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# Design, synthesis and biological evaluation of exiguamine A analogues as IDO1 inhibitors

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ABSTRACT

A series of exiguamine A analogues were designed and synthesized via 15 steps. Their inhibitory activities against IDO1 were tested and the structure-activity relationships were studied. Most compounds exhibited potent IDO1 inhibitory activities with $IC_{50}$ values at the level of $10^{-7}$–$10^{-8}$ M. Compound **21f** was the most potent IDO1 inhibitor with an $IC_{50}$ value of 65.3 nM, which was comparable with the positive control drug epacadostat ($IC_{50}$ = 46 nM). Moreover, compound **21f** showed higher selectivity for IDO1 over tryptophan 2,3-dioxygenase (TDO) and no cytotoxicity at its effective concentration, rending it justifiable for further optimization and evaluation.



## 1. Introduction

Indoleamine 2,3-dioxygenase 1 (IDO1) is a heme-containing enzyme that catalyzes the initial and rate limiting step in the oxidative catabolism of tryptophan along the kynurenine pathway, leading to the generation of biologically active metabolites such as kynurenine, kynurenic acid, excitotoxin quinolinic acid, and nicotinamide adenine dinucleotide ($NAD^+$) [1,2]. The depletion of tryptophan and the production of metabolites result in apoptosis of effector T cell and promotion of Treg differentiation, both responsible for local immunosuppression [3]. Many studies have shown that IDO1 is up-regulated in a variety of cancer cells, such as breast cancer, cervical cancer and brain cancer, which is related to the invasion of tumor and poor prognosis of patients [4,5]. Hence, IDO1 has been regarded as an attractive target for cancer immunotherapy.

Since the discovery of 4-phenylimidazole as a weak IDO inhibitor [6], intense efforts to develop small-molecule IDO1 inhibitors are ongoing in academic and pharmaceutical companies. To date, several IDO1 inhibitors including indoximod [7], epacadostat (INCB024360) [8], BMS-986205 [9] and PF-06840003 [10], navoximod [11] have been subjected to clinical trials (either alone or in combination with other anti-cancer drugs).

Exiguamine A (Fig. 1), which was isolated from the marine Sponge *Neopetrosia exigua* [12], was found to be a potent IDO1 inhibitor ($K_i$ = 41 nM). It is a racemate and possesses an unusual hexacyclic skeleton including an indolequinone and an *N,N*-dimethyl dihydroindolinium moiety, a spirobicyclic system linking of hydantoin and a pyran ring [13]. Its unique structure and potent IDO1 inhibitory activity made exiguamine A an appealing lead structure for developing novel IDO1 inhibitors. So far, there have been only three reports about the total synthesis and structural modification of exiguamine A [13–15]. The preliminary structure-activity relationship study demonstrated that a charged quaternary ammonium ion and a closed pyran ring were not necessary for its bioactivity. However, the quinone functionality was critical for its activity.

In this work, we designed a series of novel exiguamine A analogues based on the bioisosterism approach. The 31-*O* atom was replaced with methylene group, and meanwhile, the unnecessary quaternary ammonium ion was removed (Fig. 1). This series of novel exiguamine A analogues were relatively simple in structure and easy to be synthesized. A total of thirty-nine analogues with different amino side chains were synthesized and their IDO1 inhibitory activities were evaluated.

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0223-5234/

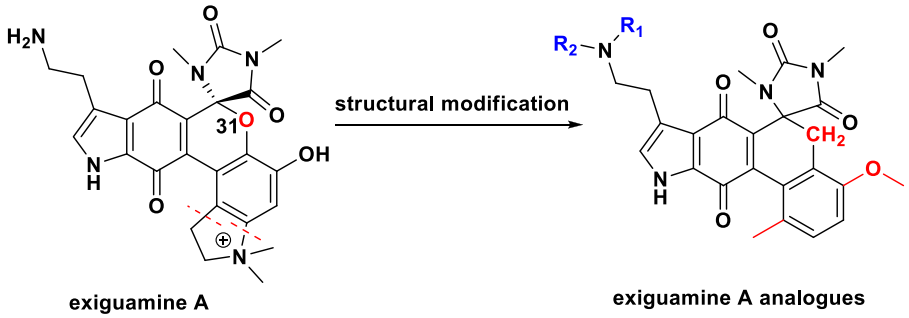


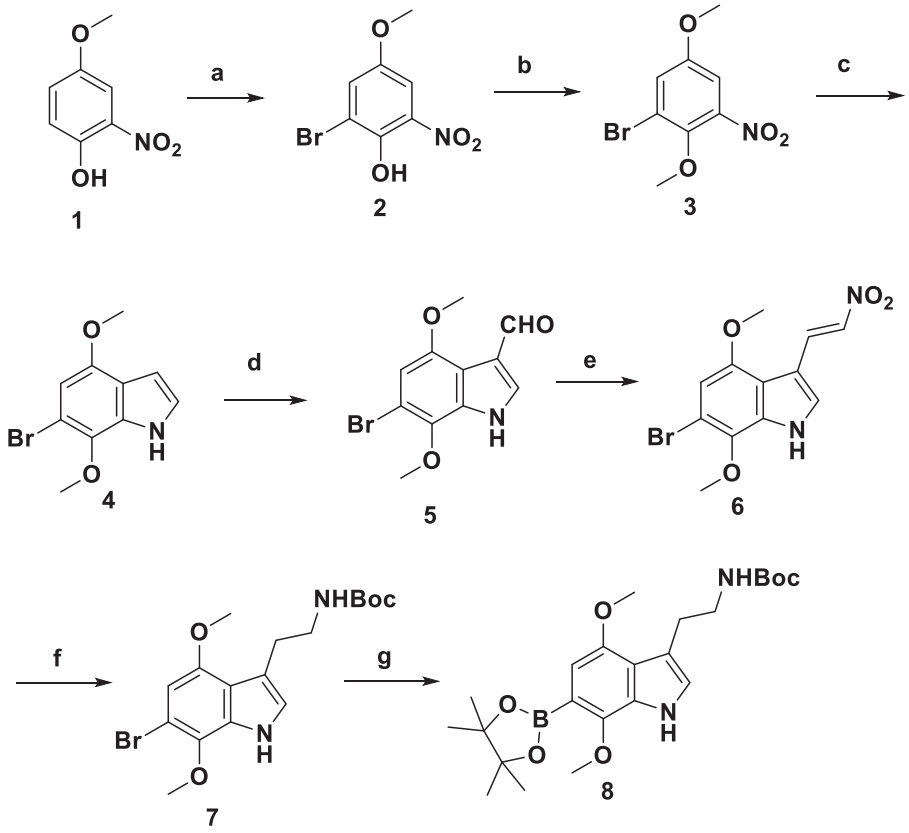

**Fig. 1.** Structural modification of exiguamine A.

## 2. Results and discussions

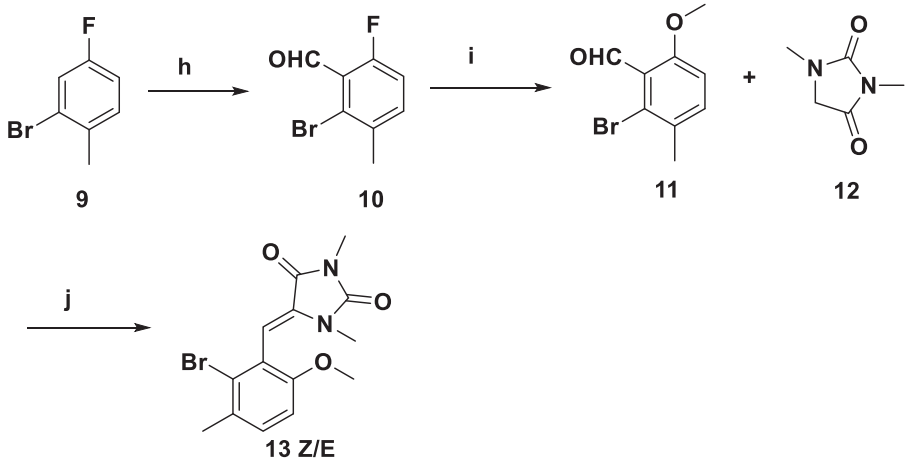
### 2.1. Chemistry

Exiguamine A analogues were synthesized over 15 setps. The key steps were the Suzuki-Miyaura cross-coupling and the intramolecular Michael addition reactions. The detailed synthetic routes were depicted in Schemes 1–4. The synthesis commenced with commercially available 4-methoxy-2-nitrophenol **1**, which was converted to 1-bromo-2,5-dimethoxy-3-nitrobenzene **3** over two-steps as reported in literature [16]. Compound **3** was reacted with isopropenyl magnesium bromide at −60 °C to yield 6-bromo-4,7-dimethoxy-1H-indole **4** according to Bartoli indole synthetic method. Formylation of indole **4** under Vilsmeier-Haack conditions gave 6-bromo-4,7-dimethoxy-1H-indole-3-carbaldehyde **5**, which underwent Henry reaction to produce (*E*)-6-bromo-4,7-dimethoxy-3-(2-nitrovinyl)-1*H*-indole **6**. Compound **6** was then completely reduced by borane in THF, followed by protection with $(BOC)_2O$ to afford compound **7**. Miyaura palladium-catalyzed borylation of compound **7** was conducted, resulting in the formation of compound **8** in good yield.

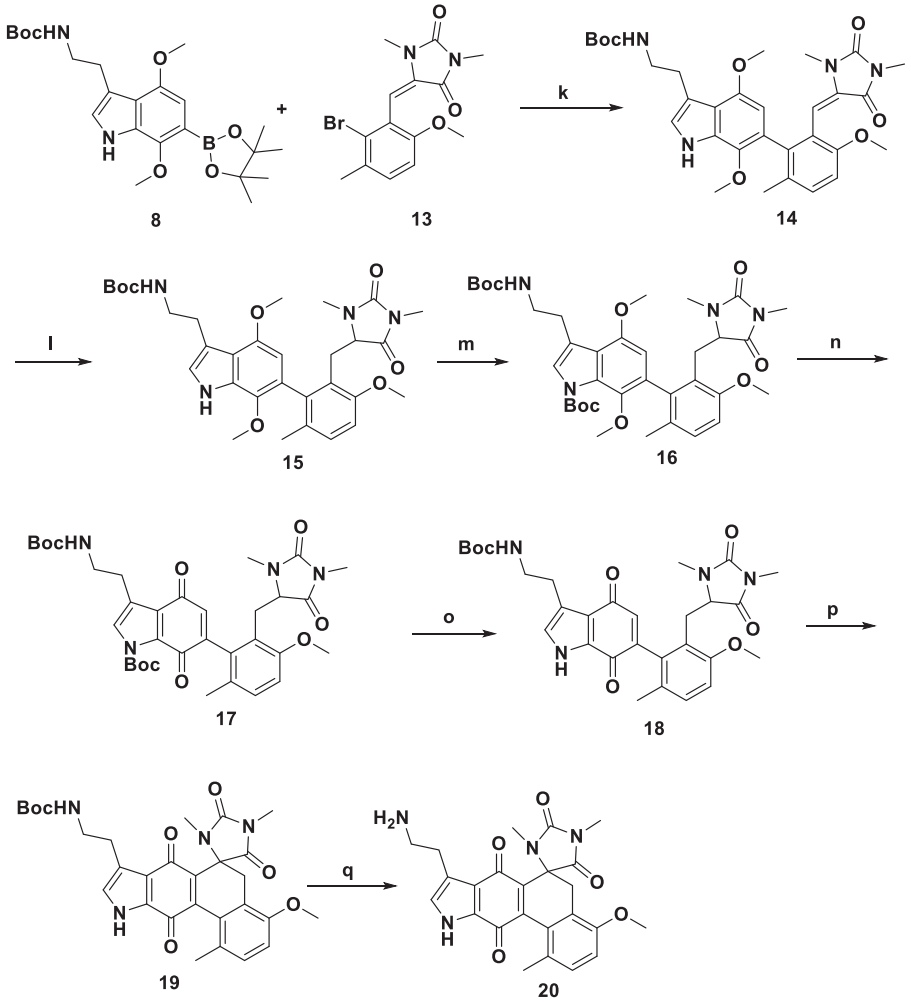
Formylation of commercially available 2-bromo-4-fluoro-1-methylbenzene **9** with LDA and DMF at −78 °C generated 2-bromo-6-fluoro-3-methylbenzaldehyde **10**, which was then subjected to a nucleophilic substitution reaction with $CH_3ONa$ to produce 2-bromo-6-methoxy-3-methylbenzaldehyde **11**. Condensation of *N*, *N*-dimethylhydantoin **12** and compound **11** in acetic acid and *n*-butylamine mixture afforded compound **13** in 78% yield.




**Scheme 1.** Reagents and conditions: (a) $Br_2$, KBr, $CH_3COOH/H_2O$, 70 °C, 89%; (b) $Me_2SO_4$, $K_2CO_3$, reflux, acetone, 80%; (c) vinylmagnesium bromide, THF, −65 °C, 55%; (d) $POCl_3$, DMF, 0 °C–40 °C, 93%; (e) $CH_3NO_2$, $CH_3COONH_4$, 100 °C, 82%; (f) i. $BH_3$•THF, THF, reflux; ii. $(Boc)_2O$, $Et_3N$, DCM, rt, 65% over two steps. (g) $Pd_2(dba)_3$, Xphos, HBPin, $Et_3N$, 1.4-dioxane, reflux, 72%.

