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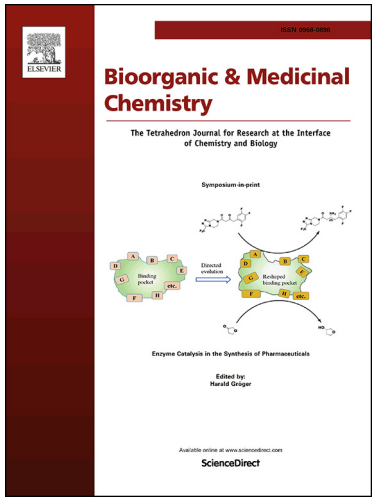

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Design, synthesis and structure-activity relationship study of novel naphthoindolizine and indolizinoquinoline-5,12-dione derivatives as IDO1 inhibitors

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Abstract

Indoleamine 2,3-dioxygenase 1 (IDO1) is regarded as a promising target for cancer immunotherapy. Many naphthoquinone derivatives have been reported as IDO1 inhibitors so far. Herein, two series of naphthoquinone derivatives, naphthoindolizine and indolizinoquinoline-5,12-dione derivatives, were synthesized and evaluated for their IDO1 inhibitory activity. Most of the target compounds showed significant inhibition potency and high selectivity for IDO1 over tryptophan 2,3-dioxygenase (TDO). The structure-activity relationship was also summarized. The most potent compounds **5c** (IC_{50} 23 nM, IDO1 enzyme), and **5b'** (IC_{50} 372 nM, HeLa cell) were identified as promising lead compounds.

Keywords

Indoleamine 2,3-dioxygenase 1; cancer immunotherapy; Naphthoindolizine; Indolizinoquinoline-5,12-dione derivatives.

1. Introduction

L-tryptophan is one of the essential amino acids necessary for protein synthesis and nitrogen balance in human body. Over 90% of L-tryptophan utilized by humans is processed through kynurenine pathway [1]. The heme-containing enzyme indoleamine 2,3-dioxygenase 1 (IDO1) catalyzes the first and rate-limiting step of kynurenine pathway, leading to the depletion of tryptophan and the production of its metabolites, such as kynurenine, kynurenic acid, and quinolinic acid [2]. IDO1 was first discovered in rabbit intestine in 1967 [3], and it is expressed ubiquitously throughout human body except in liver.

IDO1 plays an essential role in the regulation of tryptophan homeostasis. Its activation depletes the local tryptophan concentration and accumulates the metabolites of kynurenine pathway. The deficit of tryptophan leads to starvation of cytotoxic T-cells in the microenvironment and the metabolites of tryptophan can activate regulatory T-cells, which further suppresses the immune response to tumors. Numerous studies have demonstrated that

the expression of IDO1 is up-regulated in many human cancers and associated with poor prognosis [4]. IDO1 helps tumors to induce tolerance from the host's immune system and it is overexpressed in many human cancers, which suggest that it might be a promising target for cancer immunotherapy.

Indeed, several groups have discovered that blockade of IDO1 can directly increase the ability of tumor-bearing mice to reject tumors [5, 6]. Furthermore, IDO1 inhibitors combined with chemotherapeutic agents or established immunotherapeutic strategies, like anti-PD1/PD-L1 therapy, seem to have better efficacy and produce superior response rates compared with single agent therapy [4, 7-12]. These studies demonstrate that inhibition of IDO1 can reactivate the immune system to break tumor-induced immune resistance.

Many naphthoquinone derivatives have been reported as IDO1 inhibitors. Andersen et al. [13] reported that several natural products isolated from a marine invertebrate showed potent inhibitory activities against IDO1 and the most potent one was Annulin B with a K_i = 0.12 μM (**Fig. 1**). Since most of the marine natural product inhibitors contain a naphthoquinone core, Muller et al [14] hypothesized that the naphthoquinone core was the relevant pharmacophore in Annulin B. They screened commercially available compounds containing a quinone structure for IDO1 inhibitory activity and several compounds demonstrated micromolar levels of inhibitory potency, such as menadione (IC_{50} = 0.72 μM) and Juglone (IC_{50} = 1.0 μM) (**Fig. 1**). Based on menadione's structure, they synthesized several pyranonaphthoquinone derivatives (PNQs) and the most potent ones demonstrated nanomolar level of inhibition against IDO1, such as PNQ 1 (K_i = 70 nM), PNQ 2 (K_i = 61 nM), PNQ 3 (K_i = 66 nM) (**Fig. 1**).

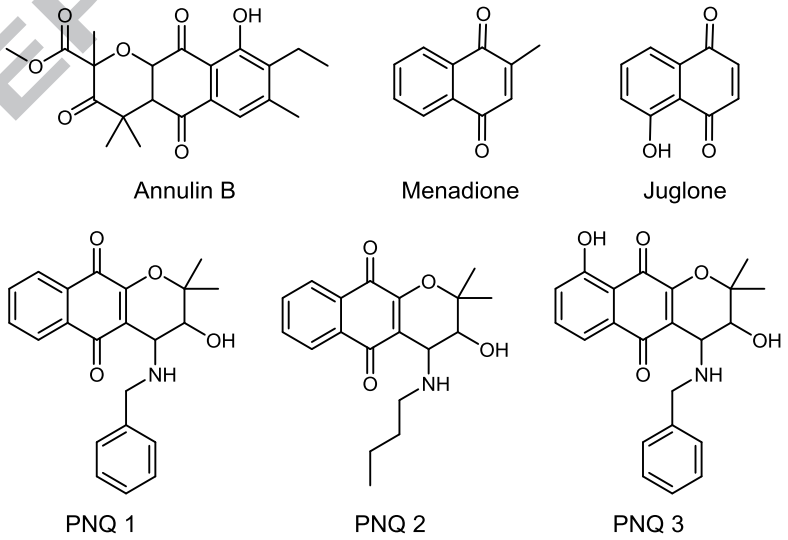
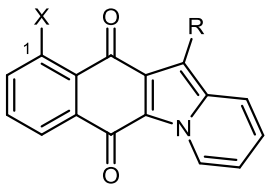


Figure 1. Structure of naphthoquinone derivatives as IDO1 inhibitors.

Here, we designed and synthesized two new series of novel naphthoquinone derivatives,

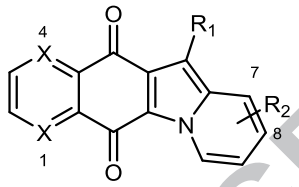
naphthoindolizine (**1-2**) and indolizinoquinoline-5,12-dione derivatives (**3-6**) (**Fig. 2**). We tested their inhibitory activity against IDO1 and found several compounds exhibited high inhibition activity. The structure-activity relationship of the target molecules was summarized. The most potent compound **5c** showed nanomolar level of inhibition against IDO1 (IC_{50} = 23 nM) and low cytotoxicity.



