI): *m/z,* calcd. for $C_{19}H_{17}N_2O_6$ $[M+H]^+$: 369.1074, found 369.1081.

*4.1.11. General procedure for synthesis of 4-substituted indole-2-carboxylate derivatives **(11a–m)***

Taking **11a** as an example: To a solution of **4e** (250 mg, 0.68 mmol) in toluene (12 mL), $Pd_2(dba)_3$ (62 mg, 0.068 mmol), Xantphos (79 mg, 0.14 mmol), Na_2CO_3 (216 mg, 2.03 mmol) and phenylboronic acid (248 mg, 2.03 mmol) were sequentially added. The reaction mixture was heated to 100 °C 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 by silica gel column chromatography to afford product **11a** as yellow solid (256 mg, 77%).

*4.1.11.1. Ethyl 3-(3-ethoxy-3-oxopropyl)-7-nitro-4-phenyl-1H-indole-2-carboxy -late **(11a)**.* Starting from compound **4e** (250 mg, 0.68 mmol) and phenylboronic acid (248 mg, 2.03 mmol), compound **11a** was afforded as yellow solid (256 mg, 77%). mp: 110–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.46 (s, 1H), 8.31 (d,

J = 8.4 Hz, 1H), 7.46–7.47 (m, 3H), 7.40 (m, 2H), 7.08 (d, J = 8.4 Hz, 1H), 4.45 (q, J = 7.2 Hz, 2H), 3.98 (q, J = 7.2 Hz, 2H), 2.97 (t, J = 8.4 Hz, 2H), 2.19 (t, J = 8.4 Hz, 2H), 1.43 (t, J = 7.2 Hz, 3H), 1.16 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{22}H_{23}N_2O_6$ $[M+H]^+$: 411.1511, found 411.1511.

4.1.11.2. Ethyl 3-(3-ethoxy-3-oxopropyl)-4-(2-fluorophenyl)-7-nitro-1H-indole-2 -carboxylate ***(11b)****.* Starting from compound **4e** (250 mg, 0.68 mmol) and 2-fluorophenylboronic acid (285 mg, 2.03 mmol), compound **11b** was afforded as light yellow solid (248 mg, 86%). mp: 117–119 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.45 (s, 1H), 8.34 (d, J = 8.1 Hz, 1H), 7.44–7.50 (m, 1H), 7.18–7.38 (m, 3H), 7.11 (d, J = 8.1 Hz, 1H), 4.45 (q, J = 7.2 Hz, 2H), 4.00 (q, J = 6.9 Hz, 2H), 2.90–3.00 (m, 2H), 2.21–2.28 (m, 2H), 1.43 (t, J = 7.2 Hz, 3H), 1.18 (t, J = 6.9 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{22}H_{22}N_2O_6F$ $[M+H]^+$: 429.1456, found 429.1450.

4.1.11.3. Ethyl 3-(3-ethoxy-3-oxopropyl)-4-(3-fluorophenyl)-7-nitro-1H-indole-2 -carboxylate ***(11c)****.* Starting from compound **4e** (200 mg, 0.54 mmol) and 3-fluorophenylboronic acid (227 mg, 1.62 mmol), compound **11c** was afforded as light yellow solid (222 mg, 77%). mp: 136–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.47 (s, 1H), 8.31 (d, J = 8.0 Hz, 1H), 7.45 (dd, J_1 = 7.6 Hz, J_2 = 14.0 Hz, 1H), 7.17–7.19 (m, 2H), 7.12 (d, J = 9.2 Hz, 1H), 7.08 (d, J = 8.0 Hz, 1H), 4.46 (q, J = 7.2 Hz, 2H), 4.00 (q, J = 7.2 Hz, 2H), 2.95–2.97 (m, 2H), 2.24 (t, J = 7.2 Hz, 2H), 1.43 (t, J = 7.2 Hz, 3H), 1.18 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{22}H_{22}N_2O_6F$ $[M+H]^+$: 429.1456, found 429.1458.

4.1.11.4. Ethyl 3-(3-ethoxy-3-oxopropyl)-4-(4-fluorophenyl)-7-nitro-1H-indole-2 -carboxylate ***(11d)****.* Starting from compound **4e** (250 mg, 0.68 mmol) and 4-fluorophenylboronic acid (285 mg, 2.03 mmol), compound **11d** was afforded as light yellow solid (210 mg, 72%). mp: 134–135 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.46 (s, 1H), 8.31 (d, J = 8.4 Hz, 1H), 7.38 (dd, J_1 = 5.4 Hz, J_2 = 8.7 Hz, 2H), 7.17 (t, J = 8.7 Hz, 2H), 7.06 (d, J = 8.1 Hz, 1H), 4.46 (q, J = 7.2 Hz, 2H), 4.01 (q, J = 7.2 Hz, 2H), 2.98 (t, J = 8.7 Hz, 2H), 2.19 (t, J = 8.7 Hz, 2H), 1.43 (t, J = 7.2 Hz, 3H), 1.18 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{22}H_{22}N_2O_6F$ $[M+H]^+$: 429.1456, found 429.1450.