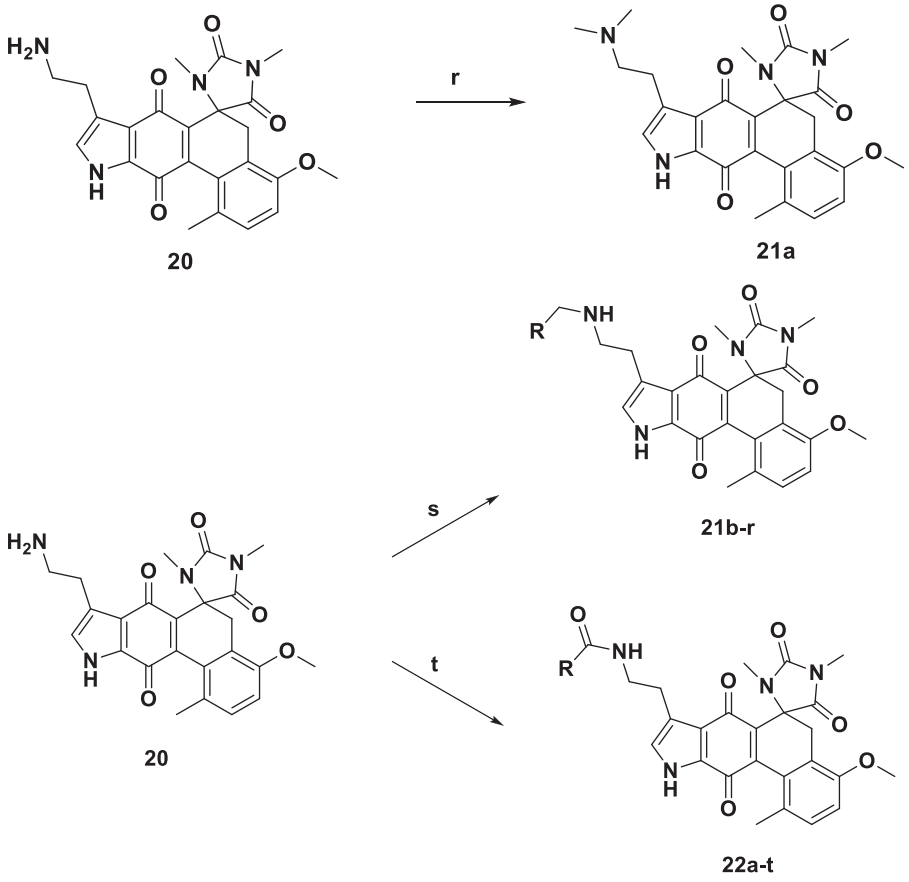



**Scheme 2.** Reagents and conditions: (h) LDA, DMF, THF, −78 °C, 79%; (i) $CH_3ONa$, $CH_3OH$, reflux, 70%; (j) $CH_3(CH_2)_3NH_2$, $CH_3COOH$, reflux, 78%.




**Scheme 3.** Reagents and conditions: (k) $Pd(OAc)_2$, BI-DIME, $K_3PO_4$, 1.4-dioxane reflux, 78%; (l) Pd(C), $H_2$, $CH_3OH$, rt, 83%; (m) $(Boc)_2O$, DMAP, $Et_3N$, DCM, rt, 96%; (n) CAN, $CH_3CN$/$H_2O$, 0 °C, 50%; (o) TFA, DCM, 75%; (p) *n*-BuLi, THF, −78 °C; $O_2$; 41%; (q) 6 M HCl, MeOH.

Suzuki-Miyaura cross-coupling was conducted between borate ester **8** and compound **13** to yield compound **14**, which was then catalytically hydrogenated to deliver compound **15**. Protection of the indolyl nitrogen of compound **15** by $(Boc)_2O$ afforded compound **16**. Treatment of compound **16** with ceric ammonium nitrate (CAN) afforded the expected quinone **17**. The Boc-group on indolyl




**Scheme 4.** Reagents and conditions: (r) HCHO, $NaBH_3CN$, $CH_3COOH$, $CH_3OH$, rt; (s) RCHO, $NaBH_3CN$, $CH_3COOH$, $CH_3OH$, rt; (t) RCOOH, EDCI, DMAP, rt.

nitrogen of quinone **17** was removed by $CF_3COOH$ at room temperature. Finally, compound **18** was treated with *n*-BuLi in THF at −78 °C. After quenching with ammonium chloride, the reaction mixture was exposed to air and stirred at room temperature for 30 min to form compound **19** in 40.5% yield. Removal of the Boc-group of compound **19** with 6 N HCl in $CH_3OH$ provided amine precursor **20**.

The synthesis of compounds **21a-r**, **22a-t** was outlined in Scheme 4. Bis-methyl compound **21a** was prepared by the reaction between amine precursor **20** and HCHO. Reductive alkylation of amine precursor **20** with other aldehydes afforded amine derivatives **21b-r**. Amine precursor **20** was acylated with different acids to afford the corresponding amide derivatives **22a-t**.

### 2.2. Inhibitory activities against IDO1 enzyme

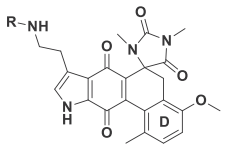
All synthesized compounds were tested for their inhibitory activities against IDO1 enzyme. Epacadostat was selected as the control compound. The results are listed in Table 1.

Most compounds exhibited potent IDO1 inhibitory activities with the $IC_{50}$ values at the level of $10^{-7}$–$10^{-8}$ M. Compound **21f** with 2′- bromine benzyl group exhibited the highest inhibitory activity ($IC_{50}$ = 65.3 nM), which was comparable with epacadostat ($IC_{50}$ = 46 nM).

Generally, the inhibitory activity of the amine analogues was more potent than that of the amide analogues, which indicated that carbonyl group of the amide analogues might be unfavorable for the IDO binding. Compound **21c** ($IC_{50}$ = 172.9 nM) with an unsubstituted benzyl group had a lower micromolar level inhibitory activity against IDO1. Other substituted benzene rings (**21d-21m**) were explored as well. Their inhibitory activities were similar to compounds **21c** and compounds with electron-withdrawing groups exhibited an improved activity compared with compounds with electron-donating groups. Compound **21n** ($IC_{50}$ = 158.7 nM) with a longer chain also showed better IDO1 inhibitory activity. However, replacement of phenyl group in compound **21c** with benzothiophenyl (**21o**, $IC_{50}$ = 621.4 nM), thienyl (**21p**, $IC_{50}$ = 369.6 nM) or biphenyl group (**21q**, $IC_{50}$ = 953.9 nM) led to a significantly decrease in inhibitory activity. In addition to aromatic rings, alkyl substituents had been explored as well. Compound **21a** with bis-methyl groups and compound **21b** with *tert*-butyl group displayed comparable activity to compound **21f**, the $IC_{50}$ values of which were respectively 79.3 nM and 100.8 nM.

In addition, the inhibitory activities of different amides were also studied. Compound **22r** ($IC_{50}$ = 209.4 nM) bearing a furan-2′-carbonyl group and **22d** ($IC_{50}$ = 210.2 nM) bearing a benzoyl group exhibited the best IDO1 inhibitory activities in this series. Three compounds with cinnamic amide were explored, and only compound **22a** ($IC_{50}$ = 578.1 nM) had a weak inhibitory activity. Compound **22g** ($IC_{50}$ = 249.9 nM) with the same 2′-bromine substituted group as compound **21f** retained moderate activity. Lengthening the distance between carbonyl and the aromatic ring (compounds **22k**, **22l**) improved inhibitor activity against IDO1. Among the heterocyclic aromatic acid analogues, the replacement of pyridyl (**22s**, $IC_{50}$ = 429.5 nM) with furyl **22r** or thienyl **22q** ($IC_{50}$ = 220.5 nM) resulted in a slightly increase in potency. And they all displayed better activity against their corresponding 2-

**Table 1**
Enzyme inhibitory activities for compounds **19**, **21a~21r**, **22a~22t**.



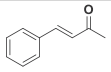
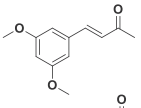
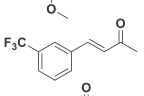
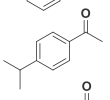
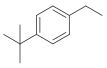
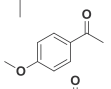
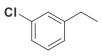
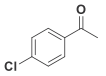
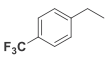
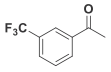
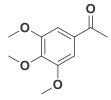
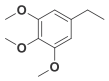
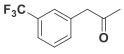
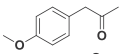
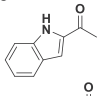
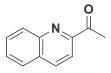
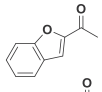
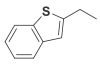
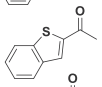
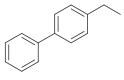
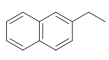
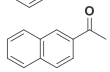
| Compounds | R | $IC_{50}$ (nM) ± SD | Compounds | R | $IC_{50}$ (nM) ± SD |
|---|---|---|---|---|---|
| **Epacadostat** | - | 46 ± 3.53 | 22a |  | 578.1± 37.6 |
| 21a | - | 100.8 ± 6.2 | 22b |  | >42000 |
| 21b |  | 79.3 ± 2.5 | 22c |  | >42000 |
| 21c |  | 172.9 ± 8.2 | 22d |  | 210.2 ± 19.8 |
| 21d |  | 242.1 ± 13.4 | 22e |  | >42000 |
| 21e |  | 265.5 ± 12.2 | 22f |  | 2500 ± 100 |
| 21f |  | 65.3 ± 0.1 | 22g |  | 249.9 ± 20 |
| 21g |  | 150.0 ± 2.3 | 22h |  | >42000 |
| 21h |  | 161.4 ± 15.5 | 22i |  | >42000 |
| 21i |  | 251.8 ± 23.2 | 22j |  | >42000 |
| 21j |  | 180.9 ± 10.1 | 22k |  | 441.4 ± 33.1 |
| 21k |  | 247.5 ± 29.0 | 22l |  | 330.3 ± 25.2 |
| 21l |  | 233.8 ± 10.6 | 22m |  | >42000 |
| 21m |  | 212.7 ± 3.6 | 22n |  | >42000 |
| 21n |  | 158.7 ± 7.8 | 22o |  | 631.2 ± 44.0 |
| 21o |  | 621.4 ± 2.6 | 22p |  | >42000 |
| 21p |  | 369.6 ± 37.0 | 22q |  | 220.5 ± 14.3 |
| 21q |  | 953.9 ± 172 | 22r |  | 209.4 ± 8.7 |
| 21r |  | 270.5 ± 23.0 | 22s |  | 429.5 ± 31.0 |
| | | | 22t |  | >42000 |
| | | | 19 |  | 466.7 ± 23.4 |

**Table 2**
Cytotoxicity of compounds against five cell lines.

| Compound | IDO $IC_{50}$ (nM) | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|
| | | HepG2 | U87 | HGC27 | HCT-116 | MCF-7 |
| Epacadostat | 46 ± 3.5 | >50 | >50 | >50 | >50 | >50 |
| 21a | 100.8 ± 6.2 | >50 | >50 | >50 | >50 | >50 |
| 21b | 79.3 ± 2.5 | >50 | >50 | >50 | >50 | >50 |
| 21f | 65.3 ± 0.1 | 1.56 ± 0.05 | 1.22 ± 0.04 | 2.08 ± 0.15 | 1.35 ± 0.05 | 1.52 ± 0.07 |
| 21g | 150.0 ± 2.3 | >50 | >50 | >50 | >50 | >50 |
| 22d | 210.2 ± 19.8 | 3.53 ± 0.33 | 2.74 ± 0.18 | 4.00 ± 0.40 | 1.70 ± 0.08 | 5.35 ± 0.13 |
| 22r | 209.4 ± 8.7 | 4.18 ± 0.32 | 2.59 ± 0.17 | 3.16 ± 0.55 | 2.15 ± 0.15 | 6.91 ± 0.36 |

benzopyridine, 2-benzofuran and 2-benzothiophene analogues (**22n-22p**). What's more, compound **22t** with sterically hindered aromatic amide side chain had no inhibition. Finally, compound **19** with *tert*-butyl group also displayed a weak $IC_{50}$ value of 466.7 nM against IDO1.

### 2.3. Inhibitory activities against TDO enzyme

The same oxidative catabolism of tryptophan along the kynurenine pathway is also catalyzed by tryptophan 2,3- dioxygenase (TDO), a related enzyme with different substrate specificity and tissue distribution [17]. Emerging evidence reveals a substantial role for TDO in immune tolerance and tumor progression and developing IDO1/TDO dual inhibitors may broaden impact in cancer treatment [18,19]. Taking this into consideration, representative compound **21a**, **21b**, **21f**, **21g, 22d**, **22r** were tested on their inhibitory activity against TDO. However, the inhibitory percentage at 1 μM of these potent compounds was 0% (results were not shown), demonstrating their preferential selectivity for IDO1 over TDO enzyme.

### 2.4. MTT assay of cell viability

In IDO1 enzyme assay, alkylamine **21a**, **21b**, **21f**, **21g** and amide **22d**, **22r** exhibited better inhibitory activity. Since the inhibition of IDO1 could be affected by cytotoxicity, we investigated their cytotoxicity on five human cancer cell lines including liver cancer cells (HepG2), brain cancer cells (U87), gastric cancer cells (HGC27), colorectal cancer cells (HCT-116), breast cancer cells (MCF-7). Cells were treated (72h) with each compound and cell viability was measured by MTT assay. Results are reported in Table 2. All the compounds displayed an $IC_{50}$ value of micromolar level (most $IC_{50}$ values were over 50 μM), which was significantly higher than their $IC_{50}$ values against IDO1. These results revealed that all the compounds did not cause influences on cells at their effective concentration against IDO1.