1-2

X = H, OH;

R = COOMe, COOEt, $COOCH(CH_3)_2$, COOH, $CONH(CH_2)_2N(CH_3)_2$, $CONH(CH_2)_3N(CH_3)_2$, $CONH(CH_2)_2OH$, $CONH(CH_2)_3OH$



3-6 (3'-6')

X = N, CH;

R_1 = COOMe, COOEt, COOH, $CONH(CH_2)_2N(CH_3)$, CONHAr, $CONHNHC(=S)NH_2$, CH_2Ph;

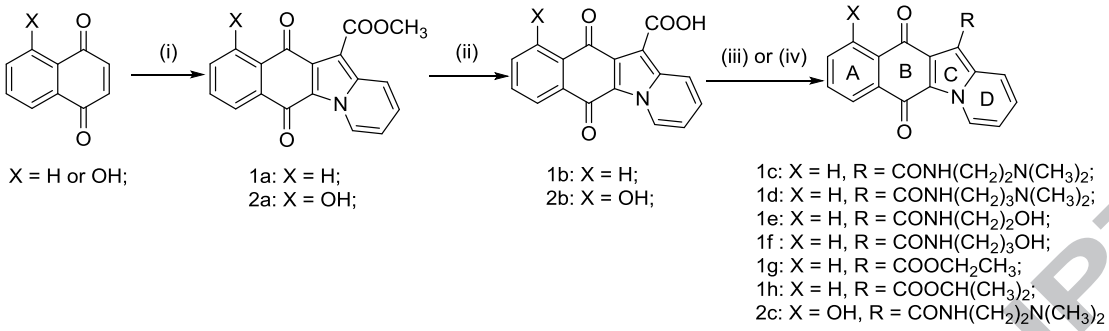
R_2 = 8-H, 8-CH_3, 8-OCH_3, 8-SCH_3, 8-I, 8-NH_2, 8-COOMe, 8-COOEt, 8-COOH, 7-F

Figure 2. Structures of the naphthoindolizine derivatives (compounds **1-2**) and indolizinoquinoline-5,12-dione derivatives (compounds **3-6**).

2. Results and Discussion

2.1. Chemistry

The naphthoindolizine derivatives (NIs) were synthesized following the multi-step strategy shown in **Scheme 1**. The core heterocyclic skeleton of **1a** was synthesized through an one-pot three-component reaction using 1,4-naphthoquinone, pyridine, and dimethyl butynedioate in DMF in the presence of a catalytic amount of hydrated copper(II) chloride heated at 80°C for 16h under 1 atm of O_2 [15]. Compound **1a** subsequently underwent hydrolysis to give the carboxylic acid **1b**. Finally, **1b** was converted into amide **1c-f** via reaction with amines by treatment with EDCI and HOBT in dichloromethane. Ester **1g** and **1h** were obtained by the reaction of **1b** and ethanol or isopropanol by DCC and DMAP. Similarly, **2a-c** were prepared using 5-hydroxy-1,4-naphthalenedione. No regioisomers were obtained and the hydroxyl group's position of **2a** was characterized by NMR analysis. The H-H COSY spectrum indicated that the signal at δ = 7.74 ppm could be assigned to H-4. Furthermore, the HMBC spectrum demonstrated that the resonance at δ = 179.63 ppm was attributed to C-5 by long-range hetero-correlation with H-4. The signal at δ = 162.10 ppm was attributed to C-1 by correlation with hydrogen of hydroxyl (δ = 12.54 ppm) and the resonance at δ = 180.22 ppm was assigned to correlation between C-12 and OH (δ = 12.54 ppm) (**Fig. 3**).



Scheme 1. Synthesis of naphthoindolizine derivatives **1a-2c**. Reagents and conditions: (i) DMF, pyridine, dimethyl butynedioate, $CuCl_2$, 1atm O_2, refluxing; (ii) MeOH/H_2O, K_2CO_3; (iii) DCM, amines, EDCI, HOBt; (iv) DCM, DCC, DMAP, EtOH/isopropanol.

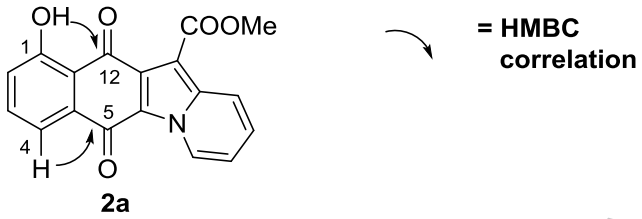
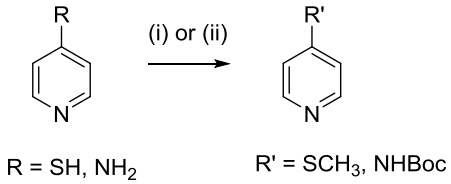
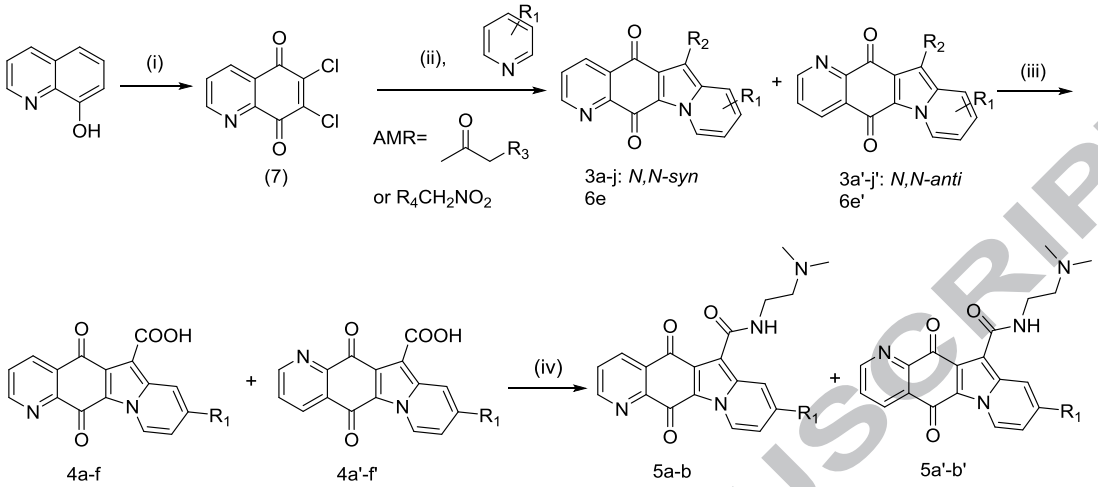


Figure 3. Structural characterization of **2a**.