4.1.11.5. Ethyl 3-(3-ethoxy-3-oxopropyl)-4-(3-methoxyphenyl)-7-nitro-1H-indole-2 -carboxylate ***(11e)****.* Starting from compound **4e** (200 mg, 0.54 mmol) and 3-methoxyphenylboronic acid (246 mg, 1.62 mmol), compound **11e** was afforded as yellow solid (245 mg, 82%). mp: 124–125 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.45 (s, 1H), 8.31 (d, J = 8.4 Hz, 1H), 7.38 (t, J = 7.8 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 6.95–7.02 (m, 2H), 6.91 (m, 1H), 4.45 (q, J = 6.9 Hz, 2H), 4.00 (q, J = 7.2 Hz, 2H), 3.85 (s, 3H), 2.99 (t, J = 8.4 Hz, 2H), 2.24 (t, J = 8.4 Hz, 2H), 1.43 (t, J = 6.9 Hz, 3H), 1.17 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{23}H_{25}N_2O_7$ $[M+H]^+$: 441.1656, found 441.1655.

4.1.11.6. Ethyl 3-(3-ethoxy-3-oxopropyl)-4-(4-methoxyphenyl)-7-nitro-1H-indole-2-carboxylate ***(11f)****.* Starting from compound **4e** (250 mg, 0.68 mmol) and 4-methoxyphenylboronic acid (309 mg, 2.03 mmol), compound **11f** was afforded as light yellow solid (266 mg, 89%). mp: 119–120 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.44 (s, 1H), 8.29 (d, J = 8.1 Hz, 1H), 7.32 (d, J = 8.7 Hz, 2H), 7.06 (d, J = 8.1 Hz, 1H), 6.99 (d, J = 8.7 Hz, 2H), 4.45 (q, J = 7.5 Hz, 2H), 3.99 (q, J = 6.9 Hz, 2H), 3.88 (s, 3H), 3.04 (t, J = 8.4 Hz, 2H), 2.19 (t, J = 8.4 Hz, 2H), 1.43 (t, J = 7.5 Hz, 3H), 1.16 (t, J = 6.9 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{23}H_{25}N_2O_7$ $[M+H]^+$: 441.1656, found 441.1649.

4.1.11.7. Ethyl 3-(3-ethoxy-3-oxopropyl)-4-(3-nitrophenyl)-7-nitro-1H-indole-2 -carboxylate ***(11g)****.* Starting from compound **4e** (400 mg, 1.08 mmol) and 3-nitrophenylboronic acid (541 mg, 3.24 mmol), compound **11g** was afforded as yellow solid (148 mg, 27%). mp: 148–149 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.51 (s, 1H), 8.34–8.38 (m, 2H), 8.30 (s, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.70 (t, J = 7.8 Hz, 1H), 7.10 (d, J = 8.1 Hz, 1H), 4.47 (q, J = 6.9 Hz, 2H), 3.95 (q, J = 6.9 Hz, 2H), 2.81 (m, 2H), 2.25–2.28 (m, 2H), 1.43 (t, J = 6.9 Hz, 3H), 1.13 (t, J = 6.9 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{22}H_{22}N_3O_8$ $[M+H]^+$: 456.1401, found 456.1400.

4.1.11.8. Ethyl 3-(3-ethoxy-3-oxopropyl)-4-(4-nitrophenyl)-7-nitro-1H-indole-2 -carboxylate ***(11h)****.* Starting from compound **4e** (250 mg, 0.68 mmol) and 4-nitrophenylboronic acid (340 mg, 2.03 mmol), compound **11g** was afforded as yellow solid (160 mg, 52%). mp: 134–135 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.51 (s, 1H), 8.33–8.37 (m, 3H), 7.61 (d, J = 8.4 Hz, 2H), 7.08 (d, J = 8.4 Hz, 1H), 4.47 (q, J = 7.2 Hz, 2H), 3.95 (q, J = 7.2 Hz, 2H), 2.92 (t, J = 8.1 Hz, 2H), 2.21 (t, J = 8.1 Hz, 2H), 1.44 (t, J = 7.2 Hz, 3H), 1.13 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{22}H_{22}N_3O_8$ $[M+H]^+$: 456.1391, found 456.1401.