### 2.5. Molecular docking study of compound *21f*

In order to elucidate the possible binding mode of compound **21f** and IDO1, a docking study was performed with the Discovery Studio 2018 software package. A crystal structure of IDO1 (PDB: 4PK5) was selected as the receptor [20]. The docking calculation was carried out with the CDOCKER protocol and used default settings. As shown in Fig. 2, the methyl group of D ring was stuck deeper into pocket B and formed π-alkyl interaction with the key residue Arg231. What's more, the alkyl amine side chain of **21f** occupied the pocket A. The *NH* formed hydrogen bonds with amino acid residue Gly262. Benzene ring formed π-π interaction with amino acid residues Phe163, Tyr126, and formed π-alkyl interaction with amino acid residue Val130, Ala264. Although a general structure-activity relationship could not be summarized from these data, it is apparent that the novel analogue **21f** had potent inhibitory activity against IDO1 and the amino side chain had much important influence on its activity.

## 3. Conclusion

In conclusion, a series of novel exiguamine A analogues with simpler structure were designed and synthesized. The key steps included the Suzuki-Miyaura cross-coupling and the intramolecular Michael addition reactions. This convergent approach

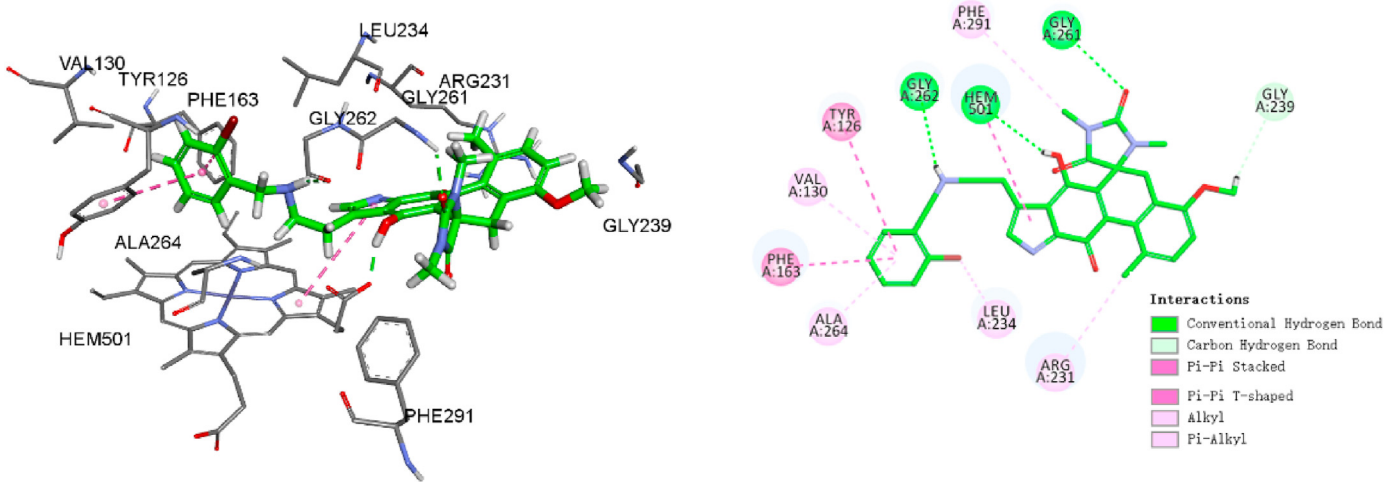



**Fig. 2.** A The docking mode of compound **21f** (shown in green) to IDO1 (PDB: 4PK5). B The schematic 2D diagram of the key interactions.

was easy to operate and thus beneficial to synthesize more structurally diversified derivatives. A total of thirty-nine analogues were synthesized and their inhibitory activity against IDO1 were evaluated. Most of the analogues exhibited potent IDO1 inhibitory activity with $IC_{50}$ values at the level of $10^{-7}$–$10^{-8}$ M. Compound **21f** was the most potent IDO1 inhibitor with an $IC_{50}$ value of 65.3 nM. The preliminary SARs demonstrated that the 31-*O* of the spiro ring and a charge quaternary ammonium ion were not essential to maintain the activity. And the amide side chain played a crucial role for inhibitory activity against IDO1. Molecular docking studies provided insight into the observed SARs. Additionally, compound **21f** showed stronger IDO1 inhibitory activity in comparison with TDO and weak cytotoxicity at its effective concentration in MTT assay. Further structural optimization and biological studies of **21f** are in progress and will be reported in due course.

## 4. Experimental section

### 4.1. General

Commercially available reagents and solvents were used without further purification if not stated otherwise. $^1H$ and $^{13}C$ NMR spectra were recorded on a Varian Mercury 400 spectrometer (400 MHz, $^1H$; 100 MHz, $^{13}C$) or a Bruker AV 500 spectrometer (500 MHz, $^1H$; 125 MHz, $^{13}C$) or a Varian INOVA 600 spectrometer (600 MHz, $^1H$; 150 MHz, $^{13}C$) in $CDCl_3$, $CD_3OD$ using tetramethylsilane (TMS) as an internal standard. Chemical shifts were expressed in ppm. High resolution mass spectra were taken on Thermo Exactive Plus spectrometer. Melting points were obtained on Yanaco MP-500D melting point apparatus and were uncorrected.

### 4.2. 2-Bromo-4-methoxy-6-nitrophenol **2**

Compound **2** was prepared according to the reported procedures [16]. To a solution of 4-methoxy-2-nitrophenol **1** (1g, 5.9 mmol), KBr (0.7g, 5.9 mmol) in $H_2O$ (5 mL) and AcOH (15 mL), bromine (0.32 mL, 5.9 mmol) was added dropwise. After completion of the addition, the reaction mixture was heated at 70 °C for a period of 16 h. Then, the reaction was quenched with saturated solution of $Na_2S_2O_3 \cdot 5H_2O$ and extracted with EtOAc (50 mL × 3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was obtained as a yellow solid (1.3g) and used in the next step without purification.

### 4.3. 1-Bromo-2,5-dimethoxy-3-nitrobenzene **3**

Compound 3 was prepared according to the reported procedures [16]. To the solution of compound **2** (500 mg, 2.01 mmol), $K_2CO_3$ (415 mg, 3.01 mmol) in acetone (10 mL), $Me_2SO_4$ (0.3 mL, 3.01 mmol) was added dropwise. The reaction mixture was refluxed. After completion of the reaction as indicated by TLC, the reaction mixture was cooled to room temperature. After filtration, the filtrate was evaporated in vacuo. The residue was dissolved with ethyl acetate (50 mL), washed with water (50 mL), brine, dried over anhydrous $Na_2SO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 20:1) to afford compound **3** (421 mg, 80%) as a yellow solid. Mp: 84–85 °C; $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.35 (d, $J$ = 3.2Hz, 1H), 7.28 (d, $J$ = 3.2Hz, 1H), 3.96 (s, 3H), 3.83 (s, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 155.6, 145.1, 144.6, 123.9, 120.2, 109.3, 62.8, 56.3; HRMS calcd. For $C_8H_9O_4NBr$ $[M+H]^+$ 261.9709, found 261.9711.

### 4.4. 6-Bromo-4,7-dimethoxy-1H-indole **4**

To a solution of compound **3** (5g, 19.1 mmol) in anhydrous THF (50 mL), vinylmagnesium bromide in THF (1 M, 57.3 mL) was added dropwise at −60 °C under argon atmosphere. The mixture was stirred for 2 h at this temperature and then quenched with saturated aqueous $NH_4Cl$ (100 mL), and extracted with ethyl acetate (100 mL × 3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/EtOAc = 10:1) to afford compound **4** (2.69g, 55%) as a brown solid. Mp: 94–96 °C; $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.10 (t, $J$ = 2.4Hz, 1H), 6.62 (t, $J$ = 2.4Hz, 1H), 6.59 (s, 1H), 3.94 (s, 3H), 3.91 (s, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 149.9, 137.7, 130.7, 123.2, 119.9, 107.7, 104.1, 101.3, 61.2, 55.9; HRMS calcd. for $C_{10}H_{11}BrNO_2$ $[M+H]^+$ 255.9968, found 255.9961.

### 4.5. 6-Bromo-4,7-dimethoxy-1H-indole-3-carbaldehyde **5**

To 5 mL of DMF at 0 °C, freshly distilled $POCl_3$ (0.27 mL, 11.7 mmol) was added. The reaction mixture was stirred for 15 min, and then a solution of compound **4** (1 g, 3.9 mmol) in 5 mL of DMF was added dropwise over 10 min. The resultant mixture was stirred for 30 min at 0 °C, then heated to 40 °C for 1 h. The reaction mixture was then cooled to 0 °C, and 1.0 N aqueous NaOH was added dropwise until the mixture became basic. The reaction mixture was diluted with water (200 mL), and extracted with EtOAc (2 × 200 mL). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/EtOAc = 5:2) to afford compound **5** (1.02g,93%) as a brown solid. Mp: 56–58 °C; $^1H$ NMR (400 MHz, $CDCl_3$) δ 10.43 (s, 1H), 9.20 (s, 1H), 7.90 (d, $J$ = 2.8Hz, 1H), 6.78 (s, 1H), 3.96 (s, 3H), 3.96 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 187.9, 150.8, 137.9, 131.2, 128.1, 120.6, 117.1, 109.0, 106.8, 61.5, 55.9; HRMS calcd. For $C_{11}H_{11}O_3NBr$ $[M+H]^+$ 283.9917, found 283.9912.

### 4.6. (E)-6-bromo-4,7-dimethoxy-3-(2-nitrovinyl)-1H-indole **6**

To a solution of compound **5** (0.8g, 2.82 mmol) in nitromethane (10 mL), ammonium acetate (261 mg, 3.38 mmol) was added. The reaction mixture was heated to 100 °C for 1 h, then cooled to room temperature. The reaction was quenched with $H_2O$ and extracted with $CH_2Cl_2$ (100 mL × 3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/EtOAc = 3:1) to afford compound **6** (0.75g, 82%) as an orange yellow solid. Mp: 159–161 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 12.59 (s, 1H), 8.51 (d, $J$ = 13.2Hz, 1H), 8.29 (s, 1H), 8.11 (d, $J$ = 13.2Hz, 1H), 6.84 (s, 1H), 3.94 (s, 3H), 3.84 (s, 3H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 150.6, 138.5, 135.0, 133.7, 132.6, 132.3, 117.2, 109.3, 109.2, 106.6, 61.7, 56.6; HRMS calcd. For $C_{12}H_{12}O_4N_2Br$ $[M+H]^+$ 326.9975, found 326.9969.

### 4.7. Tert-butyl (2-(6-bromo-4,7-dimethoxy-1H-indol-3-yl)ethyl) carbamate **7**

To a solution of compound **6** (1g, 3.06 mmol) in anhydrous THF (10 mL) at 0 °C, $BH_3$ in THF (1 M, 18.4 mL) was added dropwise over 15 min. Then reaction mixture was refluxed for 48 h, then cooled to 0 °C, and slowly quenched by addition of small pieces of ice followed by 1.0 M aqueous HCl (100 mL). The mixture was then stirred at 60 °C for 1 h, cooled to 0 °C and 2.5 N aqueous NaOH (50 mL) was added dropwise until the mixture became basic. The reaction mixture was quenched with $H_2O$ and extracted with EtOAc

(3 × 200 mL). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was obtained (0.9g) as a brown solid and carried forward to the next step without purification.

To a solution of ensuing unprotected tryptamine (0.9g) in $CH_2Cl_2$ (15 mL), $(Boc)_2O$ (1.06 mL, 4.59 mmol), TEA (0.64 mL, 4.59 mmol) were added. The solution was stirred at room temperature under argon for 2 h, and then washed with saturated aq saturated $NaHCO_3$, brine, and dried over anhydrous $Na_2SO_4$. The residue was purified by column chromatography (petroleum ether/EtOAc = 5:1) to afford compound **7** (0.79 g, 65% over two steps) as a white solid. Mp: 70–72 °C; $^1$H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 6.86 (s, 1H), 6.54 (s, 1H), 4.83 (s, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.42 (d, $J$ = 5.6Hz, 2H), 2.99 (t, $J$ = 5.6Hz, 1H), 1.40 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 156.2, 151.0, 137.6, 131.6, 121.8, 118.5, 114.9, 107.6, 103.6, 79.0, 61.1, 55.7, 42.0, 28.5 (3C), 26.8. HRMS calcd. For $C_{17}H_{24}O_4N_2Br$ $[M+H]^+$ 339.0914, found 399.0910.

### 4.8. Tert-butyl (2-(4,7-dimethoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indol-3-yl)ethyl)carbamate **8**

An oven-dried 50 mL Schlenk flask was charged with compound **7** (500 mg, 1.25 mmol), $Pd_2(dba)_3$ (17 mg, 0.0182 mmol) and XPhos (35 mg, 0.073 mmol). The flask was evacuated and refilled with argon three times. Anhydrous 1.4-dioxane (10 mL), triethylamine (0.52 mL, 3.75 mmol) and pinacolborane (0.66 mL, 3.75 mmol) were added and the flask was equipped with a reflux condenser. The reaction mixture was refluxed for 40 min. After cooling to RT, the reaction was quenched with water and extracted with EtOAc (100 mL × 2). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/EtOAc = 6:1) to afford compound **8** (400 mg, 72%) as a white solid. Mp: 56–58 °C; $^1$H NMR (500 MHz, $CDCl_3$) δ 8.37 (s, 1H), 6.94 (s, 1H), 6.72 (s, 1H), 4.89 (s, 1H), 3.93 (s, 3H), 3.93 (s, 3H), 3.42 (s, 2H), 3.02 (t, $J$ = 5.5Hz, 2H), 1.39 (s, 9H), 1.38 (s, 12H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 156.2, 150.2, 147.2, 131.5, 122.6, 121.7, 114.7, 104.3, 101.5, 83.4 (2C), 78.8, 62.9, 55.3, 42.1, 28.4 (3C), 26.8, 24.9 (3C), 24.6; HRMS calcd. for $C_{23}H_{36}N_2O_6B$ $[M+H]^+$ 447.2661, found 447.2645.