The indolizinoquinoline-5,12-dione derivatives (IQDs) were obtained as shown in **Scheme 2-5**. Pyridine scaffolds were purchased or prepared as shown in **Scheme 2**. 6,7-Dichloroquinoline-5,8-dione (**7**) was obtained by oxidizing 8-hydroxyquinoline with sodium chlorate in concentrated HCl solution [16]. The heterocyclic skeletons of **3a,a'-3j,j'** and **6e,e'** were formed through a single-step reaction of substrate **7**, pyridine derivatives and an active methylene reagent (AMR). Two regioisomers (*N,N-syn* and *N,N-anti* isomers) were obtained in the cyclization, which gave the same results as by Defant et al. [17]. Some of the isomers could be isolated in pure form by chromatography, such as **3a**/**3a'**, **3i**/**3i'**, and **6e**/**6e'**. But most of the isomers were inseparable due to the similarity of their structure. **3a,a'-3f,f'** underwent hydrolysis to give **4a,a'-4f,f'**. The regioisomers **4a,a'-4e,e'** could be isolated readily by chromatography, whereas **4f,f'** were not isolated due to their poor solubility. Finally, **4a,a'** and **4b,b'** were converted into **5a,a'** and **5b,b'** by reaction with N,N-dimethyl-1,2-ethanediamine in the presence of HBTU in CH_2Cl_2 (**Scheme 3**). **6a,a'-6c,c'** were obtained through the reaction of **4a,a'** and aniline derivatives as shown in **Scheme 4**. **6d,d'** were prepared by the reaction of ethyl-m-aminobenzoate with **4a,a'** and subsequent hydrolysis of ester group (**Scheme 4**). **4a** was treated with N,N'-carbonyldiimidazole and thiosemicarbazide in DMF to afford **5c** (**Scheme 5**) [18].



Scheme 2. Preparation of pyridine derivatives. Reagents and conditions: (i) MeOH, CH_3I, KOH; (ii) DCM, Di-tert-butyl dicarbonate.



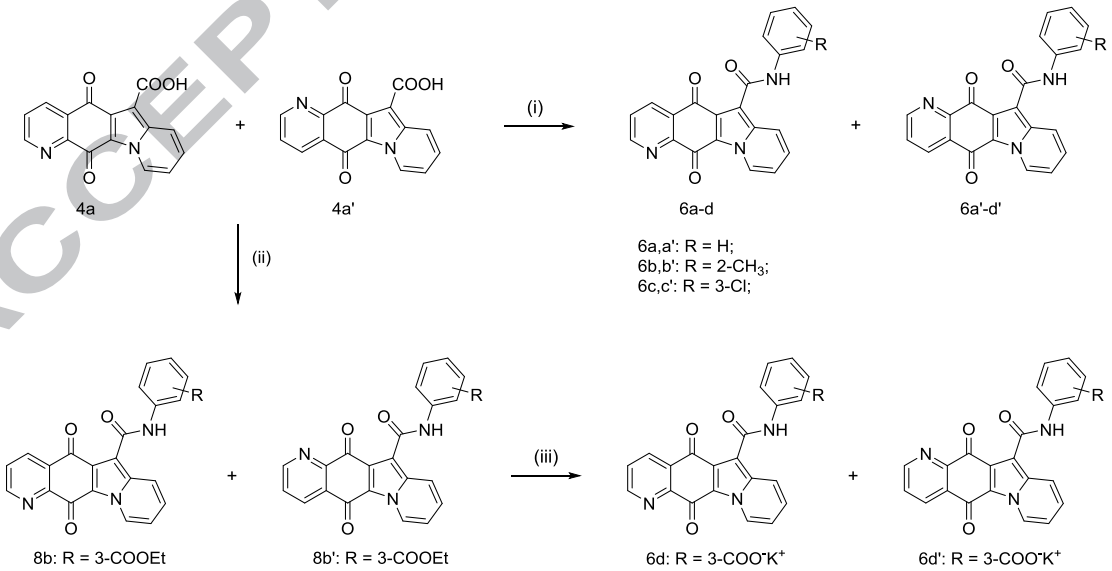
3a,a': R_1 = 4-H, R_2 = R_3 = COOEt;
3b,b': R_1 = 4-SCH_3, R_2 = R_3 = COOEt;
3c,c': R_1 = 4-OCH_3, R_2 = R_3 = COOEt;
3d,d': R_1 = 4-CH_3, R_2 = R_3 = COOEt;
3e,e': R_1 = 4-I, R_2 = R_3 = COOEt;
3f, f': R_1 = 4-COOMe, R_2 = R_3 = COOEt;
3g,g': R_1 = 4-COOEt, R_2 = R_3 = COOEt;
8a,a': R_1 = 4-NHBoc, R_2 = R_3 = COOEt;
3i, i': R_1 = 4-H, R_2 = R_3 = COOMe;
3j, j': R_1 = 3-F, R_2 = R_3 =COOEt;
6e,e': R_1 = 4-H, R_2 = R_4 = CH_2Ph;

(v) →

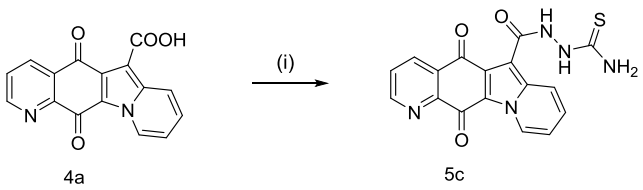
4a,a': R_1 = H;
4b,b': R_1 = SCH_3;
4c,c': R_1 = OCH_3;
4d,d': R_1 = CH_3;
4e,e': R_1 = I;
4f, f': R_1 = COOH;
3h,h': R_1 = 4-NH_2, R_2 = COOEt;

5a,a': R_1 = H;
5b,b': R_1 = SCH_3;

Scheme 3. Synthesis of indolizinoquinoline-5,12-dione derivatives 3a,a'-5b,b' & 6e,e'. Reagents and conditions: (i) $NaClO_3$, concd HCl, 50°C; (ii) EtOH, pyridine derivatives, AMR, refluxing; (iii) MeOH/H_2O, K_2CO_3; (iv) DCM, N,N-Dimethyl-1,2-ethanediamine, HBTU, DIPEA; (v) DCM, CF_3COOH.



Scheme 4. Synthesis of indolizinoquinoline-5,12-dione derivatives 6a,a'-6d,d'. Reagents and conditions: (i) DCM, aniline derivatives, HBTU, DIPEA; (ii) DCM, ethyl-m-aminobenzoate, HBTU, DIPEA; (iii) MeOH/H_2O, KOH.



Scheme 5. Synthesis of indolizinoquinoline-5,12-dione derivatives 5c. Reagents and conditions: (i) DMF, thiosemicarbazide, N,N'-carbonyldiimidazole.

The structures of regioisomers **3a/3a'** and **3i/3i'** were confirmed by the NMR spectra, ESI-MS spectrum, and melting point, in their pure form. The results were similar to those of Defant's [17, 19]. The structures of regioisomers **4a,a'-4e,e'** were confirmed by R_f value in TLC. Since the R_f value of **4a** (*N,N-syn*) is larger than that of **4a'** (*N,N-anti*) in TLC (DCM/MeOH = 10: 1), we hypothesized that the regioisomer with a larger R_f value was *N,N-syn* and the other regioisomer with a smaller R_f value was *N,N-anti.* The NMR spectra of **4b** and **4b'** confirmed our hypothesis. The HMBC spectrum of **4b** showed correlation of C-5 at δ = 185.45 ppm with H-4 at δ = 8.61 ppm while the HMBC spectrum of **4b'** indicated correlation between C-5 at δ = 172.59 ppm and H-4 at δ = 8.63 ppm. The structures of regioisomers **4c,c'-4e,e'** were assigned according to the pattern of R_f value.