4.1.11.9. Ethyl 3-(3-ethoxy-3-oxopropyl)-4-ethyl-7-nitro-1H-indole-2-carboxylate ***(11i)****.* To a solution of **4e** (250 mg, 0.68 mmol) in toluene (15 mL), $Pd(OAc)_2$ (15 mg, 0.068 mmol), $(tBu)_3P{\cdot}HBF_4$ (39 mg, 0.14 mmol), K_3PO_4 (720 mg, 3.40 mmol, 1 mL H_2O) and ethylboronic acid (451 mg, 2.04 mmol) were added in order. The reaction mixture was heated to 90 °C for 4 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 by column chromatography to afford product **11i** as light yellow solid (162 mg, 55%). mp: 85–87 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.39 (s, 1H), 8.22 (d, J = 8.0 Hz, 1H), 7.05 (d, J = 8.0 Hz, 1H), 4.47 (q, J = 7.2 Hz, 2H), 4.16 (q, J = 7.2 Hz, 2H), 3.59 (t, J = 8.4 Hz, 2H), 3.18 (q, J = 7.6 Hz, 2H), 2.66 (t, J = 8.4 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H), 1.39 (t, J = 7.2 Hz, 3H), 1.26 (t, J = 7.6 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{18}H_{23}N_2O_6$ $[M+H]^+$: 363.1551, found 363.1542.

4.1.11.10. Ethyl 3-(3-ethoxy-3-oxopropyl)-4-isobutyl-7-nitro-1H-indole-2–carboxy -late ***(11j)****.* Following the procedure of **4.1.11.9**, starting from compound **4e** (200 mg, 0.54 mmol) and isobutylboronic acid (165 mg, 2.62 mmol), compound **11j** was afforded as light yellow solid (150 mg, 71%). mp: 73–75 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.40 (s, 1H), 8.20 (d, J = 8.4 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 4.47 (q, J = 6.9 Hz, 2H), 4.17 (q, J = 6.9 Hz, 2H), 3.57 (t, J = 8.4 Hz, 2H), 2.96 (d, J = 7.2 Hz, 2H), 2.64 (t, J = 8.4 Hz, 2H), 1.91–2.00 (m, 1H), 1.46 (t, J = 6.9 Hz, 3H), 1.26 (t, J = 6.9 Hz, 3H), 1.00 (d, J = 6.6 Hz, 6H). HRMS (ESI): *m/z,* calcd. for $C_{20}H_{27}N_2O_6$ $[M+H]^+$: 391.1864, found 391.1859.

4.1.11.11. Ethyl 3-(3-ethoxy-3-oxopropyl)-7-nitro-4-phenylamino-1H-indole-2 -carboxylate ***(11k)****.* Starting from compound **4e** (250 mg, 0.68 mmol) and aniline (189 mg, 2.03 mmol), compound **11k** was afforded as orange solid (164 mg, 57%). mp: 144–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.51 (s, 1H), 9.55 (s, 1H), 8.12 (d, J = 9.2 Hz, 1H), 7.38–7.44 (m, 4H), 7.17 (t, J = 6.8 Hz, 1H), 6.91 (d, J = 9.2 Hz, 1H), 4.46 (q, J = 7.2 Hz, 2H), 4.16 (q, J = 7.2 Hz, 2H), 3.53 (t, J = 5.6 Hz, 2H), 3.00 (t, J = 5.6 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H), 1.23 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{22}H_{24}N_3O_6$ $[M+H]^+$: 426.1660, found 426.1658.

4.1.11.12. Ethyl 3-(3-ethoxy-3-oxopropyl)-7-nitro-4-(3-methoxyphenylamino)-1H -indole-2-carboxylate ***(11l)****.* Starting from compound **4e** (250 mg, 0.68 mmol) and 3-methoxyaniline (250 mg, 2.03 mmol), compound **11l** was afforded as yellow solid (270 mg, 87%). mp: 126–128 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.50 (brs, 1H), 9.57 (s, 1H), 8.13 (d, J = 9.3 Hz, 1H), 7.32 (t, J = 8.1 Hz, 1H),

6.96–7.00 (m, 3H), 6.71 (dd, J_1 = 1.8 Hz, J_2 = 8.1 Hz, 1H), 4.46 (q, J = 7.2 Hz, 2H), 4.15 (q, J = 7.2 Hz, 2H), 3.84 (s, 3H), 3.52 (t, J = 6.0 Hz, 2H), 3.00 (t, J = 6.0 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H), 1.23 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{23}H_{26}N_3O_7$ $[M+H]^+$: 456.1765, found 456.1765.

*4.1.11.13. Ethyl 3-(3-ethoxy-3-oxopropyl)-7-nitro-4-(4-methoxyphenylamino)-1H -indole-2-carboxylate **(11m)**.* Starting from compound **4e** (250 mg, 0.68 mmol) and 4-methoxyaniline (251 mg, 2.04 mmol), compound **11m** was afforded as orange solid (252 mg, 82%). mp: 85–87 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.51 (s, 1H), 9.30 (s, 1H), 8.09 (d, J = 9.3 Hz, 1H), 7.31 (d, J = 8.7 Hz, 2H), 6.97 (d, J = 8.7 Hz, 2H), 6.66 (d, J = 9.3 Hz, 1H), 4.45 (q, J = 7.2 Hz, 2H), 4.15 (q, J = 7.2 Hz, 2H), 3.85 (s, 3H), 3.53 (t, J = 5.4 Hz, 2H), 2.98 (t, J = 5.4 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H), 1.22 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{23}H_{26}N_3O_7$ $[M+H]^+$: 456.1765, found 456.1758.