### 4.9. 2-Bromo-6-fluoro-3-methylbenzaldehyde **10**

Compound **10** was prepared according to the reported procedures [21]. To a solution of 2-bromo-4-fluoro-1-methylbenzene **9** (10g, 52.9 mmol) in anhydrous THF (100 mL), LDA (31.8 mL, 2 M) was added dropwise at −78 °C under argon and the reaction mixture was stirred at −78 °C for 2h. Then DMF (4.9 mL, 63.5 mmol) was added to the mixture, and then the reaction mixture was stirred for 1 h at this temperature. The reaction mixture was quenched with saturated aqueous $NH_4Cl$ (100 mL) and extracted with EtOAc (200 mL × 3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/EtOAc = 50:1) to afford compound **10** (9.1g, 79%) as a yellow oil. Mp: 63–65 °C; $^1$H NMR (500 MHz, $CDCl_3$) 10.39 (s, 1H), 7.42 (t, $J$ = 9 Hz, 1H), 7.05 (t, $J$ = 9 Hz, 1H), 2.44 (s, 3H); 13C NMR (125 MHz, CDCl3) δ 189.5, 161.0 (d, $J$ = 262.5Hz), 135.9 (d, $J$ = 8.75Hz), 135.6 (d, $J$ = 3.75Hz), 127.3 (d, $J$ = 2.5Hz), 123.2 (d, $J$ = 8.75Hz), 115.6 (d, $J$ = 21.25Hz), 22.6; HRMS calcd. for $C_8H_7OFBr$ $[M+H]^+$ 216.9659, found 216.9654.

### 4.10. 2-Bromo-6-methoxy-3-methylbenzaldehyde **11**

Compound **11** was prepared according to the reported procedures [21]. To a solution of NaOMe (18.4 mL, 5 M in $CH_3OH$), compound **10** (5g, 0.023mol) was added. Then reaction mixture was refluxed for 16 h, then cooled to 0 °C. 2 N aqueous HCl was added dropwise to pH = 2. The reaction mixture was concentrated in vacuum, quenched with water and extracted with EtOAc (100 mL × 3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/EtOAc = 3:1) to afford compound **11** (3.69g, 70%) as a yellow white solid. Mp: 52–54 °C; $^1$H NMR (400 MHz, $CDCl_3$) δ 10.42 (s, 1H), 7.36 (d, $J$ = 8.8Hz, 1H), 6.87 (d, $J$ = 8.8Hz, 1H), 3.88 (s, 3H), 2.39 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 191.3 159.5, 135.3, 131.3, 126.9, 124.1, 110.7, 56.2, 22.4; HRMS calcd. for $C_9H_{10}O_2Br$ $[M+H]^+$ 228.9859, found 228.9851.

### 4.11. (Z/E)-5-(2-bromo-6-methoxy-3-methylbenzylidene)-1,3-dimethylimidazolidine-2,4-dione **13**

To a solution of 1,3-dimethylimidazolidine-2,4-dione **12** (0.62g, 4.81 mmol) in $CH_3COOH$ (15 mL), *n*-Butylamine (0.95 mL, 9.62 mmol) and compound 11(1g, 4.37 mmol) were added. The reaction mixture was refluxed for 10 h, then cooled to 0 °C. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (50 mL) and extracted with DCM (50 mL × 3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/EtOAc = 10:1) to afford compound **13** (1.17g, 78%) as a white solid (*Z*:*E* = 20:11). Mp: 113–115 °C; $^1$H NMR (400 MHz, $CDCl_3$) δ 7.19–7.16 (m, 1.55H), 6.81–6.78 (m, 1.55H), 6.61 (s, 1H), 5.99 (s, 0.55H), 3.78 (s, 3H), 3.75 (s, 1.65H), 3.27 (s, 1.65H), 3.13 (s, 3H), 3.03 (s, 1.65H), 2.79 (s, 3H), 2.37 (s, 3H), 2.36 (s, 1.65H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 163.4, 156.5, 156.1, 155.5, 131.3, 131.2, 130.8, 130.5, 130.4, 130.0, 128.8, 127.5, 127.2, 123.1, 123.1, 120.0, 109.6, 109.3, 109.0, 107.9, 56.1, 55.8, 27.9, 26.4, 25.0, 24.6, 22.8, 22.8; HRMS calcd. For $C_{14}H_{16}O_3N_2Br$ $[M+H]^+$ 339.0339, found 339.0335.

### 4.12. Tert-butyl (Z/E)-(2-(6-(2-((1,3-dimethyl-2,5-dioxoimidazolidin-4-ylidene)methyl)-3-methoxy-6-methylphenyl)-4,7-dimethoxy-1H-indol-3-yl)ethyl)carbamate **14**

Dried 1,4-dioxane (20 mL) was charged to a mixture of compound **13** (760 mg, 2.24 mmol), compound **8** (1.1g, 2.46 mmol), tripotassium orthophosphate (1.43g, 6.72 mmol), $Pd(OAc)_2$ (10 mg, 44.8 μmol), ligand BI-DIME (30 mg, 89.6 μmol). The mixture was pumped and refilled with argon three times. The reaction mixture was refluxed for 24 h, and then cooled to room temperature. The reaction mixture was quenched with $H_2O$ and extracted with EtOAc (100 mL × 2). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/EtOAc = 3:1) to afford compound **14** (1.02g, 78%) as a white solid (*Z*:*E* = 2:1). Mp: 106–108 °C; $^1$H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 0.5H), 8.13 (s, 1H), 7.27–7.22 (m, 1.5H), 6.90–6.86 (m, 3H), 6.33 (s, 1H), 6.07 (s, 0.5H), 6.01 (s, 1H), 5.77 (s, 0.5H), 4.87 (s, 1H), 3.80–3.79 (m, 9H), 3.50–3.45 (m, 6H), 3.39 (s, 1.5H), 3.06–2.99 (m, 7.5H), 2.93 (s, 3H), 2.83 (s, 1.5H), 2.12 (s, 1.5H), 2.11 (s, 3H), 1.42 (s, 9H), 1.42 (s, 4.5H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 163.4, 161.3, 156.2 (2C), 155.6, 155.6, 154.8, 154.2, 150.5, 149.9, 140.7, 139.7, 137.4, 131.5, 131.4, 130.8, 130.7, 130.4, 129.3, 128.8, 123.8, 123.5, 122.3, 121.8, 121.6, 121.5, 118.9, 118.3, 114.4, 114.2, 109.9, 109.6, 109.5, 108.5, 78.8 (2C), 60.3, 60.3, 55.8, 55.4 (2C), 55.4, 42.0, 41.9, 29.7, 28.5, 27.9, 27.0, 26.2, 24.8, 24.4, 19.9, 19.8; HRMS calcd. for $C_{31}H_{39}N_4O_7$ $[M+H]^+$ 579.2813, found 579.2791.

*4.13. Tert-butyl (2-(6-(2-((1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-3-methoxy-6-methylphenyl)-4,7-dimethoxy-1H-indol-3-yl)ethyl)carbamate* ***15***

To a solution of compound **14** (1.02g, 1.76 mmol) in MeOH (10 mL) at room temperature, Pd/C (100 mg) was added and then the mixture was hydrogenated for 10h. The reaction mixture was filtered through Celite, washed with MeOH, and concentrated under vacuum. The residue was purified by column chromatography (petroleum ether/EtOAc = 3:1) to afford compound **15** (0.84g, 83%) as a white solid (isomer ratio = 5:3). Mp: 109–111 °C; $^1$H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 0.6H), 8.17 (s, 1H), 7.19 (d, *J* = 8.4Hz, 1.6H), 6.92 (d, *J* = 1.2Hz, 1.6H), 6.86–6.83 (m, 1.6H), 6.09 (s, 0.6H), 6.03 (s, 1H), 4.87 (s, 1H), 4.27 (dd, *J* = 9.6, 5.6Hz, 1H), 4.13 (dd, *J* = 8, 7.2Hz, 0.6H), 3.87 (s, 3H), 3.85 (s, 1.8H), 3.84 (s, 1.8H), 3.82 (s, 3H), 3.49–3.45 (m, 5H), 3.43 (s, 3H), 3.20 (dd, *J* = 13.6, 5.6Hz, 1H), 3.10–3.02 (m, 3.8H), 2.90 (s, 3H), 2.87 (s, 1.8H), 2.82 (dd, *J* = 13.6, 8Hz, 0.6H), 2.75 (dd, *J* = 13.6, 9.6Hz, 1H), 2.68 (s, 1.8H), 2.46 (s, 3H), 2.07 (s, 1.8H), 2.04 (s, 3H), 1.42 (s, 14.4H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 173.3, 172.8, 157.3, 156.9, 156.2, 156.0, 150.6, 150.2, 140.5, 140.2, 137.1, 131.5, 131.4, 129.6, 129.3, 129.2, 123.9, 123.7, 123.7, 123.2, 121.6, 118.4, 114.3, 109.5, 109.3, 102.0, 100.9, 78.9, 60.3, 60.2, 60.1, 59.8, 55.5, 55.4, 55.4, 42.0, 41.9, 30.6, 29.7, 29.6, 29.2, 28.5, 28.2, 27.0, 24.8, 24.6, 20.5, 20.4; HRMS calcd. for $C_{31}H_{41}N_4O_7$ $[M+H]^+$ 581.2970, found 581.2955.

*4.14. Tert-butyl 3-(2-((tert-butoxycarbonyl)amino)ethyl)-6-(2-((1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-3-methoxy-6-methylphenyl)-4,7-dimethoxy-1H-indole-1-carboxylate* ***16***

To a solution of compound **15** (0.8g, 1.38 mmol) in $CH_2Cl_2$ (10 mL), $(Boc)_2O$ (0.45g, 2.07 mmol), DMAP (17 mg, 0.14 mmol), TEA (0.29 mL, 2.07 mmol) were added. The solution was stirred at room temperature under argon for 2 h, and then washed with saturated aqueous $NaHCO_3$, brine, and dried over anhydrous $Na_2SO_4$. The residue was purified by column chromatography (petroleum ether/EtOAc = 5:1) to afford compound **16** (0.9 g, 96%) as a white solid (isomer ratio = 2:1). Mp: 85–87 °C; $^1$H NMR (400 MHz, $CDCl_3$) δ 7.22 (s, 1.5H), 7.17 (d, *J* = 8.4Hz, 1.5H), 6.85–6.82 (m, 1.5H), 6.37 (s, 0.5H), 6.27 (s, 1H), 4.77 (s, 1H), 4.27–4.20 (m, 1.5H), 3.86–3.84 (m, 6H), 3.80 (s, 3H), 3.46 (dd, *J* = 13.6, 6.4Hz, 3H), 3.35 (s, 1.5H), 3.34 (s, 3H), 3.22–3.13 (m, 1.5H), 3.02–2.99 (m, 3H), 2.91 (s, 3H), 2.85 (s, 1.5H), 2.77–2.71 (m, 2.5H), 2.66 (dd, *J* = 13.6, 9.2Hz, 0.5H), 2.48 (s, 3H), 2.04 (s, 1.5H), 2.03 (s, 3H), 1.60 (s, 4.5H), 1.59 (s, 9H), 1.44 (s, 13.5H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 173.2, 172.6, 157.4, 156.7, 156.2, 156.1, 155.8, 150.3, 149.8, 148.9, 140.6, 140.3, 139.5, 129.8, 129.6, 129.3, 129.2, 129.0, 125.4, 123.5, 122.9, 122.2, 122.1, 117.7, 109.6, 109.3, 107.1, 105.5, 83.4, 83.3, 79.0 (2C), 60.4, 60.1, 60.0, 59.8, 55.7 (2C), 55.6, 55.4, 41.0, 30.7, 29.7, 29.3, 28.5, 28.0, 28.0, 27.2, 24.8, 24.5, 20.6, 20.5; HRMS calcd. for $C_{36}H_{49}N_4O_9$ $[M+H]^+$ 681.3494, found 681.3464.

*4.15. Tert-butyl 3-(2-((tert-butoxycarbonyl)amino)ethyl)-6-(2-((1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-3-methoxy-6-methylphenyl)-4,7-dioxo-4,7-dihydro-1H-indole-1-carboxylate* ***17***

To a solution of compound **16** (0.5g, 0.73 mmol) in a mixture of acetonitrile (3.5 mL) and water (1.5 mL), a solution of CAN (1.0g, 1.83 mmol) in a mixture of acetonitrile (2.5 mL) and water (2.5 mL) was added at 0 °C. Then the reaction mixture was stirred for 1h at this temperature. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (50 mL) and extracted with DCM (50 mL × 3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/EtOAc = 3:1) to afford compound **17** (0.23g, 50%) as a yellow solid (isomer ratio = 10:7). Mp: 132–134 °C; $^1$H NMR (400 MHz, $CDCl_3$) δ 7.34 (s, 1.7H), 7.15 (d, *J* = 8.4Hz, 1.7H), 6.85 (d, *J* = 8.4Hz, 1.7H), 6.46 (s, 0.7H), 6.44 (s, 1H), 4.76 (s, 1H), 4.28 (t, *J* = 6.8Hz, 0.7H), 3.99 (dd, *J* = 10, 4.4Hz, 1H), 3.86 (s, 2.1H), 3.83 (s, 3H), 3.45–3.41 (m, 3.4H), 3.26 (dd, *J* = 14, 4.4Hz, 1H), 3.18 (dd, *J* = 14, 6.8Hz, 0.7H), 3.03–2.95 (m, 6.4H), 2.93 (s, 2.1H), 2.88–2.82 (m, 3.1H), 2.64–2.59 (m, 3.7H), 2.10 (s, 3H), 2.09 (s, 2.1H), 1.57 (s, 6.3H). 1.56 (s, 9H), 1.44 (s, 15.3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 184.2, 184.0, 175.4, 175.0, 172.8, 172.5, 157.2, 156.8, 156.0,155.9, 155.6, 148.0, 147.9, 147.5, 135.9, 135.5, 135.3, 135.1, 131.2, 129.7, 129.6, 128.5, 128.3, 128.2, 128.0, 127.9, 123.4, 122.8, 122.6, 122.4, 110.8, 110.5, 86.4, 86.3, 79.2 (2C), 61.1, 59.9, 55.5, 55.4, 40.0, 30.5, 30.0, 29.3, 28.4, 28.1, 28.0, 27.5, 26.0, 24.9, 24.7, 20.0, 20.0; HRMS calcd. for $C_{34}H_{43}N_4O_9$ $[M+H]^+$ 651.3025, found 651.3032.