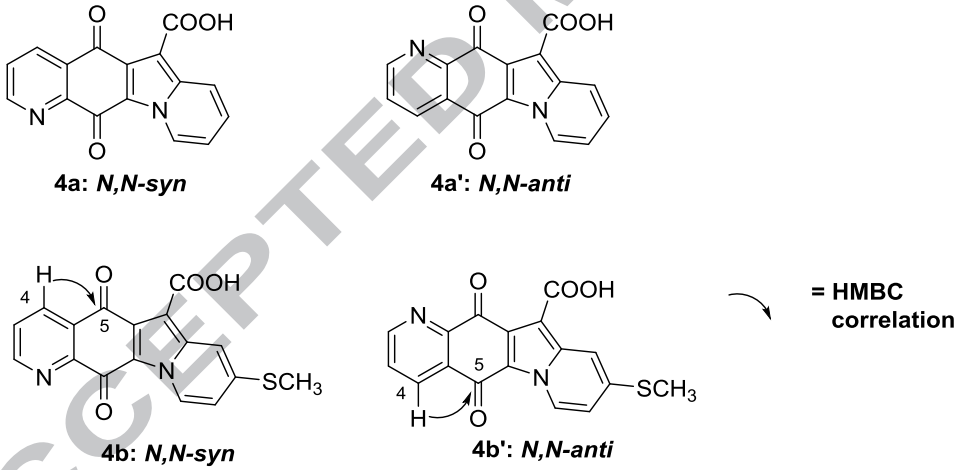


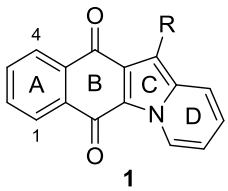
Figure 4. Structural assignments of the *N,N-syn* and *N,N-anti* regioisomers.

2.2. IDO1 Inhibitory Activities

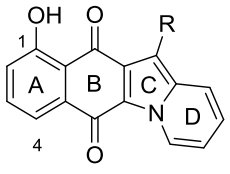
The IDO1 inhibitory activities of **1-6** along with INCB024360 [20] were assessed by IDO1 inhibition assay. Under the conditions, the IC_{50} of INCB024360 was 0.073 μM. The results shown in **Table 1-4** indicated that many of our compounds strongly inhibited the catalytic activity of IDO1.

As shown in **Table 1**, compounds **1a**, **1b**, **1g**, **2a** and **2b** exhibited high inhibitory activity. Ester side chains attached to the pyrrole ring had superior activity than carboxylic acids and amides. Long and bulky ester side chains (**1g** and **1h**) showed weaker inhibition. In addition, hydroxyl group at C-1 in the A ring had no impact on the inhibition activity.

Table 1. IDO1 Inhibitory activities of **1a-h** and **2a-c**.



1



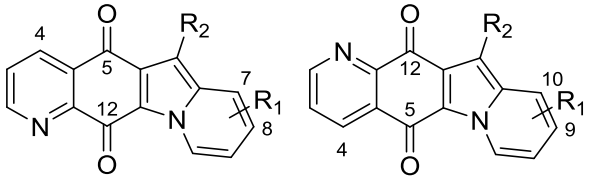
2

Compd	R	Inhibition Rate (% inhibition at 1 μM)	IC_{50} (μM)	SD(μM)
1a	$COOCH_3$	77	0.156	0.0297
1b	COOH	61	0.714	0.1209
1c	$CONH(CH_2)_2N(CH_3)_2$	27	ND	-
1d	$CONH(CH_2)_3N(CH_3)_2$	24	ND	-
1e	$CONH(CH_2)_2OH$	22	ND	-
1f	$CONH(CH_2)_3OH$	15	ND	-
1g	$COOCH_2CH_3$	68	0.332	0.0728
1h	$COOCH(CH_3)_2$	13	ND	-
2a	$COOCH_3$	77	0.087	0.0099
2b	COOH	60	ND	-
2c	$CONH(CH_2)_2N(CH_3)_2$	29	ND	-
INCB024360	-	-	0.073	0.0028

ND: Not detected.

Interestingly, the introduction of N atom in the A ring had dual effect. Compared with **1a**, **1b** and **1c**, the activities of **3i** and **3i'** decreased, while the activities of **4a**, **4a'**, **5a**, and **5a'** significantly increased (**Table 2**). The position of N atom in the A ring also showed variable effect. For example, the inhibition rate of **3a** is lower than its regioisomer **3a'** while that of **4a** is higher than **4a'**, which were obtained respectively through hydrolysis of **3a** and **3a'**. But in general, *N,N-syn* regioisomers were more potent IDO1 inhibitors. Modifications with smaller ester side chains (**3i** and **3i'**) led to stronger inhibition activities than the larger ones (**3a** and **3a'**). But modifications of IQD with carboxyl and amine side chains at the C ring demonstrated superior inhibitory activity than ester group, which was different from NIs. Moreover, electron-donating substituent groups at C-8, such as **3c,c'**, **3d,d'**, **3e,e'**, **3h,h'**, **4b,b'** and **5b,b'**, generally showed stronger inhibitory activity than electron-withdrawing groups (**3f,f'** and **3g,g'**). Compound **5b** (IC_{50} = 50 nM) and **5b'** (IC_{50} = 39 nM) demonstrated strong inhibition, which suggested that SCH_3 at C-8 and amine side chain at the pyrrole ring were beneficial to the activity. Compound **5c**, carrying a hydrophilic thiourea group as the side chain of C ring, showed the highest inhibition activity (IC_{50} = 23 nM).

Table 2. IDO1 Inhibitory activity of **3a,a'-5b,b'** and **5c**.