*4.1.12. Synthesis of 4-substituted indole-2-carboxylic acid derivatives **(12a–m)***

*4.1.12.1. 3-(2-Carboxyethyl)-7-nitro-4-phenyl-1H-indole-2-carboxylic acid **(12a)**.* Following the procedure of **4.1.5**, and starting from compound **11a** (130 mg, 0.32 mmol), compound **12a** was afforded as light yellow solid (103 mg, 92%). mp: > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.70 (brs, 1H), 11.81 (brs, 1H), 10.78 (s, 1H), 8.30 (d, J = 8.4 Hz, 1H), 7.47–7.48 (m, 5H), 7.12 (d, J = 8.4 Hz, 1H), 2.80 (t, J = 8.0 Hz, 2H), 2.06 (t, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.95, 162.07, 145.70, 138.79, 132.11, 128.62, 128.44, 128.34, 128.15, 127.85, 127.57, 123.11, 121.85, 121.66, 34.29, 19.73. HRMS (ESI): *m/z,* calcd. for $C_{18}H_{15}N_2O_6$ $[M+H]^+$: 355.0925, found 355.0923.

*4.1.12.2. 3-(2-Carboxyethyl)-4-(2-fluorophenyl)-7-nitro-1H-indole-2-carboxylic acid **(12b)**.* Following the procedure of **4.1.5**, and starting from compound **11b** (120 mg, 0.28 mmol), compound **12b** was afforded as light yellow solid (99 mg, 95%). mp: >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.82 (s, 1H), 8.31 (d, J = 8.4 Hz, 1H), 7.54 (dd, J_1 = 6.8 Hz, J_2 = 13.2 Hz, 1H), 7.48 (t, J = 7.2 Hz, 1H), 7.32–7.38 (m, 2H), 7.16 (d, J = 8.4 Hz, 1H), 2.80–2.85 (m, 1H), 2.65–2.73 (m, 1H), 2.07 (t, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.87, 162.01, 158.67 (J_{CF} = 242.1 Hz), 138.32, 132.76, 131.10 (J_{CF} = 8.0 Hz), 130.93, 128.43, 128.38, 127.80, 126.04 (J_{CF} = 16.0 Hz), 124.56, 122.71, 122.30, 121.61, 115.58 (J_{CF} = 21.0 Hz), 34.40, 19.38. HRMS (ESI): *m/z,* calcd. for $C_{18}H_{14}N_2O_6F$ $[M+H]^+$: 373.0830, found 373.0821.

*4.1.12.3. 3-(2-Carboxyethyl)-4-(3-fluorophenyl)-7-nitro-1H-indole-2-carboxylic acid **(12c)**.* Following the procedure of **4.1.5**, and starting from compound **11c** (110 mg, 0.26 mmol), compound **12c** was afforded as light yellow solid (90 mg, 94%). mp: >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.82 (s, 1H), 8.30 (d, J = 8.0 Hz, 1H), 7.51–7.54 (m, 1H), 7.29–7.38 (m, 3H), 7.14 (d, J = 8.0 Hz, 1H), 2.80 (t, J = 8.0 Hz, 2H), 2.12 (t, J = 8.0 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.96, 162.04, 161.67 (J_{CF} = 243.8 Hz), 143.90, 141.01 (J_{CF} = 7.9 Hz), 132.35, 130.27 (J_{CF} = 8.5 Hz), 128.50, 127.88, 127.80, 124.75, 122.73, 121.73, 121.51, 115.44 (J_{CF} = 22.1 Hz), 115.27 (J_{CF} = 20.9 Hz), 34.28, 19.76. HRMS (ESI): *m/z,* calcd. for $C_{18}H_{14}N_2O_6F$ $[M+H]^+$: 373.0830, found 373.0828.

*4.1.12.4. 3-(2-Carboxyethyl)-4-(4-fluorophenyl)-7-nitro-1H-indole-2-carboxylic acid **(12d)**.* Following the procedure of **4.1.5**, and starting from compound **11d** (120 mg, 0.28 mmol), compound **12d** was afforded as light yellow solid (78 mg, 75%). mp: >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.76 (s, 1H), 8.28 (d, J = 8.0 Hz, 1H), 7.52 (dd, J_1 = 5.6 Hz, J_2 = 8.4 Hz, 2H), 7.32 (t, J = 8.4 Hz, 2H), 7.12 (d, J = 8.0 Hz, 1H), 2.81 (t, J = 8.4 Hz, 2H), 2.08 (t, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.03, 162.23 (J_{CF} = 244.4 Hz), 162.06, 144.56, 135.10, 132.22, 130.55 (J_{CF} = 8.4 Hz), 128.58, 127.95, 127.60, 122.96, 121.99, 121.63, 115.12 (J_{CF} = 21.7 Hz), 34.29, 19.78. HRMS (ESI): *m/z,* calcd. for $C_{18}H_{14}N_2O_6F$ $[M+H]^+$: 373.0830, found 373.0824.