*4.16. Tert-butyl (2-(6-(2-((1,3-dimethyl-2,5-dioxoimidazolidin-4-yl)methyl)-3-methoxy-6-methylphenyl)-4,7-dioxo-4,7-dihydro-1H-indol-3-yl)ethyl)carbamate* ***18***

To a solution of compound **17** (500 mg, 7.87 mmol) in $CH_2Cl_2$, trifluoroacetic acid (0.6 mL) was added at 0 °C. The reaction mixture was stirred at room temperature for 2 h, then cooled to 0 °C. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with DCM (50 mL × 3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/EtOAc = 3:1) to afford compound **18** (323 mg, 75%) as an orange solid (isomer ratio = 10:7). Mp: 126–128 °C; $^1$H NMR (400 MHz, $CDCl_3$) δ 9.60 (s, 1.7H), 7.16 (d, *J* = 8.4Hz, 1.7H), 6.93 (s, 1H), 6.90 (s, 0.7H), 6.85 (d, *J* = 8.4Hz, 1.7H), 6.42 (s, 0.7H), 6.40 (s, 1H), 4.80 (s, 1.7H), 4.28 (t, *J* = 7.2Hz, 0.7H), 4.05 (dd, *J* = 9.2, 4.8Hz, 1H), 3.86 (s, 2.1H), 3.84 (s, 3H), 3.46–3.41 (m, 3.4H), 3.24 (dd, *J* = 14.4, 4.8. Hz, 1H), 3.09 (dd, *J* = 13.6, 7.6Hz, 0.7H), 3.04–2.97 (m, 8.5H), 2.84 (dd, *J* = 14.4, 9.2Hz, 1H), 2.76 (dd, *J* = 13.6, 7.2Hz, 0.7H), 2.73 (s, 2.1H), 2.59 (s, 3H), 2.09 (s, 3H), 2.09 (s, 2.1H), 1.43 (s, 15.3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 184.0, 183.9, 176.4, 176.3, 173.1, 172.8, 157.3, 157.0, 156.3, 156.2, 155.9, 155.7, 146.3, 146.3, 138.2, 137.5, 135.2, 134.8, 131.2, 131.2, 129.8, 129.6, 128.5, 128.4, 125.3, 125.2, 123.8, 123.6, 123.3, 123.2, 122.4, 110.6, 110.4, 79.3 (2C), 60.8, 60.0, 55.6, 55.5, 40.7 (2C), 30.7, 30.3, 29.8, 29.4, 28.8, 28.5, 26.2, 25.0, 24.9, 20.1 (2C); HRMS calcd. for $C_{29}H_{35}N_4O_7$ $[M+H]^+$ 551.2500 found 551.2488.

*4.17. Tert-butyl (2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)carbamate* ***19***

To a solution of compound **18** (150 mg,0.27 mmol) in THF (5 mL), *n*-BuLi (0.2 mL, 1.6 M) was added dropwise at −78 °C under argon. The reaction mixture was stirred for 12 h at this temperature and then was quenched with saturated aqueous $NH_4Cl$ (10 mL). After stirring 30 min at room temperature, the mixture was extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/EtOAc = 5:1) to afford compound **19** (60 mg, 41%) as an orange solid. Mp: 168–170 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.22 (d, *J* = 8.4Hz, 1H), 7.06 (d, *J* = 8.4Hz, 1H), 7.01 (s, 1H), 3.86 (s, 3H), 3.39 (d, *J* = 16.8Hz, 1H), 3.29–3.21 (m, 2H), 3.14 (s, 3H), 2.99 (d, *J* = 16.8Hz, 1H), 2.93–2.86 (m, 1H), 2.82 (dd, *J* = 13.6, 6.8Hz, 1H), 2.24 (s, 3H), 2.21 (s, 3H), 1.40 (s, 9H); $^{13}$C NMR (125 MHz, $CD_3OD$) δ 181.0, 176.6, 175.2, 157.0, 155.8, 154.2, 146.8, 137.5, 131.4, 131.0, 129.2, 129.0, 125.4, 123.6, 122.1, 122.0, 112.8, 78.5, 61.9, 55.0, 40.1, 32.0, 27.5 (3C), 25.8, 25.7, 24.5, 21.9; HRMS calcd. for

$C_{29}H_{33}N_4O_7$ $[M+H]^+$ 549.2344 found 549.2347.

### 4.18. 8'-(2-Aminoethyl)-4′-methoxy-1,1′,3-trimethylspiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone **20**

To a solution of compound **19** (500 mg, 7.87 mmol) in anhydrous methanol (5 mL), 6 M aqueous HCl was added at 0 °C, and then the reaction mixture was stirred overnight at room temperature. 2.0 M aqueous NaOH was added dropwise until the mixture became basic. The reaction was extracted with DCM (50mL × 2). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give the crude compound **20** (380 mg, 93%) as a yellow solid.

### 4.19. 8'-(2-(Dimethylamino)ethyl)-4′-methoxy-1,1′,3-trimethylspiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone **21a**

To a solution of compound **20** (10 mg, 0.022 mmol) in anhydrous methanol, HCHO (37%, 2.7 μL), $NaBH_3CN$ (3.4 mg, 0.055 mmol), and acetic acid (0.02 mL) were added and then the mixture was stirred at room temperature for 30 min. The mixture was concentrated under reduced pressure and then dissolved in EtOAc, washed with saturated anhydrous $NaHCO_3$ and brine, dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure to give the crude product. Purification by column chromatography on silica gel (DCM:MeOH = 15:1) afforded compounds **21a** (7 mg, 70%) as a yellow solid. Mp: 157–159 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.23 (d, $J$ = 8.8 Hz, 1H), 7.17 (s, 1H), 7.08 (d, $J$ = 8.8 Hz, 1H), 3.87 (s, 3H), 3.42 (d, $J$ = 16.8 Hz, 1H), 3.28–3.21 (m, 2H), 3.12–3.07 (m, 5H), 3.00 (d, $J$ = 16.8 Hz, 1H), 2.86 (s, 6H), 2.24 (s, 3H), 2.22 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 181.3, 176.6, 175.2, 155.7, 154.3, 147.0, 137.1, 131.0, 129.1, 128.9, 125.6, 122.0, 121.8, 119.8, 112.9, 61.8, 57.3, 54.9, 42.3, 31.8, 25.7, 24.3, 21.8, 20.9; HRMS calcd. for $C_{26}H_{29}N_4O_5$. $[M+H]^+$477.2132, found 477.2132.

### 4.20. General procedure for the synthesis of compounds **21b-r**

To a solution of compound **20** (1.0 equiv) in anhydrous methanol, the corresponding aldehyde (1eq), $NaBH_3CN$ (1.25eq), and acetic acid (0.02 mL) were added, and then the mixture was stirred at room temperature for 30 min. The mixture was concentrated under reduced pressure and then dissolved in EtOAc, washed with saturated anhydrous $NaHCO_3$ and brine, dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure to give the crude product. Purification by column chromatography on silica gel (DCM:MeOH = 15:1) afforded compounds **21b**-**21r** (65%–83%) as a yellow solid.

#### 4.20.1. 4′-Methoxy-1,1′,3-trimethyl-8'-(2-(neopentylamino)ethyl)spiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone **21b**

Mp: 180–182 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.23 (d, $J$ = 8.4 Hz, 1H), 7.15 (s, 1H), 7.08 (d, $J$ = 8.4 Hz, 1H), 3.86 (s, 3H), 3.42 (d, $J$ = 16.8 Hz, 1H), 3.26–3.19 (m, 2H), 3.15–3.11 (m, 5H), 3.01 (d, $J$ = 16.8 Hz, 1H), 2.88 (s, 2H), 2.24 (s, 3H), 2.22 (s, 3H), 1.06 (s, 9H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.9, 178.0, 176.7, 157.2, 155.8, 148.7, 138.6, 132.5, 130.6, 130.4, 127.1, 123.6, 123.3, 121.7, 114.5, 63.3, 60.5, 56.4, 50.3, 33.4, 31.6, 27.6 (3C), 27.2, 25.8, 23.3; HRMS calcd. for $C_{29}H_{35}N_4O_5$. $[M+H]^+$ 519.2602, found 519.2600.

#### 4.20.2. 8'-(2-(Benzylamino)ethyl)-4′-methoxy-1,1′,3-trimethylspiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone **21c**

Mp: 161–163 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.45–7.40 (m, 5H), 7.23 (d, $J$ = 8.8 Hz, 1H), 7.11–7.07 (m, 2H), 4.14 (s, 2H), 3.87 (s, 3H), 3.42 (d, $J$ = 16.8 Hz, 1H), 3.24–3.18 (m, 2H), 3.10–3.06 (m, 5H), 3.00 (d, $J$ = 16.8 Hz, 1H), 2.24 (s, 3H), 2.22 (s, 3H); $^{13}$C NMR (175 MHz, $CD_3OD$) δ 182.8, 178.0, 176.8, 157.2, 155.8, 148.6, 138.6, 133.5, 132.5, 130.8, 130.6, 130.5, 130.3, 127.1, 123.6, 123.3, 114.4, 63.3, 56.4, 52.5, 33.4, 27.2, 25.8, 23.9, 23.3; HRMS calcd. for $C_{31}H_{31}N_4O_5$ $[M+H]^+$ 539.2289, found 539.2284.

#### 4.20.3. 4′-Methoxy-1,1′,3-trimethyl-8'-(2-((2-methylbenzyl)amino)ethyl)spiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone **21d**

Mp: 152–154 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.33–7.30 (m, 1H), 7.24–7.22 (m, 4H), 7.09–7.06 (m, 2H), 4.02 (s, 2H), 3.86 (s, 3H), 3.41 (d, $J$ = 16.8 Hz, 1H), 3.16–3.11 (m, 5H), 3.06–2.98 (m, 3H), 2.36 (s, 3H), 2.23 (s, 3H), 2.22 (s, 3H); $^{13}$C NMR (150 MHz, $CD_3OD$) δ 182.7, 178.0, 176.8, 157.3, 155.8, 148.5, 138.8, 138.1, 133.3, 132.5, 131.8, 130.9, 130.6, 130.5, 130.5, 129.7, 127.8, 127.6, 127.0, 123.6, 123.4, 123.3, 114.4, 63.4, 56.5, 50.6, 33.4, 27.2, 25.8, 23.8, 23.3, 19.2; HRMS calcd. for $C_{32}H_{33}N_4O_5$. $[M+H]^+$ 553.2445, found 553.2446.

#### 4.20.4. 4′-Methoxy-1,1′,3-trimethyl-8'-(2-(neopentylamino)ethyl)spiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone **21e**

Mp: 194–196 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.49 (d, $J$ = 8.4 Hz, 2H), 7.37 (d, $J$ = 8.4 Hz, 2H), 7.23 (d, $J$ = 8.4 Hz, 1H), 7.11 (s, 1H), 7.08 (d, $J$ = 8.4 Hz, 1H), 4.12 (s, 2H), 3.87 (s, 3H), 3.43 (d, $J$ = 16.8 Hz, 1H), 3.23–3.19 (m, 2H), 3.11–3.07 (m, 5H), 3.01 (d, $J$ = 16.8 Hz, 1H), 2.24 (s, 3H), 2.21 (s, 3H), 1.32 (s, 9H); $^{13}$C NMR (175 MHz, $CD_3OD$) δ 182.9, 178.0, 176.8, 157.2, 155.8, 153.9, 148.6, 138.6, 133.4, 132.5, 130.5, 130.4, 127.3, 127.1, 123.6, 123.4, 114.5, 63.3, 56.4, 54.9, 52.2, 35.6, 33.4, 31.7 (3C), 27.2, 25.9, 23.8, 23.3; HRMS calcd. for $C_{35}H_{39}N_4O_5$. $[M+H]^+$ 595.2915, found 595.2912.

#### 4.20.5. 8'-(2-((2-Bromobenzyl)amino)ethyl)-4′-methoxy-1,1′,3-trimethylspiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone **21f**

Mp: 165–167 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.54 (dd, $J$ = 8, 0.8 Hz, 1H), 7.39 (dd, $J$ = 7.6, 1.6 Hz, 1H), 7.33 (dt, $J$ = 7.6, 0.8 Hz, 1H), 7.22 (d, $J$ = 8.8 Hz, 1H), 7.16 (dt, $J$ = 8, 1.6 Hz, 1H), 7.07 (d, $J$ = 8.8 Hz, 1H), 7.01 (s, 1H), 3.87 (s, 2H), 3.86 (s, 3H), 3.39 (d, $J$ = 16.8 Hz, 1H), 3.12 (s, 3H), 3.00 (d, $J$ = 16.8 Hz, 1H), 2.96–2.91 (m, 2H), 2.88–2.84 (m, 2H), 2.23 (s, 3H), 2.22 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.4, 178.0, 176.7, 173.9, 155.8, 148.3, 139.2, 138.9, 134.0, 133.1, 132.4, 132.0, 131.0, 130.6, 130.5, 130.3, 128.9, 126.8, 125.0, 123.5, 123.4, 114.3, 63.4, 56.4, 53.8, 33.4, 27.2, 26.3, 25.9, 23.3; HRMS calcd. for $C_{31}H_{30}N_4O_5Br$ $[M+H]^+$ 617.1394, found 617.1380.