3, 4, 5: *N,N-syn* **3', 4', 5': *N,N-anti***

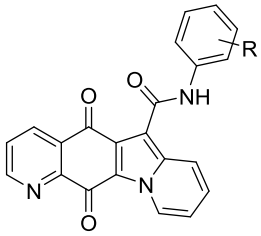
Compd	R_1	R_2	Inhibition Rate (% inhibition at 1 μM)	IC_{50} (μM)	SD(μM)
3a	8-H	COOEt	35	ND	-
3a'	8-H	COOEt	61	0.977	0.027
3b,b'[a]	8-SCH_3	COOEt	23	ND	-
3c,c' [a]	8-OCH_3	COOEt	63	ND	-
3d,d' [a]	8-CH_3	COOEt	66	ND	-
3e,e' [a]	8-I	COOEt	66	ND	-
3f,f' [a]	8-COOMe	COOEt	56	ND	-
3g,g' [a]	8-COOEt	COOEt	24	ND	-
3h,h' [a]	8-NH_2	COOEt	52	ND	-
3i	8-H	COOMe	65	0.438	0.129
3i'	8-H	COOMe	69	0.448	0.112
3j,j' [a]	7-F	COOEt	71	0.314	0.049
4a	8-H	COOH	70	0.288	0.051
4a'	8-H	COOH	58	1.192	0.318
4b,b' [a]	8-SCH_3	COOH	70.5	ND	-
4c	8-OCH_3	COOH	69	0.299	0.064
4c'	8-OCH_3	COOH	47	ND	-
4d	8-CH_3	COOH	70	0.394	0.028
4d'	8-CH_3	COOH	54	ND	-
4e	8-I	COOH	67	0.117	0.020
4e'	8-I	COOH	67	0.420	0.105
4f,f' [a]	8-COOH	COOH	56	ND	-
5a	8-H	$CONH(CH_2)_2N(CH_3)_2$	78	0.127	0.032
5a'	8-H	$CONH(CH_2)_2N(CH_3)_2$	77	0.188	0.013
5b	8-SCH_3	$CONH(CH_2)_2N(CH_3)_2$	72.5	0.050	0.001
5b'	8-SCH_3	$CONH(CH_2)_2N(CH_3)_2$	80.5	0.039	0.003
5c	8-H	$CONHNHC(=S)NH_2$	74.5	0.023	0.006
INCB024360	-	-	-	0.073	0.003

ND: Not detected. [a] The mixture was used to determine the inhibition rate.

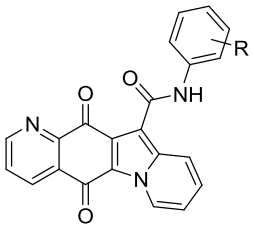
When side chains at the C ring contained different anilines, the inhibition activities were moderately increased (**Table 3**). Comparison of the two regioisomers, the *N,N-syn* isomers generally showed more potent activity than the *N,N-anti* isomers. When the side chain was benzyl group (**6e** and **6e'**), the inhibition rate dropped significantly (**Table 4**), which suggested

that a flexible side chain might not be desirable and a conjugated system might be necessary.

Table 3. IDO1 Inhibitory activity of **6a,a'-6d,d'**.



6: ***N,N-syn***

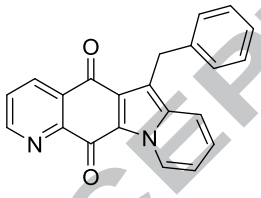


6': ***N,N-anti***

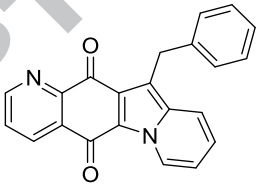
Compd	R	Inhibition Rate (% inhibition at 1 μM)	IC_{50} (μM)	SD(μM)
6a	H	68	0.361	0.075
6a'	H	62	0.119	0.020
6b	2-CH_3	74	0.427	0.110
6b'	2-CH_3	64	0.134	0.026
6c	3-Cl	29.5	ND	-
6c'	3-Cl	34	ND	-
6d	3-COO^-K^+	74.5	0.148	0.025
6d'	3-COO^-K^+	71.5	0.390	0.115
INCB024360	-	-	0.073	0.004

ND: Not detected.

Table 4. IDO1 Inhibitory activity of **6e** and **6e'**.



6e: ***N,N-syn***



6e': ***N,N-anti***

Compd	Inhibition Rate (% inhibition at 1 μM)	IC_{50} (μM)
6e	34	ND
6e'	56	ND
INCB024360	96	0.073

ND: Not detected.

2.3. Selectivity over TDO

Tryptophan 2,3-dioxygenase (TDO), which is a distantly related tetrameric enzyme mainly expressed in the liver, catalyzes the same reaction in kynurenine pathway. We also evaluated our compounds for TDO inhibition [25] (**Table 5**). The results indicated that the compounds that showed strong IDO1 inhibition activity also exhibited high selectivity for IDO over TDO.

Table 5. TDO Inhibition of several NIs and IQDs

Compd	Inhibition Rate (% inhibition at 10 μM)	IC50 (μM)	SD(μM)
1a	40	ND	-
3a'	14	ND	-
4a	28	ND	-
5a	91	3.35	1.4
5a'	88	7.15	2.7
5c	ND	1.2	2.2
6a	ND	7.3	1.5
6a'	ND	>100	-
6b	ND	>100	-
6b'	ND	>100	-
6d	ND	41.6	10.0
6d'	ND	>100	-

ND: Not detected.

2.4. Cytotoxicity Study

An et al [21] evaluated the cytotoxic activities of similar indolizinoquinoline-5,12-dione derivatives and they found that most of their compounds showed significant cytotoxic activities against a wide variety of tumor cell lines, such as lung adenocarcinoma cells, large-cell lung carcinoma cells, promyelocytic leukemia cells and breast carcinoma cells. Considering the similarity between the core structure of their compounds and ours, we also tested the cytotoxicity of the most potent compound, **5c**, by MTT using two human cancer cell lines, human cervical carcinoma cells (HeLa), breast carcinoma cells (MCF-7), and embryonic kidney cells (HEK293T). Doxorubicin was used as the positive control. According to **Table 6**, **5c** showed weak cytotoxic activities against the three human cell lines.

Table 6. Cytotoxic activities of **5c**

Compd	Cytotoxic activities IC_{50} (μM)		
	HeLa	MCF-7	HEK293T
5c	>20	>20	>20
Doxorubicin	0.66±0.04	0.37±0.03	1.13±0.11

2.5. Cellular IDO-1 Inhibitory Activities of NIs and IQDs

To study the cellular IDO1 inhibitory activity, HeLa cells expressing human IDO1 were created and used for assays with NIs and IQDs. Several compounds displayed significant IDO1 inhibitory activity, such as **5a**, **5a'**, **5b** and **5b'** (shown in **Table 7**). However, some compounds that showed high inhibition against IDO1 in enzymatic assays did not demonstrated similar inhibition in

cellular assays, which might result from membrane permeability, or the complexity of kynurenine pathway.

Table 7. Cellular IDO1 Inhibitory Activities

Compd	Inhibition Rate (% inhibition at 1 μM)	IC_{50} (μM) HeLa
1a	24	-
2a	12	-
4e	12	-
5a	43	0.738
5a'	81	1.235
5b	42	1.083
5b'	77	0.372
5c	22	-
6a'	25	-
6b'	38	2.662
6d	9	-
INCB024360	96	0.007

2.6. Molecular Docking study of 5c

Because of the large structural difference between **5c** and the original ligand of 4PK5 [16], there is no suitable binding conformation for docking 5c to 4PK5 directly. Alternatively, we used Schrodinger software Induced Fit Docking (IFD) to expand the 4PK5 binding site gradually by docking a series of smaller IDO inhibitors in sequence, during which the docking result of the previous step was used to generate the next grid. In this manner, the IDO1 protein can be docked with **5c** by Schrodinger glide (Released Jan, 2017). Compound **5c** was docked inside the IDO1 binding cleft (Fig. 5) by three hydrogen-bonds (amino acid L234, G236, and G263 with the side chain, respectively), $\pi-\pi$ interaction (amino acid F163 with pyridine moiety).