*4.1.12.5. 3-(2-Carboxyethyl)-4-(3-methoxyphenyl)-7-nitro-1H-indole-2-carboxylic acid **(12e)**.* Following the procedure of **4.1.5**, and starting from compound **11e** (140 mg, 0.32 mmol), compound 12e was afforded as light yellow solid (112 mg, 91%). mp: 220–222 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.76 (s, 1H), 8.29 (d, J = 8.1 Hz, 1H), 7.40 (t, J = 8.1 Hz, 1H), 7.13 (d, J = 8.1 Hz, 1H), 6.98–7.05 (m, 3H), 3.79 (s, 3H), 2.81 (t, J = 8.4 Hz, 2H), 2.13 (t, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.03, 162.08, 158.88, 145.51, 140.13, 129.33, 128.59, 127.88, 127.59, 123.15, 121.69, 121.60, 120.68, 114.28, 113.77, 55.20, 34.38, 19.79. HRMS (ESI): *m/z,* calcd. for $C_{19}H_{17}N_2O_7$ $[M+H]^+$: 385.1030, found 385.1027.

*4.1.12.6. 3-(2-Carboxyethyl)-4-(4-methoxyphenyl)-7-nitro-1H-indole-2-carboxylic acid **(12f)**.* Following the procedure of **4.1.5**, and starting from compound **11f** (125 mg, 0.28 mmol), compound **12f** was afforded as light yellow solid (93 mg, 85%). mp: >250 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.74 (s, 1H), 8.28 (d, J = 8.1 Hz, 1H), 7.39 (d, J = 8.4 Hz, 2H), 7.10 (d, J = 8.1 Hz, 1H), 7.04 (d, J = 8.4 Hz, 2H), 3.82 (s, 3H), 2.86 (t, J = 8.1 Hz, 2H), 2.08 (t, J = 8.1 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.11, 162.10, 159.47, 145.82, 131.80, 130.93, 129.69, 128.72, 127.96, 127.41, 123.27, 122.02, 121.70, 113.69, 55.25, 34.24, 19.82. HRMS (ESI): *m/z,* calcd. for $C_{19}H_{17}N_2O_7$ $[M+H]^+$: 385.1030, found 385.1022.

*4.1.12.7. 3-(2-Carboxyethyl)-7-nitro-4-(3-nitrophenyl)-1H-indole-2-carboxylic acid **(12g)**.* Following the procedure of **4.1.5**, and starting from compound **11g** (100 mg, 0.32 mmol), compound **12g** was afforded as yellow solid (75 mg, 85%). mp: 134–136 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.87 (s, 1H), 8.32–8.37 (m, 3H), 7.97 (d, J = 7.6 Hz, 1H), 7.82 (t, J = 8.0 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 3.34 (t, J = 7.2 Hz, 2H), 2.13 (t, J = 7.2 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.89, 161.97, 147.34, 142.57, 140.21, 135.14, 132.73, 130.00, 128.54, 128.04, 127.82, 123.27, 123.04, 122.41, 121.97, 121.61, 34.16, 19.90. HRMS (ESI): *m/z,* calcd. for $C_{18}H_{14}N_3O_8$ $[M+H]^+$: 400.0775, found 400.0775.

*4.1.12.8. 3-(2-Carboxyethyl)-7-nitro-4-(4-nitrophenyl)-1H-indole-2-carboxylic acid **(12h)**.* Following the procedure of **4.1.5**, and starting from compound **11h** (100 mg, 0.32 mmol), compound **12h** was affoarded as yellow solid (75 mg, 85%). mp: >250 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.91 (s, 1H), 8.35 (d, J = 8.1 Hz, 3H), 7.80 (d, J = 8.4 Hz, 2H), 7.19 (d, J = 8.1 Hz, 1H), 2.77 (t, J = 7.8 Hz, 2H), 2.08 (t, J = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.98, 161.99, 147.37, 145.57, 142.89, 132.77, 130.10, 128.50, 128.05, 127.59, 123.30, 122.46, 121.59, 121.54, 34.25, 19.89. HRMS (ESI): *m/z,* calcd. for $C_{18}H_{14}N_3O_8$ $[M+H]^+$: 400.0775, found 400.0767.