#### 4.20.6. 8'-(2-((3-Chlorobenzyl)amino)ethyl)-4′-methoxy-1,1′,3-trimethylspiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone **21g**

Mp: 160–162 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.46 (s, 1H), 7.40–7.33 (m, 3H), 7.22 (d, $J$ = 8.4 Hz, 1H), 7.09–7.06 (m, 2H), 4.05 (s, 2H), 3.86 (s, 3H), 3.41 (d, $J$ = 16.8 Hz, 1H), 3.14–3.09 (m, 5H), 3.05–2.98 (m, 3H), 2.23 (s, 3H), 2.22 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.7, 178.0, 176.7, 157.2, 155.7, 148.5, 138.7, 137.8, 135.8, 133.3, 132.5, 131.6, 130.5, 130.5, 129.9, 128.9, 127.0, 123.5, 123.3, 122.7, 114.4, 63.3, 56.4, 52.3, 33.4, 27.2, 25.8, 24.6, 23.3; HRMS calcd. for $C_{31}H_{30}N_4O_5Cl$. $[M+H]^+$ 573.1899, found 573.1890.

*4.20.7. 4′-Methoxy-1,1′,3-trimethyl-8'-(2-((4-(trifluoromethyl) benzyl)amino)ethyl)spiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone* ***21h***

Mp: 166–168 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.69 (d, $J$ = 8 Hz, 2H), 7.57 (d, $J$ = 8 Hz, 2H), 7.22 (d, $J$ = 8.4 Hz, 1H), 7.08–7.06 (m, 2H), 4.04 (s, 2H), 3.86 (s, 3H), 3.41 (d, $J$ = 16.4 Hz, 1H), 3.11 (s, 3H), 3.02–2.98 (m, 5H), 2.23 (s, 3H), 2.21 (s, 3H); $^{13}$C NMR (175 MHz, $CD_3OD$) δ 182.7, 178.0, 176.7, 157.3, 155.8, 148.5, 138.7, 133.2, 132.5, 130.7, 130.5, 126.9, 126.8, 126.8, 126.4, 124.9, 123.6, 123.5, 123.4, 114.3, 63.4, 56.4, 54.9, 52.7, 33.4, 27.1, 25.8, 25.2, 23.3; HRMS calcd. for $C_{32}H_{30}N_4O_5F_3$. $[M+H]^+$ 607.2163, found 607.2151.

*4.20.8. Methyl 3-(((2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f]indol]-8′-yl)ethyl)amino)methyl)benzoate* ***21i***

Mp: 160–162 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 8.11 (t, $J$ = 1.6 Hz, 1H), 8.04 (dt, $J$ = 7.6, 1.2Hz, 1H), 7.67 (dd, $J$ = 7.6, 1.2Hz, 1H), 7.56 (t, $J$ = 7.6Hz, 1H), 7.23 (d, $J$ = 8.4 Hz, 1H), 7.10–7.06 (m, 2H), 4.16 (s, 2H), 3.91 (s, 3H), 3.86 (s, 3H), 3.41 (d, $J$ = 16.8 Hz, 1H), 3.22–3.13 (m, 2H), 3.12–3.06 (m, 5H), 3.00 (d, $J$ = 16.8 Hz, 1H), 2.23 (s, 3H), 2.22 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.7, 178.0, 176.7, 168.0, 157.2, 155.7, 148.5, 138.7, 135.7, 135.3, 133.4, 132.5, 132.2, 131.6, 130.9, 130.6, 130.5, 127.0, 123.5, 123.3, 122.5, 114.4, 63.3, 56.4, 52.8, 52.4, 33.4, 27.2, 25.8, 24.5, 23.3; HRMS calcd. for $C_{33}H_{33}N_4O_7$. $[M+H]^+$ 597.2344, found 597.2334.

*4.20.9. 4′-Methoxy-1,1′,3-trimethyl-8'-(2-((3,4,5-trimethoxybenzyl) amino)ethyl)spiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone* ***21j***

Mp: 188–190 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.22 (d, $J$ = 8.8 Hz, 1H), 7.08–7.06 (m, 2H), 6.71 (s, 2H), 3.93 (d, $J$ = 2.8 Hz, 2H), 3.86 (s, 3H), 3.84 (s, 6H), 3.74 (s, 3H), 3.41 (d, $J$ = 16.4 Hz, 1H), 3.10 (s, 3H), 3.08–2.97 (m, 5H), 2.24 (s, 3H), 2.22 (s, 3H); $^{13}$C NMR (150 MHz, $CD_3OD$) δ 182.7, 178.0, 176.8, 157.3, 155.8, 154.9, 148.5, 139.4, 138.8, 133.3, 132.5, 130.6, 130.5, 126.9, 123.6, 123.4, 114.5, 107.7, 63.4, 61.1, 56.8 (2C), 56.5, 53.5, 33.4, 27.2, 25.8, 25.0, 23.2; HRMS calcd. for $C_{34}H_{37}N_4O_8$ $[M+H]^+$ 629.2606, found 629.2613.

*4.20.10. 8'-(2-((2,3-Dimethoxybenzyl)amino)ethyl)-4′-methoxy-1,1′,3-trimethylspiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone* ***21k***

Mp: 165–167 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.23 (d, $J$ = 8.4 Hz, 1H), 7.13–7.11 (m, 3H), 7.08 (d, $J$ = 8.4 Hz, 1H), 6.96 (dd, $J$ = 6.4, 2.8 Hz, 1H), 4.18 (s, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.42 (d, $J$ = 16.4 Hz, 1H), 3.26–3.21 (m, 2H), 3.11–3.07 (m, 5H), 3.00 (d, $J$ = 16.4 Hz, 1H), 2.24 (s, 3H), 2.22 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.8, 178.0, 176.8, 157.2, 155.7, 154.1, 149.1, 148.6, 138.6, 132.5, 130.6, 130.5, 127.2, 126.3, 125.7, 123.5, 123.3, 121.6, 115.6, 114.4, 63.3, 61.4, 56.4, 47.7, 33.4, 27.2, 25.8, 23.6, 23.3; HRMS calcd. for $C_{33}H_{35}N_4O_7$. $[M+H]^+$ 599.2500, found 599.2490.

*4.20.11. 8'-(2-((3,4-Dimethylbenzyl)amino)ethyl)-4′-methoxy-1,1′,3-trimethylspiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone* ***21l***

Mp: 174–176 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.24–7.19 (m, 3H), 7.14 (dd, $J$ = 7.6, 1.2Hz, 1H), 7.10–7.07 (m, 2H), 4.06 (s, 2H), 3.87 (s, 3H), 3.42 (d, $J$ = 16.8 Hz, 1H), 3.23–3.16 (m, 2H), 3.11–3.07 (m, 5H), 3.00 (d, $J$ = 16.8 Hz, 1H), 2.29 (s, 3H), 2.27 (s, 3H), 2.24 (s, 3H), 2.21 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.7, 178.0, 176.8, 157.2, 155.8, 148.5, 139.0, 138.7, 133.4, 132.5, 131.8, 131.4, 130.5, 130.5, 128.1, 127.0, 123.5, 123.3, 122.0, 114.4, 63.3, 56.4, 52.5, 33.4, 27.2, 25.8, 24.1, 23.3, 19.8, 19.6; HRMS calcd. for $C_{33}H_{35}N_4O_5$. $[M+H]^+$ 567.2602, found 567.2591.

*4.20.12. 8'-(2-((3,5-Difluorobenzyl)amino)ethyl)-4′-methoxy-1,1′,3-trimethylspiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone* ***21m***

Mp: 151–153 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.44–7.38 (m, 1H), 7.22 (d, $J$ = 8.8 Hz, 1H), 7.07 (d, $J$ = 8.8 Hz, 1H), 7.04 (s, 1H), 6.98–6.92 (m, 2H), 3.88 (s, 2H), 3.86 (s, 3H), 3.40 (d, $J$ = 16.8 Hz, 1H), 3.12 (s, 3H), 3.00 (d, $J$ = 16.8 Hz, 1H), 2.96–2.87 (m, 4H), 2.23 (s, 3H), 2.21 (s, 3H); $^{13}$C NMR (125 MHz, $CD_3OD$) δ 182.5, 178.0, 176.7, 157.3, 155.7, 148.4, 138.8, 133.4 (d, $J$ = 15 Hz), 133.4 (d, $J$ = 3.8 Hz), 133.1, 132.5, 130.6, 130.5, 125.6 (d, $J$ = 288.8 Hz), 123.5, 123.3, 114.3, 112.5 (d, $J$ = 17.5 Hz), 104.9, 104.7, 63.4, 56.4, 46.3, 33.4, 27.1, 25.9, 25.8, 23.3; HRMS calcd. for $C_{31}H_{29}N_4O_5F_2$. $[M+H]^+$ 575.2101, found 575.2101.

*4.20.13. 4′-Methoxy-1,1′,3-trimethyl-8'-(2-(phenethylamino)ethyl) spiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone* ***21n***

Mp: 133–135 °C; $^1$H NMR (500 MHz, $CD_3OD$) δ 7.36–7.33 (m, 2H), 7.28 (d, $J$ = 7.5 Hz, 1H), 7.23 (d, $J$ = 8.5 Hz, 1H), 7.13 (s, 1H), 7.09 (d, $J$ = 8.5 Hz, 1H), 3.87 (s, 3H), 3.43 (d, $J$ = 16.5 Hz, 1H), 3.28–3.25 (m, 4H), 3.14–3.09 (m, 2H), 3.07 (s, 3H), 3.03–2.97 (m, 3H), 2.24 (s, 3H), 2.22 (s, 3H); $^{13}$C NMR (175 MHz, $CD_3OD$) δ 182.9, 178.0, 176.8, 157.2, 155.8, 148.6, 138.6, 137.8, 133.5, 132.5, 130.6, 130.4, 130.1, 129.8, 128.4, 127.2, 123.6, 123.3, 121.3, 114.5, 63.3, 56.4, 49.9, 33.4, 33.3, 27.2, 25.8, 23.6, 23.3; HRMS calcd. for $C_{32}H_{33}N_4O_5$. $[M+H]^+$ 553.2445, found 553.2443.

*4.20.14. 8'-(2-((Benzo[b]thiophen-2-ylmethyl)amino)ethyl)-4′-methoxy-1,1′,3-trimethylspiro[imidazolidine-4,6′-naphtho [2,1-f] indole]-2,5,7′,11′(5′H,10′H)-tetraone* ***21o***

Mp: 168–170 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.90–7.88 (m, 1H), 7.85–7.83 (m, 1H), 7.46 (s, 1H), 7.39–7.36 (m, 2H), 7.23 (d, $J$ = 8.4 Hz, 1H), 7.12 (s, 1H), 7.08 (d, $J$ = 8.4 Hz, 1H), 4.44 (s, 2H), 3.87 (s, 3H), 3.42 (d, $J$ = 16.8 Hz, 1H), 3.27–3.24 (m, 2H), 3.11–3.08 (m, 2H), 3.06 (s, 3H), 3.00 (d, $J$ = 16.8 Hz, 1H), 2.22 (s, 3H), 2.21 (s, 3H); $^{13}$C NMR (175 MHz, $CD_3OD$) δ 182.8, 178.0, 176.7, 157.2, 155.8, 148.6, 141.9, 140.9, 138.7, 133.4, 132.5, 130.5, 130.5, 127.1, 126.3, 125.9, 125.1, 123.6, 123.5, 123.4, 114.4, 63.3, 56.4, 47.6, 33.4, 30.8, 27.2, 25.8, 23.3; HRMS calcd. For $C_{33}H_{31}O_5N_4S$ $[M+H]^+$ 595.2010 found 595.2016.

*4.20.15. 4′-Methoxy-1,1′,3-trimethyl-8'-(2-((thiophen-2-ylmethyl) amino)ethyl)spiro[imidazolidine-4,6′-naphtho [2,1-f]indole]-2,5,7′,11′(5′H,10′H)-tetraone* ***21p***

Mp: 172–174 °C; $^1$H NMR (500 MHz, $CD_3OD$) δ 7.54 (d, $J$ = 5 Hz, 1H), 7.26–7.22 (m, 2H), 7.12–7.08 (m, 3H), 4.43 (s, 2H), 3.87 (s, 3H), 3.42 (d, $J$ = 17 Hz, 1H), 3.25–3.16 (m, 2H), 3.12–3.07 (m, 5H), 3.01 (d, $J$ = 17 Hz, 1H), 2.24 (s, 3H), 2.22 (s, 3H); $^{13}$C NMR (175 MHz, $CD_3OD$) δ 182.8, 178.0, 176.8, 157.2, 155.8, 148.6, 138.6, 133.5, 132.5, 132.5, 130.5, 130.5, 128.7, 127.1, 123.6, 123.3, 114.4, 63.3, 56.4, 48.2, 46.4, 33.4, 27.2, 25.9, 23.9, 23.3; HRMS calcd. for $C_{29}H_{29}N_4O_5S$ $[M+H]^+$ 545.1853, found 545.1848.