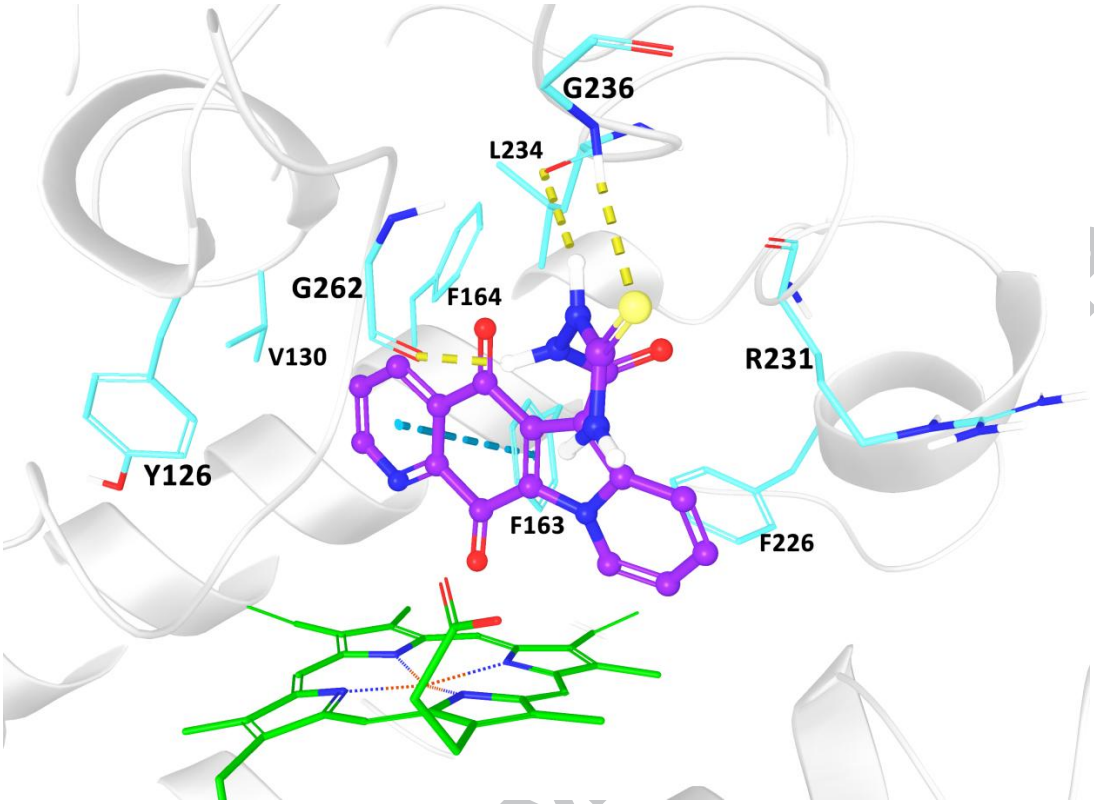


Figure 5. View of compound **5c** docked inside the IDO1 binding cleft.

3. Conclusions

Approximately fifty naphthoindolizine and indolizinoquinoline-5,12-dione derivatives were synthesized and many of them demonstrated significant inhibitory activity against IDO1. For NIs, introduction of small ester side chain at the C ring led to superior inhibitory activity and introduction of a hydroxyl group at C-1 had no impact on inhibitory activity. For IQDs, carboxylic acids and amides (generated from aliphatic amine or anilines) side chains exhibited higher inhibitory activity than esters. Electron-donating groups at C-8, such as SCH_3, were beneficial to the activity. Besides, *N,N-syn* isomers generally showed more potent inhibitory activity than *N,N-anti* isomers. Several compounds that had strong IDO1 inhibitory activity also exhibited high selectivity over TDO. **5c** was identified as the most potent compound (IC_{50} = 23 nM) with weak cytotoxicity, and **5b'** was the most potent one in cellular assay, which are promising leads for developing potent and highly selective IDO1 inhibitors.

4. Experimental protocols

4.1. General methods and materials

All starting materials and common laboratory chemicals were purchased from commercial sources and used without further purification. All solvents were distilled and dried according to standard procedures. Chemical reactions were monitored by thin layer chromatography using silica gel 60 F_{254} aluminum supported plate (layer thickness 0.2 mm). Flash column

chromatography was performed with silica gel 200–300 meshes. Melting points were determined using the X-5 apparatus. ^{1}H and ^{13}C NMR spectra were measured on a Bruker ARX400 instrument, using TMS as internal standard [chemical shifts (δ) in ppm, coupling constants (J) in Hz]. High resolution mass spectra were obtained by Electro Spray Ionization (ESI).

4.2. General preparation of NIs

1,4-Naphthaquionone or 5-hydroxyl-1,4-naphthalenedione (1.0 mmol), pyridine (3.0 mmol), butynedioates (1.0 mmol) and hydrated copper(II) chloride (0.3 mmol) were mixed in 15 mL DMF and stirred at 80 °C for 16 h under 1 atm of O_2 [15]. After completion of the reaction, the reaction mixture was cooled to room temperature and concentrated in vacuum. The residue was subsequently subjected to flash column chromatography (ethyl acetate/petroleum ether, 1:6) to give the corresponding target compound **1a** or **2a**. The methyl ester group of **1a** and **2a** was hydrolyzed using K_2CO_3 in MeOH/H_2O. When the hydrolysis was complete, 1M HCl was added and the mixture was extracted with CH_2Cl_2. The organic phase was dried with $MgSO_4$ and concentrated in vacuum to give the product **1b** and **2b**. **1b** or **2b** (1.0 mmol) was dissolved in DCM and EDCI (1.5 mmol), HOBT (1.5 mmol) were added to the solution. The mixture was stirred at room temperature for 1h, followed by the addition of different amines (1.5 mmol) and the reaction mixture was stirred for 5h. The solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography to give the target compound **1c-1f** and **2c**. **1b** or **2b** (1.0 mmol) was dissolved in DCM and DMAP (1.5 mmol), DCC (1.5 mmol) were added to the solution. The mixture was stirred at room temperature for 1h, followed by the addition of different alcohols (1.5 mmol) and the reaction mixture was stirred for 5h at room temperature. The solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (DCM/MeOH, 200:1) to give the target compound **1g** and **1h**.