*4.1.12.9. 3-(2-Carboxyethyl)-4-ethyl-7-nitro-1H-indole-2-carboxylic acid **(12i)**.* Following the procedure of **4.1.5**, and starting from compound **11i** (110 mg, 0.29 mmol), compound **12i** was afforded as light yellow solid (40 mg, 45%). mp: 197–198 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.62 (s, 1H), 8.22 (d, J = 8.4 Hz, 1H), 7.16 (d, J = 8.4 Hz, 1H), 3.44 (t, J = 7.2 Hz, 2H), 3.15 (q, J = 7.5 Hz, 2H), 2.50–2.56 (m, 2H), 1.31 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.48, 162.10, 149.30, 131.16, 128.54, 127.75, 126.74, 123.17, 122.40, 120.01, 35.71, 25.73, 20.72, 15.25. HRMS (ESI): *m/z,* calcd. for $C_{14}H_{15}N_2O_6$ $[M+H]^+$: 307.0925, found 307.0919.

*4.1.12.10. 3-(2-Carboxyethyl)-4-isobutyl-7-nitro-1H-indole-2-carboxylic acid **(12j)**.* Following the procedure of **4.1.5**, and starting from compound **11j** (110 mg, 0.28 mmol), compound **12j** was afforded as light yellow solid (82 mg, 88%). mp: 199–200 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.62 (brs, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 3.42 (t, J = 8.0 Hz, 2H), 2.95 (d, J = 6.8 Hz, 2H), 2.50–2.54 (m, 2H), 1.89–1.92 (m, 1H), 0.95 (d, J = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.45, 162.09, 146.65, 131.32, 128.74, 128.11, 126.90, 123.01, 122.19, 121.82, 41.88, 35.67, 30.19, 22.03, 20.62. HRMS (ESI): *m/z,* calcd. for $C_{16}H_{19}N_2O_6$ $[M+H]^+$: 335.1238, found 335.1231.

*4.1.12.11. 3-(2-Carboxyethyl)-7-nitro-4-phenylamino-1H-indole-2-carboxylic acid **(12k)**.* Following the procedure of **4.1.5**, and starting from compound **11k** (103 mg, 0.24 mmol), compound **12k** was afforded as yellow solid (85 mg, 95%). mp: 235–237 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.52 (s, 1H), 9.34 (s, 1H), 8.11 (d, J = 9.2 Hz, 1H), 7.45 (t, J = 7.6 Hz, 2H), 7.36 (d, J = 7.6 Hz, 2H), 7.19 (t, J = 7.2 Hz, 1H), 6.87 (d, J = 9.2 Hz, 1H), 3.50 (t, J = 6.8 Hz, 2H), 2.74 (t, J = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.70, 161.93, 149.00, 140.11, 130.89, 129.60, 125.96, 124.68, 124.58, 124.21, 123.99, 122.15, 117.32, 104.56, 35.23, 19.94. HRMS (ESI): *m/z,* calcd. for $C_{18}H_{16}N_3O_6$ $[M+H]^+$: 370.1034, found 370.1033.

*4.1.12.12. 3-(2-Carboxyethyl)-7-nitro-4-(3-methoxyphenylamino)-1H-indole-2-carboxylic acid **(12l)**.* Following the procedure of **4.1.5**, and starting from compound **11l** (160 mg, 0.35 mmol), compound **12l** was afforded as yellow solid (130 mg, 93%). mp: 248–250 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.53 (s, 1H), 9.32 (s, 1H), 8.13 (d, J = 9.3 Hz, 1H), 7.34 (t, J = 8.1 Hz, 1H), 6.85–6.94 (m, 3H), 6.76 (d, J = 7.8 Hz, 1H), 3.77 (s, 3H), 3.49 (t, J = 6.6 Hz, 2H), 2.73 (t, J = 6.6 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 175.71, 161.93, 160.29, 148.71, 141.43, 130.84, 130.36, 125.86, 124.78, 124.75, 123.91, 117.59, 114.00, 109.55, 107.52, 105.13, 55.15, 35.22, 19.93. HRMS (ESI): *m/z,* calcd. for $C_{19}H_{18}N_3O_7$ $[M+H]^+$: 400.1139, found 400.1138.

*4.1.12.13. 3-(2-Carboxyethyl)-7-nitro-4-(4-methoxyphenylamino)-1H-indole-2-carboxylic acid **(12m)**.* Following the procedure of **4.1.5**, and starting from compound **11m** (148 mg, 0.33 mmol), compound **12m** was afforded as yellow solid (90 mg, 69%). mp: >250 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.49 (s, 1H), 9.11 (s, 1H), 8.06 (d, J = 9.3 Hz, 1H), 7.30 (d, J = 8.7 Hz, 2H), 7.05 (d, J = 8.7 Hz, 2H), 6.49 (d, J = 9.3 Hz, 1H), 3.80 (s, 3H), 3.52 (t, J = 6.9 Hz, 2H), 2.72 (t, J = 6.9 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 175.35, 161.93, 156.86, 150.44, 132.32, 131.00, 126.28, 125.44, 124.14, 123.63, 116.00, 114.86, 103.56, 55.34, 35.26, 19.98. HRMS (ESI): *m/z,* calcd. for $C_{19}H_{18}N_3O_7$ $[M+H]^+$: 400.1139, found 400.1131.