*4.20.16. 8'-(2-(([1,1′-Biphenyl]-4-ylmethyl)amino)ethyl)-4′-methoxy-1,1′,3-trimethylspiro[imidazolidine-4,6′-naphtho [2,1-f] indole]-2,5,7′,11′(5′H,10′H)-tetraone* ***21q***

Mp: 168–170 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.72 (d, $J$ = 8.4 Hz, 2H), 7.65–7.63 (m, 2H), 7.52 (d, $J$ = 8.4 Hz, 2H), 7.44 (t, $J$ = 7.2 Hz, 2H), 7.37–7.33 (m, 1H), 7.23 (d, $J$ = 8.4 Hz, 1H), 7.13 (s, 1H), 7.08 (d, $J$ = 8.4 Hz, 1H), 4.20 (s, 2H), 3.86 (s, 3H), 3.42 (d, $J$ = 16.8 Hz, 1H), 3.28–3.18 (m, 2H), 3.13–3.10 (m, 2H), 3.08 (s, 3H), 3.00 (d, $J$ = 16.8 Hz, 1H), 2.23 (s, 3H), 2.21 (s, 3H); $^{13}$C NMR (150 MHz, $CD_3OD$) δ 182.9, 178.0, 176.8, 157.3, 155.8, 148.6, 143.5, 141.5, 138.7, 132.5, 131.2, 130.6, 130.5, 130.0, 129.9, 128.9, 128.8, 128.2, 128.1, 128.0, 127.1, 123.6, 123.4, 114.5, 63.4, 56.5, 52.3, 33.4, 30.8, 27.2, 25.8, 24.1, 23.3; HRMS calcd. for $C_{37}H_{35}N_4O_5$. $[M+H]^+$ 615.2602, found

615.2587.

*4.20.17. 4′-Methoxy-1,1′,3-trimethyl-8'-(2-((naphthalen-2-ylmethyl)amino)ethyl)spiro[imidazolidine-4,6′-naphtho [2,1-f] indole]-2,5,7′,11′(5′H,10′H)-tetraone* ***21r***

Mp: 156–158 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.89–7.85 (m, 4H), 7.51–7.47 (m, 3H), 7.22 (d, *J* = 8.8 Hz, 1H), 7.08–7.05 (m, 2H), 4.12 (s, 2H), 3.86 (s, 3H), 3.40 (d, *J* = 16.8 Hz, 1H), 3.09–2.97 (m, 8H), 2.21 (s, 3H), 2.20 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.6, 178.0, 176.7, 157.3, 155.7, 148.4, 138.8, 134.9, 134.6, 133.2, 132.5, 130.5, 129.7, 129.3, 129.0, 128.8, 127.7, 127.5, 127.4, 126.9, 123.6, 123.5, 123.4, 114.3, 63.4, 56.4, 53.5, 33.4, 27.1, 25.8, 25.2, 23.3; HRMS calcd. for $C_{35}H_{33}N_4O_5$. $[M+H]^+$ 589.2445, found 589.2431.

*4.21. General procedure for the synthesis of compounds* ***22a-t***

To a solution of compound **20** (1.0 equiv) in $CH_2Cl_2$, the corresponding acids (1.2 equiv), DMAP (3 equiv) and EDCI (1.3 equiv) were added, and then the mixture was stirred at room temperature for 30 min. The mixture was diluted with $CH_2Cl_2$, washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $Na_2SO_4$, concentrated under reduced pressure to give the crude product. Purification by column chromatography on epacadastasilica gel (PET/EtOAc = 5:1) afforded compounds **22a-t** (70%–88%) as a yellow solid.

*4.21.1. (E)-N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)cinnamamide* ***22a***

Mp: 187–189 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.56 (dd, *J* = 8, 2 Hz, 2H), 7.49 (d, *J* = 15.6 Hz, 1H), 7.40–7.34 (m, 3H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.07–7.05 (m, 2H), 6.55 (d, *J* = 15.6 Hz, 1H), 3.86 (s, 3H), 3.54 (t, *J* = 7.2 Hz, 2H), 3,40 (d, *J* = 16.8 Hz, 1H), 3.13 (s, 3H), 3.02–2.96 (m, 3H), 2.22 (s, 3H), 2.21 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.7, 178.1, 176.7, 168.7, 157.3, 155.7, 148.4, 141.8, 138.8, 136.3, 133.0, 132.4, 130.8, 130.6, 130.5, 130.0, 128.9, 126.9, 124.8, 123.6, 123.5, 121.9, 114.3, 63.4, 56.4, 41.1, 33.4, 27.1, 26.5, 25.9, 23.3; HRMS calcd. for $C_{33}H_{31}N_4O_6$ $[M+H]^+$ 579.2238, found 579.2239.

*4.21.2. (E)-3-(3,5-dimethoxyphenyl)-N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro [imidazolidine-4,6′-naphtho [2,1-f]indol]-8′-yl)ethyl)acrylamide* ***22b***

Mp: 215–217 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.40 (d, *J* = 15.6 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.07–7.05 (m, 2H), 6.71 (d, *J* = 2.4 Hz, 2H), 6.52 (d, *J* = 15.6 Hz, 1H), 6.48 (t, *J* = 2.4 Hz, 1H), 3.86 (s, 3H), 3.79 (s, 6H), 3.54 (t, *J* = 6.8 Hz, 2H), 3.39 (d, *J* = 16.8 Hz, 1H), 3.13 (s, 3H), 3.00–2.96 (m, 3H), 2.22 (s, 3H), 2.21 (s, 3H). $^{13}$C NMR (100 MHz, $CD_3OD$) δ 178.1, 168.7, 162.7, 155.7, 146.1, 144.5, 141.8, 138.3, 138.3, 133.0, 132.4, 130.6, 126.9, 124.8, 123.6, 123.5, 122.4, 114.3, 106.8, 103.0, 56.4, 56.0, 55.9, 54.9, 41.2, 33.4, 27.1, 26.4, 25.9, 23.3; HRMS calcd. for $C_{35}H_{35}N_4O_8$ $[M+H]^+$ 639.2449, found 639.2440.

*4.21.3. (E)-N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)-3-(3-(trifluoromethyl)phenyl)acrylamide* ***22c***

Mp: 162–164 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.88 (d, *J* = 0.8 Hz, 1H), 7.83 (dd, *J* = 8, 0.8 Hz, 1H), 7.65 (dd, *J* = 8, 0.8 Hz, 1H), 7.59 (d, *J* = 8 Hz, 1H), 7.54 (d, *J* = 15.6 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.07–7.05 (m, 2H), 6.66 (d, *J* = 15.6 Hz, 1H), 3.86 (s, 3H), 3.55 (t, *J* = 6.8 Hz, 2H), 3.40 (d, *J* = 16.8 Hz, 1H), 3.13 (s, 3H), 3.01–2.97 (m, 3H), 2.22 (s, 3H), 2.21 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.8, 176.6, 175.2, 166.6, 155.8, 154.2, 146.9, 138.4, 137.3, 136.0, 130.9, 130.9, 129.4, 129.1, 129.0, 125.6, 125.6, 125.4, 123.9, 123.8, 123.3, 122.6, 122.0, 112.8, 61.9, 54.9, 39.8, 31.9, 25.6, 24.9, 24.3, 21.8; HRMS calcd. for $C_{34}H_{30}F_3N_4O_6$ $[M+H]^+$ 647.2112, found 647.2110.

*4.21.4. N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)benzamide* ***22d***

Mp: 229–231 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.75–7.73 (m, 2H), 7.50–7.48 (m, 1H), 7.46–7.41 (m, 2H), 7.21 (d, *J* = 8.8 Hz, 1H), 7.07–7.05 (m, 2H), 3.86 (s, 3H), 3.67–3.57 (m, 2H), 3.40 (d, *J* = 16.8 Hz, 1H), 3.14 (s, 3H), 3.07–2.98 (m, 3H), 2.21 (s, 3H), 2.20 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 178.1, 170.3, 157.3, 155.7, 148.4, 148.1, 138.9, 132.6, 132.4, 130.6, 129.7, 129.6, 128.3, 128.2, 127.0, 124.9, 123.5, 114.3, 63.4, 56.4, 41.4, 33.5, 27.2, 26.4, 25.8, 23.2; HRMS calcd. for $C_{31}H_{29}N_4O_6$ $[M+H]^+$ 553.2082, found 553.2072.

*4.21.5. 4-Isopropyl-N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f]indol]-8′-yl)ethyl)benzamide* ***22e***

Mp: 213–215 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.69–7.66 (m, 2H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.21 (d, *J* = 8.8 Hz, 1H), 7.07–7.04 (m, 2H), 3.86 (s, 3H), 3.65–3.57 (m, 2H), 3.40 (d, *J* = 16.8 Hz, 1H), 3.14 (s, 3H), 3.08–2.90 (m, 4H), 2.21 (s, 3H), 2.20 (s, 3H), 1.25 (s, 3H), 1.24 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.7, 178.1, 176.7, 170.3, 157.3, 155.7, 154.1, 148.4, 138.9, 133.2, 133.0, 132.4, 130.6, 130.6, 128.4, 127.7, 127.0, 125.0, 123.5, 114.3, 63.4, 56.4, 41.5, 35.4, 33.5, 27.2, 26.4, 25.8, 24.2, 23.3; HRMS calcd. for $C_{34}H_{35}N_4O_6$ $[M+H]^+$ 595.2551, found 595.2540.

*4.21.6. 4-Methoxy-N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f]indol]-8′-yl)ethyl)benzamide* ***22f***

Mp: 213–215 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.71 (d, *J* = 9.2 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.07–7.05 (m, 2H), 6.96 (d, *J* = 9.2 Hz, 2H), 3.86 (s, 3H), 3.83 (s, 3H), 3.64–3.56 (m, 2H), 3.40 (d, *J* = 16.4 Hz, 1H), 3.14 (s, 3H), 3.07–2.98 (m, 3H), 2.21 (s, 3H), 2.20 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 180.5, 175.9, 172.0, 167.7, 153.5, 130.7, 130.2, 128.5, 128.4, 128.4, 128.0, 127.9, 124.8, 122.8, 121.3, 112.6, 112.1, 61.2, 54.2, 53.7, 39.3, 31.3, 25.0, 24.3, 23.6, 21.0; HRMS calcd. for $C_{32}H_{31}N_4O_7$ $[M+H]^+$ 583.2187, found 583.2193.

*4.21.7. 2-Bromo-N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f]indol]-8′-yl)ethyl)benzamide* ***22g***

Mp: 154–156 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.60–7.58 (m, 1H), 7.41–7.37 (m, 1H), 7.33–7.28 (m, 2H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.12 (s, 1H), 7.06 (d, *J* = 8.4 Hz, 1H), 3.86 (s, 3H), 3.71–3.64 (m, 1H), 3.61–3.56 (m, 1H), 3.39 (d, *J* = 16.8 Hz, 1H), 3.12 (s, 3H), 3.06–2.97 (m, 3H), 2.22 (s, 3H), 2.22 (s, 3H); $^{13}$C NMR (125 MHz, $CD_3OD$) δ 181.1, 176.6, 175.3, 169.6, 155.8, 154.3, 146.9, 138.6, 137.4, 132.8, 131.6, 131.0, 130.7, 129.2, 129.1, 128.3, 127.2, 125.7, 123.3, 122.1, 122.1, 118.9, 112.8, 61.9, 54.9, 39.5, 32.0, 25.7, 24.9, 24.4, 21.9; HRMS calcd. for $C_{31}H_{28}N_4O_6Br$ $[M+H]^+$ 631.1187, found 631.1177.

*4.21.8. 4-Chloro-N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f]indol]-8′-yl)ethyl)benzamide* ***22h***

Mp: 143–145 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.74–7.71 (m, 2H), 7.46–7.44 (m, 2H), 7.21 (d, *J* = 8.8 Hz, 1H), 7.07–7.05 (m, 2H). 3.86 (s, 3H), 3.66–3.57 (m, 2H), 3.40 (d, *J* = 16.8 Hz, 1H), 3.14 (s, 3H), 3.05–2.98 (m, 3H), 2.20 (s, 3H), 2.20 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.7, 178.0, 176.7, 169.1, 157.3, 155.7, 148.4, 138.9, 138.7, 134.4, 133.0, 132.4, 130.6, 130.5, 129.9, 129.8, 127.0, 124.9, 123.5, 114.3, 63.4, 56.4, 41.6, 33.4, 27.1, 26.4, 25.8, 23.2; HRMS calcd. for $C_{31}H_{28}ClN_4O_6$ $[M+H]^+$ 587.1692, found 587.1707.

*4.21.9. N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)-3-(trifluoromethyl)benzamide* ***22i***

Mp: 179–181 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 8.09 (s, 1H), 8.00 (dd, *J* = 8, 0.8 Hz, 1H), 7.81 (dd, *J* = 8, 0.8 Hz, 1H), 7.66 (t, *J* = 8 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 1H), 7.07–7.05 (m, 2H), 3.86 (s, 3H), 3.69–3.59 (m, 2H), 3.40 (d, *J* = 16.8 Hz, 1H), 3.13 (s, 3H), 3.07–2.98 (m, 3H), 2.21 (s, 6H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.6, 178.1, 176.7, 168.5, 157.3, 156.9, 155.7, 148.4, 138.9, 136.8, 133.0, 132.4, 131.8, 130.7, 130.6, 130.6, 129.1, 126.9, 125.3, 124.8, 123.5, 114.3, 63.4, 56.4, 41.4, 33.4, 27.1, 26.4, 25.8, 23.2; HRMS calcd. for $C_{32}H_{28}F_3N_4O_6$ $[M+H]^+$ 621.1955, found 621.1964.