4.2.1. Methyl 6,11-dioxo-6,11- dihydrobenzo[*f*]pyrido [1,2-*a*]indole-12-carboxylate (**1a**)

Orange solid; yield 43%; m.p. = 190-191°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.84 (d, J = 7.0 Hz, 1H), 8.33 (d, J = 9.1 Hz, 1H), 8.27–8.16 (m, 2H), 7.77–7.66 (m, 2H), 7.44 (ddd, J = 9.0, 6.8, 1.0 Hz, 1H), 7.18 (td, J = 6.9, 1.2 Hz, 1H), 4.06 (s, 3H). Lit. [15]

4.2.2. 6,11-Dioxo-6,11-dihydrobenzo[*f*]pyrido[1,2-*a*]indole-12-carboxylic acid (**1b**)

Red solid; yield 93%; m.p. = 304-305°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.60 (s, 1H), 9.72–9.78 (m, 1H), 8.59–8.61 (m, 1H), 8.19–8.21 (m, 2H), 7.92 (d, J = 27.4 Hz, 2H), 7.72–7.73 (m, 1H), 7.51–7.52 (m, 1H). Lit. [22]

4.2.3. *N*-(2-(Dimethylamino)ethyl)-6,11-dioxo-6,11-dihydrobenzo[*f*]pyrido[1,2-*a*]indole-12-carboxamide (**1c**)

Red solid; yield 88%; m.p. = 142-143°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.32 (s, 1H), 9.69 (d, J =

6.7 Hz, 1H), 8.94 (d, *J* = 9.0 Hz, 1H), 8.04 (dd, *J* = 14.4, 7.4 Hz, 2H), 7.60 (dt, *J* = 21.6, 7.1 Hz, 2H), 7.31 (dd, *J* = 16.0, 7.9 Hz, 1H), 7.08 (t, *J* = 6.5 Hz, 1H), 3.59 (d, *J* = 5.7 Hz, 2H), 2.65 (t, *J* = 6.4 Hz, 2H), 2.37 (s, 6H). Lit. [23]

4.2.4. *N*-(3-(dimethylamino)propyl)-6,11-dioxo-6,11-dihydrobenzo[*f*]pyrido[1,2-*a*]indole-12-carboxamide (**1d**)

Red solid; yield 90%; m.p. = 148-149°C; ^{1}H NMR (400 MHz, MeOD): δ = 9.43 (d, *J* = 6.8 Hz, 1H), 8.51 (d, *J* = 9.1 Hz, 1H), 7.83 (d, *J* = 7.5 Hz, 1H), 7.76 (d, *J* = 7.5 Hz, 1H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.53 (t, *J* = 7.3 Hz, 1H), 7.37–7.27 (m, 1H), 7.11 (t, *J* = 6.9 Hz, 1H), 3.37 (t, *J* = 6.8 Hz, 2H), 2.73–2.59 (m, 2H), 2.44 (s, 6H), 1.87–1.96 (m, 2H). ^{13}C NMR (100 MHz, MeOD): δ = 182.50, 172.76, 162.38, 139.57, 133.96, 132.71, 132.65, 131.98, 127.70, 126.81, 126.38, 125.12, 123.71, 121.81, 120.59, 118.09, 108.08, 56.84, 44.14, 37.11, 26.35.Lit. [24]

4.2.5. *N*-(2-hydroxyethyl)-6,11-dioxo-6,11-dihydrobenzo[*f*]pyrido[1,2-*a*]indole-12-carboxamide (**1e**)

Red solid; yield 43%; m.p. = 160-161°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.05 (t, *J* = 5.1 Hz, 1H), 9.57 (d, *J* = 6.8 Hz, 1H), 8.72 (d, *J* = 9.1 Hz, 1H), 7.96 (dd, *J* = 15.5, 7.3 Hz, 2H), 7.75 (dt, *J* = 20.1, 7.2 Hz, 2H), 7.57–7.41 (m, 1H), 7.31 (t, *J* = 6.8 Hz, 1H), 4.83 (t, *J* = 5.1 Hz, 1H), 3.61 (dd, *J* = 11.1, 5.6 Hz, 2H), 3.41 (dd, *J* = 11.1, 5.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 183.50, 173.74, 162.40, 140.00, 135.00, 133.72, 133.63, 133.03, 128.60, 127.57, 127.43, 127.39, 126.00, 124.80, 122.59, 121.30, 119.15, 109.49, 60.67, 42.12. Lit. [25]

4.2.6. *N*-(3-hydroxypropyl)-6,11-dioxo-6,11-dihydrobenzo[*f*]pyrido[1,2-*a*]indole-12-carboxamide (**1f**)

Red solid; yield 48%; m.p. = 225-226°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.84 (t, *J* = 5.1 Hz, 1H), 9.50 (d, *J* = 6.8 Hz, 1H), 8.65 (d, *J* = 9.1 Hz, 1H), 7.91 (dd, *J* = 11.7, 7.7 Hz, 2H), 7.71 (dt, *J* = 22.3, 7.2 Hz, 2H), 7.52–7.38 (m, 1H), 7.28 (t, *J* = 6.8 Hz, 1H), 4.55 (s, 1H), 3.57 (s, 2H), 3.32–3.36 (m, 2H), 1.72–1.79 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 183.43, 173.48, 162.12, 139.82, 134.83, 133.57, 133.46, 132.84, 128.46, 127.41, 127.27, 125.84, 124.52, 122.49, 121.09, 119.08, 109.51, 58.99, 36.39, 32.77. Lit. [26]

4.2.7. Ethyl 6,11-dioxo-6,11- dihydrobenzo[*f*]pyrido [1,2-*a*]indole-12-carboxylate (**1g**)

Orange solid; yield 85%; m.p. = 157-158°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.76 (d, *J* = 7.0 Hz, 1H), 8.25 (d, *J* = 9.1 Hz, 1H), 8.22–8.07 (m, 2H), 7.67 (p, *J* = 7.5 Hz, 2H), 7.45–7.32 (m, 1H), 7.12 (t, *J* = 6.7 Hz, 1H), 4.50 (q, *J* = 7.1 Hz, 2H), 1.50 (t, *J* = 7.2 Hz, 3H). Lit. [23]

4.2.8. Isopropyl 6,11-dioxo-6,11- dihydrobenzo[*f*]pyrido[1,2-*a*]indole-12-carboxylate (**1h**)

Orange solid; yield 78%; m.p. = 131-133°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.78 (d, *J* = 7.0 Hz, 1H), 8.25 (d, *J* = 9.1 Hz, 1H), 8.22–8.11 (m, 2H), 7.68 (p, *J* = 7.6 Hz, 2H), 7.45–7.33 (m, 1H), 7.12 (t,

J = 6.9 Hz, 1H), 5.40 (dt, *J* = 12.5, 6.2 Hz, 1H), 1.49 (d, *J* = 6.3 Hz, 6H). Lit. [15]

4.2.9. Methyl 10-hydroxy-6,11-dioxo-6,11-dihydrobenzo[*f*]pyrido[1,2-*a*]indole-12-carboxylate (**2a**)

Orange solid; yield 53%; m.p. = 240-241°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.54 (s, 1H), 9.76 (d, *J* = 7.1 Hz, 1H), 8.34 (d, *J* = 9.2 Hz, 1H), 7.74 (d, *J* = 7.5 Hz, 1H), 7.55 (t, *J* = 7.9 Hz, 1H), 7.50–7.42 (m, 1H), 7.24–7.16 (m, 2H), 4.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.22, 179.63, 163.67, 162.10, 140.19, 135.48, 134.43, 129.13, 128.52, 128.28, 124.34, 121.61, 121.15, 120.08, 117.71, 115.90, 106.44, 52.13. HR-ESI-MS: Calcd for $C_{18}H_{11}NO_5$ $[M+Na]^+$: 344.0535, found: 344.0543. The structure was confirmed through H-H COSY and HMBC.