*4.1.13. General procedure for synthesis of 5-substituted indole-2-carboxylate derivatives **(13a–d)***

*4.1.13.1. Ethyl 3-(3-ethoxy-3-oxopropyl)-7-nitro-5-phenyl-1H-indole-2-carboxy -late **(13a)**.* Following the procedure of **4.1.11.9**, starting from compound **4g** (200 mg, 0.48 mmol) and phenylboronic acid (176 mg, 1.44 mmol), compound **13a** was afforded as light yellow solid (222 mg, 90%). mp: 85–87 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.18 (s, 1H), 8.55 (s, 1H), 8.32 (s, 1H), 7.68 (d, J = 7.6 Hz, 2H), 7.51 (t, J = 7.6 Hz, 2H), 7.42 (t, J = 7.2 Hz, 1H), 4.49 (q, J = 7.2 Hz, 2H), 4.08 (q, J = 7.2 Hz, 2H), 3.48 (t, J = 7.6 Hz, 2H), 2.73 (t, J = 7.6 Hz, 2H), 1.48 (t, J = 7.2 Hz, 3H), 1.17 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{22}H_{23}N_2O_6$ $[M+H]^+$: 411.1551, found 411.1543.

*4.1.13.2. Ethyl 3-(3-ethoxy-3-oxopropyl)-5-isobutyl-7-nitro-1H-indole-2-carboxy -late **(13b)**.* Following the procedure of **4.1.11.9**, and starting from compound **4g** (210 mg, 0.51 mmol) and isobutylboronic acid (156 mg, 1.53 mmol), compound **13b** was afforded as light yellow solid (188 mg, 94%). mp: 88–89 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.10 (s, 1H), 8.11 (s, 1H), 7.86 (s, 1H), 4.47 (q, J = 7.2 Hz, 2H), 4.09 (q, J = 7.2 Hz, 2H), 3.42 (t, J = 8.0 Hz, 2H), 2.65–2.70 (m, 4H), 1.92–1.98 (m, 1H), 1.46 (t, J = 7.2 Hz, 3H), 1.19 (t, J = 7.2 Hz, 3H), 0.95 (d, J = 6.4 Hz, 6H). HRMS (ESI): *m/z,* calcd. for $C_{20}H_{27}N_2O_6$ $[M+H]^+$: 391.1864, found 391.1857.

*4.1.13.3. Ethyl 3-(3-ethoxy-3-oxopropyl)-5-ethyl-7-nitro-1H-indole-2-carboxylate **(13c)**.* Following the procedure of **4.1.11.9**, and starting from compound **4g** (270 mg, 0.65 mmol) and ethylboronic acid (145 mg, 1.96 mmol), compound **13c** was afforded as light yellow solid (150 mg, 63%). mp: 95–96 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.09 (s, 1H), 8.17 (s, 1H), 7.91 (s, 1H), 4.47 (q, J = 7.2 Hz, 2H), 4.09 (q, J = 7.2 Hz, 2H), 3.42 (t, J = 7.6 Hz, 2H), 2.84 (q, J = 7.6 Hz, 2H), 2.69 (t, J = 7.6 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H), 1.34 (t, J = 7.6 Hz, 3H), 1.20 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{18}H_{23}N_2O_6$ $[M+H]^+$: 363.1551, found 363.1543.

*4.1.13.4. Ethyl 3-(3-ethoxy-3-oxopropyl)-5-phenylamino-7-nitro-1H-indole-2-car -boxylate **(13d)**.* To a solution of **4g** (250 mg, 0.56 mmol) in toluene (15 mL), $Pd_2(dba)_3$ (51 mg, 0.056 mmol), Davephos (44 mg, 0.11 mmol), K_3PO_4 (357 mg, 1.68 mmol, 2 mL H_2O) and aniline (156 mg, 1.68 mmol) were added in order. The reaction mixture was heated to 80 °C for 6 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 by column chromatography to afford product **13d** as sorrel solid (210 mg, 68%). mp: 145–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.04 (s, 1H), 8.12 (d, J = 1.6 Hz, 1H), 7.84 (s, 1H), 7.30 (t, J = 8.0 Hz, 2H), 7.03 (d, J = 8.0 Hz, 2H), 6.97 (t, J = 8.0 Hz, 1H), 4.47 (q, J = 7.2 Hz, 2H), 4.07 (q, J = 7.2 Hz, 2H), 3.36 (t, J = 7.6 Hz, 2H), 2.67 (t, J = 7.6 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H), 1.18 (t, J = 7.2 Hz, 3H). HRMS (ESI): *m/z,* calcd. for $C_{22}H_{24}N_3O_6$ $[M+H]^+$: 426.1651, found 426.1660.