*4.21.10. 3,4,5-Trimethoxy-N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f]indol]-8′-yl)ethyl)benzamide* ***22j***

Mp: 151–153 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.22 (d, *J* = 8.8 Hz, 1H), 7.11 (s, 2H), 7.07–7.04 (m, 2H), 3.87 (s, 6H), 3.86 (s, 3H), 3.79 (s, 3H), 3.65–3.56 (m, 2H), 3.39 (d, *J* = 16.8 Hz, 1H), 3.13 (s, 3H), 3.07–2.97 (m, 3H), 2.21 (s, 6H); $^{13}$C NMR (125 MHz, $CD_3OD$) δ 181.0, 176.6, 175.3, 168.2, 155.8, 154.3, 153.0, 146.9, 140.6, 137.4, 134.7, 131.6, 131.0, 129.7, 129.2, 129.1, 125.5, 125.3, 123.4, 122.1, 122.0, 112.8, 104.4, 61.9, 59.7, 55.3, 55.3, 55.0, 39.7, 32.0, 25.7, 25.1, 24.4, 21.8; HRMS calcd. for $C_{34}H_{35}N_4O_9$ $[M+H]^+$ 643.2399, found 643.2385.

*4.21.11. N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)-2-(3-(trifluoromethyl)phenyl)acetamide* ***22k***

Mp: 171–173 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.58 (s, 1H), 7.55–7.47 (m, 3H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 6.92 (s, 1H), 3.86 (s, 3H), 3.52 (s, 2H), 3.47 (dd, *J* = 13.2, 6.4 Hz, 1H), 3.42–3.37 (m, 2H), 3.14 (s, 3H), 3.00 (d, *J* = 16.8 Hz, 1H), 2.90 (t, *J* = 6.8 Hz, 2H), 2.23 (s, 3H), 2.21 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.6, 178.0, 176.7, 173.2, 157.3, 155.8, 148.4, 138.8, 138.5, 133.9, 133.0, 132.5, 130.6, 130.5, 130.3, 126.9, 126.8, 124.7, 124.6, 123.5, 123.5, 114.3, 63.4, 56.4, 43.5, 40.9, 33.4, 27.1, 26.4, 25.8, 23.3; HRMS calcd. for $C_{33}H_{30}F_3N_4O_6$ $[M+H]^+$ 635.2112, found 635.2094.

*4.21.12. N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)-2-(4-methoxyphenyl)acetamide* ***22l***

Mp: 157–159 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.22 (d, *J* = 8.4 Hz, 1H), 7.14–7.10 (m, 2H), 7.06 (d, *J* = 8.4 Hz, 1H), 6.90 (s, 1H), 6.84–6.81 (m, 2H), 3.86 (s, 3H), 3.74 (s, 3H), 3.48–3.38 (m, 3H), 3.35 (s, 2H), 3.13 (s, 3H), 3.20 (d, *J* = 16.8 Hz, 1H), 2.88 (t, *J* = 6.8 Hz, 2H), 2.23 (s, 3H), 2.21 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 181.1, 176.5, 175.2, 173.1, 158.7, 155.8, 154.2, 146.9, 137.3, 131.5, 130.9, 129.6, 129.1, 129.0, 127.5, 125.4, 123.2, 122.0, 122.0, 113.5, 112.8, 61.9, 54.9, 54.2, 41.7, 39.1, 31.9, 25.6, 24.8, 24.3, 21.8; HRMS calcd. for $C_{33}H_{33}N_4O_7$ $[M+H]^+$ 597.2344, found 597.2336.

*4.21.13. N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)-1H-indole-2-carboxamide* ***22m***

Mp: 231–233 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.62 (d, *J* = 8 Hz, 1H), 7.41 (d, *J* = 8 Hz, 1H), 7.22–7.17 (m, 2H), 7.06–7.00 (m, 3H), 6.96 (s, 1H), 3.86 (s, 3H), 3.63 (t, *J* = 7.2 Hz, 2H), 3.40 (d, *J* = 16.8 Hz, 1H), 3.15 (s, 3H), 3.10–2.98 (m, 3H), 2.20 (s, 3H), 2.18 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 178.1, 177.6, 176.7, 164.2, 157.3, 155.7, 148.4, 138.9, 138.3, 132.4, 132.2, 130.6, 130.5, 129.1, 127.0, 125.0, 124.9, 123.5, 122.9, 121.1, 114.2, 113.0, 104.2, 63.4, 56.4, 41.1, 33.4, 27.1, 26.5, 25.9, 23.2; HRMS calcd. for $C_{33}H_{30}N_5O_6$ $[M+H]^+$ 592.2191, found 592.2182.

*4.21.14. N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)quinoline-2-carboxamide* ***22n***

Mp: 180–182 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 8.44 (d, *J* = 8.4 Hz, 1H), 8.16–8.11 (m, 2H), 7.97 (d, *J* = 8.4 Hz, 1H), 7.80 (t, *J* = 7.2 Hz, 1H), 7.66 (t, *J* = 7.2 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.09 (s, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 3.85 (s, 3H), 3.76 (dd, *J* = 13.6, 6.8 Hz, 1H), 3.69 (dd, *J* = 13.6, 6.8 Hz, 1H), 3.38 (d, *J* = 16.8 Hz, 1H), 3.16–3.11 (m, 4H), 3.05 (dd, *J* = 14, 6.8 Hz, 1H), 2.99 (d, *J* = 16.8 Hz, 1H), 2.21 (s, 3H), 2.13 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.5, 178.1, 176.7, 166.9, 157.2, 155.7, 154.2, 151.1, 148.3, 148.0, 139.0, 138.9, 132.4, 131.5, 130.8, 130.8, 130.6, 130.5, 129.3, 129.0, 126.9, 124.7, 123.5, 119.5, 114.2, 63.4, 56.4, 40.8, 33.4, 27.1, 26.6, 25.8, 23.2; HRMS calcd. for $C_{34}H_{30}N_5O_6$ $[M+H]^+$ 604.2191, found 604.2177.

*4.21.15. N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)benzofuran-2-carboxamide* ***22o***

Mp: 191–193 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.70 (dd, *J* = 8, 0.8 Hz, 1H), 7.56 (dt, *J* = 8, 0.8 Hz, 1H), 7.45–7.41 (m, 2H), 7.31–7.27 (m, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.06–7.04 (m, 2H), 3.85 (s, 3H), 3.70–3.61 (m, 2H), 3.39 (d, *J* = 16.8 Hz, 1H), 3.14 (s, 3H), 3.10–2.97 (m, 3H), 2.21 (s, 3H), 2.17 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.6, 178.1, 176.7, 161.3, 157.3, 156.5, 155.7, 150.1, 148.3, 138.9, 133.0, 132.4, 130.6, 130.5, 128.9, 128.1, 126.9, 124.9, 124.7, 123.8, 123.5, 114.2, 112.9, 111.1, 63.4, 56.4, 40.7, 33.4, 27.1, 26.5, 25.8, 23.2; HRMS calcd. for $C_{33}H_{29}N_4O_7$ $[M+H]^+$ 593.2031, found 593.2035.

*4.21.16. N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)benzo[b]thiophene-2-carboxamide* ***22p***

Mp: 177–179 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.91–7.88 (m, 2H), 7.84 (d, *J* = 0.8 Hz, 1H), 7.42–7.38 (m, 2H), 7.20 (d, *J* = 8.4 Hz, 1H), 7.07–7.04 (m, 2H), 3.85 (s, 3H), 3.65–3.61 (m,2H), 3.39 (d, *J* = 16.8 Hz, 1H), 3.14 (s, 3H), 3.10–2.97 (m, 3H), 2.20 (s, 3H), 2.14 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 181.2, 176.6, 175.2, 163.3, 155.8, 154.2, 146.9, 140.9, 139.3, 138.6, 137.4, 130.9, 129.0, 125.9, 125.5, 125.1, 124.9, 124.8, 124.5, 123.3, 122.2, 122.0, 112.7, 61.9, 54.9, 40.1, 31.9, 25.6, 24.9, 24.3, 21.7; HRMS calcd. for $C_{33}H_{29}N_4O_6S$ $[M+H]^+$ 609.1802, found 609.1796.

*4.21.17. N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)thiophene-2-carboxamide* ***22q***

Mp: >250 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.59 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.56 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.09 (dd, *J* = 5.2, 3.6 Hz, 1H), 7.07–7.04 (m, 2H), 3.86 (s, 3H), 3.62–3.54 (m, 2H), 3.40 (d, *J* = 16.8 Hz, 1H), 3.14 (s, 3H), 3.07–2.95 (m, 3H), 2.20 (s, 6H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 176.5, 175.2, 163.0, 154.2, 137.4, 134.9, 131.5, 130.9, 130.0, 129.1, 128.1, 127.4, 125.5, 125.3, 123.4, 122.0, 118.7, 116.8, 115.1, 112.8, 61.9, 54.9, 40.1, 31.9, 25.7, 24.9, 24.3, 21.7; HRMS calcd. for $C_{29}H_{27}N_4O_6S$ $[M+H]^+$ 559.1646, found 559.1637.

*4.21.18. N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)furan-2-carboxamide* ***22r***

Mp: 226–228 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.62 (d, *J* = 2 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.07–7.03 (m, 3H), 6.54 (dd, *J* = 3.2, 1.6Hz, 1H), 3.86 (s, 3H), 3.63–3.54 (m, 2H), 3.40 (d, *J* = 16.8 Hz, 1H), 3.14 (s, 3H), 3.05–2.95 (m, 3H), 2.22 (s, 3H), 2.21 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.7, 178.1, 176.7, 161.0, 157.3, 155.7, 149.1, 148.4, 146.3, 138.9, 133.0, 132.4, 130.6, 130.6, 126.9, 124.8, 123.5, 123.5, 115.1, 114.3, 112.9, 63.4, 56.4, 40.7, 33.4, 27.2, 26.4, 25.8, 23.3; HRMS calcd. for $C_{29}H_{27}N_4O_7$ $[M+H]^+$ 543.1874, found 543.1870.

*4.21.19. N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)picolinamide* ***22s***

Mp: 220–222 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 8.60–8.58 (m, 1H), 8.04 (dt, *J* = 8, 1.6 Hz, 1H), 7.92 (dt, *J* = 8, 1.6 Hz, 1H), 7.52–7.49 (m, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.07–7.05 (m, 2H), 3.86 (s, 3H), 3.71–3.61 (m, 2H), 3.39 (d, *J* = 16.8 Hz, 1H), 3.14 (s, 3H), 3.08 (dd, *J* = 14.8, 7.6 Hz, 1H), 3.03–2.97 (m, 2H), 2.21 (s, 3H), 2.20 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.5, 178.1, 176.7, 157.3, 155.7, 149.9, 148.3, 138.8, 132.4, 130.6, 127.7, 126.9, 126.7, 124.7, 123.5, 123.0, 120.0, 116.5, 114.2, 63.4, 56.4, 40.8, 33.4, 27.2, 26.5, 25.9, 23.3; HRMS calcd. for $C_{30}H_{28}N_5O_6$ $[M+H]^+$ 554.2034, found 554.2042.

*4.21.20. N-(2-(4′-methoxy-1,1′,3-trimethyl-2,5,7′,11′-tetraoxo-5′,7′,10′,11′-tetrahydrospiro[imidazolidine-4,6′-naphtho [2,1-f] indol]-8′-yl)ethyl)-2-naphthamid* ***22t***

Mp: 187–189 °C; $^1$H NMR (400 MHz, $CD_3OD$) δ 8.30 (d, *J* = 1.6 Hz, 1H), 7.96–7.89 (m, 3H), 7.81 (dd, *J* = 8.8, 2 Hz, 1H), 7.57–7.54 (m, 2H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.08 (s, 1H), 7.06 (d, *J* = 8.4 Hz, 1H), 3.86 (s, 3H), 3.73–3.64 (m, 2H), 3.39 (d, *J* = 16.8 Hz, 1H), 3.14 (s, 3H), 3.11–3.04 (m, 2H), 3.00 (d, *J* = 16.8 Hz, 1H), 2.21 (s, 3H), 2.17 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 182.6, 178.1, 176.7, 170.3, 157.3, 155.7, 151.3, 148.4, 138.9, 136.3, 134.1, 133.0, 132.4, 130.6, 130.5, 130.1, 129.4, 128.8, 128.8, 127.8, 127.0, 125.3, 124.9, 124.8, 123.6, 123.5, 114.3, 63.4, 56.4, 41.4, 33.4, 27.1, 26.6, 25.8, 23.3; HRMS calcd. for $C_{35}H_{31}N_4O_6$ $[M+H]^+$ 603.2238, found 603.2252.

### *4.22. Biological evaluation*

#### *4.22.1. IDO1 and TDO inhibition assay*

Human IDO1 and TDO with an *N*-terminal His tag were expressed in *E. coli* and purified to homogeneity. The assay for IDO1 and TDO inhibition was performed according to the literature [22]. The assays were performed at room temperature as described in the literature using 20 nM hIDO1 or hTDO and 2 mM *D*-Trp in the presence of 20 mM ascorbate, 3.5 μM methylene blue and 0.2 mg/mL catalase in 50 mM potassium phosphate buffer (pH 6.5). The initial reaction rates were recorded by continuously following the absorbance increase at 321 nm due to the formation of N′-formylkynurenine. The percent inhibition at individual concentrations was calculated by the slopes and $IC_{50}$ was analyzed using the GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA, USA).

#### *4.22.2. MTT assay of cell viability*

The target compounds were evaluated in vitro against five human cell lines including HepG2, U87, HGC27, HCT-116, MCF-7. Each sample was prepared as a 50.0 mM stock solution that was dissolved in DMSO and added to the cells with less than 1% DMSO in the final drug dilution with culture medium. The cancer cell lines were cultured in RPMI1640 supplemented with 10% fetal bovine serum, at 37 °C in a 5% $CO_2$ humidified atmosphere. Briefly, cells were placed in the appropriate media on 96-well plates and were allowed to adhere for 24 h. Then all studied compounds in different concentrations were added to the culture medium and incubated for another 72 h. Afterwards, cell viability was assayed by the standard MTT assay. The results expressed as $IC_{50}$ were calculated as the concentration that reduced the absorbance of the untreated wells by 50% of vehicle in the MTT assay. Assays were performed in triplicate on these cytotoxic experiments.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2021.113631.

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