*4.1.14. Synthesis of 5-substituted indole-2-carboxylic acid derivatives **(14a–d)***

*4.1.14.1. 3-(2-Carboxyethyl)-7-nitro-5-phenyl-1H-indole-2-carboxylic acid **(14a)**.* Following the procedure of **4.1.5**, and starting from compound **13a** (120 mg, 0.29 mmol), compound **14a** was afforded as yellow solid (102 mg, 98%). mp: >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.70 (s, 1H), 8.57 (s, 1H), 8.45 (s, 1H), 7.84 (d, J = 7.6 Hz, 2H), 7.53 (t, J = 7.6 Hz, 2H), 7.42 (t, J = 7.6 Hz, 1H), 3.38 (t, J = 7.6 Hz, 2H), 2.61 (t, J = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.90, 162.31, 138.78, 133.10, 132.03, 129.07, 128.34, 127.62, 127.28, 127.05, 122.96, 120.19, 39.94, 19.46. HRMS (ESI): *m/z,* calcd. for $C_{18}H_{15}N_2O_6$ $[M+H]^+$: 355.0925, found 355.0918.

*4.1.14.2. 3-(2-Carboxyethyl)-5-isobutyl-7-nitro-1H-indole-2-carboxylic acid **(14b)**.* Following the procedure of **4.1.5**, and starting from compound **13b** (120 mg, 0.31 mmol), compound **14b** was afforded as yellow solid (75 mg, 74%). mp: >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.19 (brs, 2H), 10.35 (s, 1H), 8.03 (s, 1H), 8.01 (s, 1H), 3.29 (t, J = 7.6 Hz, 2H), 2.65 (d, J = 7.2 Hz, 2H), 2.58 (t, J = 7.6 Hz, 2H), 1.88–1.95 (m, 1H), 0.90 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.92, 163.08, 132.43, 132.11, 131.80, 130.36, 129.21, 126.20, 121.71, 119.86, 43.76, 35.12, 29.95, 21.95, 19.40. HRMS (ESI): *m/z,* calcd. for $C_{16}H_{19}N_2O_6$ $[M+H]^+$: 335.1238, found 335.1233.

*4.1.14.3. 3-(2-Carboxyethyl)-5-ethyl-7-nitro-1H-indole-2-carboxylic acid **(14c)**.* Following the procedure of **4.1.5**, and starting from compound **13c** (110 mg, 0.30 mmol), compound **14c** was afforded as light yellow solid (72 mg, 77%). mp: >250 °C; ^{1}H NMR (400 MHz,

DMSO-d_6): δ 10.55 (s, 1H), 8.13 (s, 2H), 3.29 (t, J = 7.6 Hz, 2H), 2.82 (q, J = 7.6 Hz, 2H), 2.56 (t, J = 7.6 Hz, 2H), 1.28 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.76, 162.21, 135.61, 132.56, 131.34, 128.33, 126.99, 126.73, 122.56, 121.92, 34.78, 27.62, 19.51, 15.91. HRMS (ESI): *m/z*, calcd. for $C_{14}H_{15}N_2O_6$ $[M+H]^+$: 307.0925, found 307.0918.

*4.1.14.4. 3-(2-Carboxyethyl)-5-phenylamino-7-nitro-1H-indole-2-carboxylic acid **(14d)**.* Following the procedure of **4.1.5**, and starting from compound **13d** (120 mg, 0.28 mmol), compound **14d** was afforded as sorrel solid (92 mg, 89%). mp: >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.48 (s, 1H), 8.42 (brs, 1H), 8.02 (s, 1H), 7.93 (s, 1H), 7.27 (t, J = 8.0 Hz, 2H), 7.11 (d, J = 8.0 Hz, 2H), 6.86 (t, J = 8.0 Hz, 1H), 3.24 (t, J = 7.2 Hz, 2H), 2.56 (t, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.77, 162.17, 143.71, 136.34, 132.90, 131.72, 129.37, 127.33, 123.88, 121.93, 119.85, 116.70, 115.95, 113.54, 109.30, 34.77, 19.60. HRMS (ESI): *m/z*, calcd. for $C_{18}H_{16}N_3O_6$ $[M+H]^+$: 370.1034, found 370.1028.

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 2 YT 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 × g), resuspended and lysed by sonication. The lysate was clarified by centrifugation at 31,000 × 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 [31].

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 [27]. 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) [29]. 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 **14c** in the crystal structure FBPase [28]. 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 **14c** was built and minimized using Prepare Ligands tool of DS and refined with CHARMm force field. Docking of compound **14c** 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 **14c** and FBPase.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.11.049.

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