4.2.10. 10-Hydroxy-6,11-dioxo-6,11-dihydrobenzo[*f*]pyrido[1,2-*a*]indole-12-carboxylic acid (**2b**)

Red solid; yield 89%; m.p. >320°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 13.99 (s, 1H), 12.54 (s, 1H), 9.79 (d, *J* = 6.9 Hz, 1H), 8.98 (d, *J* = 8.9 Hz, 1H), 7.89 (d, *J* = 7.2 Hz, 1H), 7.61 (q, *J* = 8.9 Hz, 2H), 7.37–7.30 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 185.44, 179.40, 162.72, 162.59, 141.80, 135.73, 132.39, 129.67, 128.37, 127.15, 126.44, 122.83, 121.56, 119.33, 115.71, 107.12. HR-ESI-MS: Calcd for $C_{17}H_9NO_5$ $[M+H]^+$: 308.0559, found: 308.0553.

4.2.11. *N*-(2-(dimethylamino)ethyl)-10-hydroxy-6,11-dioxo-6,11-dihydrobenzo[*f*]pyrido[1,2-*a*]indole-12-carboxamide (**2c**)

Red solid; yield 85%; m.p. = 185-186°C; ^{1}H NMR (400 MHz, MeOD): δ = 9.79 (d, *J* = 5.4 Hz, 1H), 8.90 (d, *J* = 10.1 Hz, 1H), 7.74 (s, 1H), 7.63 (d, *J* = 5.8 Hz, 2H), 7.40 (s, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 3.92 (s, 2H), 3.52 (s, 2H), 3.08 (s, 6H). ^{13}C NMR (100 MHz, MeOD): δ = 183.31, 179.26, 164.75, 161.96, 140.97, 135.33, 133.33, 128.84, 128.10, 125.70, 125.16, 122.14, 121.14, 120.12, 118.71, 115.86, 115.45, 108.47, 57.90, 42.72, 34.72. HR-ESI-MS: Calcd for $C_{21}H_{19}N_3O_4$ $[M+H]^+$: 378.1454, found: 378.1450.

4.3. Preparation of compound 7

6,7-Dichloroquinoline-5,8-dinone (**7**) was prepared following Shaikh's method [16] by oxidizing 8-hydroxyquinoline with sodium chlorate in concentrated HCl solution at 40°C. The reaction was diluted with water and the white precipitate was removed by filtration. The filtrate was extracted with CH_2Cl_2 and after the removal of CH_2Cl_2, the residue was recrystallized in methanol to obtain a light yellow solid 7 with a yield of 29%.

4.4. Preparation of pyridine derivatives

4-Mercaptopyridine (9 mmol) was dissolved in 100 ml of 1% methanolic KOH solution and one equivalent iodomethane was added. The reaction mixture was stirred at room temperature for 24h. After evaporating the solvent, the residue was extracted with CH_2Cl_2 and concentrated under vacuum to give 4-methylthiopyridine.

4-Aminopyridine (10 mmol) was dissolved in 100 ml of CH_2Cl_2 and di-tert-butyl dicarbonate (12 mmol) diluted with CH_2Cl_2 was added dropwise to the solution. The reaction mixture was stirred at room temperature for 24h. The solvent was removed to give 4-tert-butoxycarbonylaminopyridine.

4.5. General preparation of IQDs

The heterocyclic skeleton was prepared according to the published methods [27, 28]. Substrate **7** (1.0 mmol) was added in 15 mL absolute ethanol and heated to dissolve. Then, the AMR (1.5 mmol) and pyridine derivative (3.0 mmol) were successively added to the solution. The reaction mixture was refluxed for 16h at 80°C and concentrated in vacuum when the reaction was complete. The residue was subsequently subjected to flash column chromatography to give the corresponding target compound **3a,a'-3j,j'** and **6e,e'**. The ethyl ester group of **3a,a'-3f,f'** was hydrolyzed using K_2CO_3 in MeOH/H_2O. When the hydrolysis was complete, 1M HCl was added and the mixture was extracted with CH_2Cl_2. The organic phase was dried with $MgSO_4$ and concentrated in vacuum. The crude product was purified by silica gel column chromatography to give the target compound **4a,a'-4f,f'** and the regioisomers of **4a-e** and **4a'-e'** were separated in the meantime. **4a-b** or **4a'-b'** (1.0 mmol) was dissolved in CH_2Cl_2 and HBTU (1.5 mmol), DIPEA (1.5 mmol) was added to the solution. The mixture was stirred at room temperature for 1h. Then N,N-dimethyl-1,2-ethanediamine (1.5 mmol) was added and the reaction mixture was stirred for 5h. The solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography to give the target compound **5a-b** and **5a'-b'**.

4.5.1. Ethyl 5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylate (**3a**)

Orange solid; yield 31%; m.p. = 225-226°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.96 (d, *J* = 6.9 Hz, 1H), 9.04 (d, *J* = 3.7 Hz, 1H), 8.57 (d, *J* = 7.5 Hz, 1H), 8.37 (d, *J* = 9.0 Hz, 1H), 7.67 (dd, *J* = 7.6, 4.7 Hz, 1H), 7.62–7.42 (m, 1H), 7.33–7.18 (m, 2H), 4.54 (q, *J* = 7.1 Hz, 2H), 1.52 (t, *J* = 7.1 Hz, 4H). Lit. [17]

4.5.2. Ethyl 5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxylate (**3a'**)

Orange solid; yield 12%; m.p. = 219-220°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.74 (d, *J* = 6.7 Hz, 1H), 8.97 (s, 1H), 8.52 (d, *J* = 7.6 Hz, 1H), 8.34 (d, *J* = 9.0 Hz, 1H), 7.83–7.51 (m, 1H), 7.44 (t, *J* = 7.8 Hz, 1H), 7.18 (t, *J* = 6.5 Hz, 1H), 4.47 (q, *J* = 6.9 Hz, 2H), 1.50 (t, *J* = 7.0 Hz, 3H). Lit. [17]

4.5.3. Ethyl 8-(methylthio)-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylate (**3b**) and ethyl 9-(methylthio)-5,12-dioxo-5,12-dihydroindolizino[3,2-g]quinoline-11-carboxylate (**3b'**) mixture (**3b**/**3b'** = 86/14)

Purple solid; yield 38%; m.p. = 248-249°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.65 (d, *J* = 7.4 Hz, 0.86H), 9.48 (d, *J* = 7.3 Hz, 0.14H), 8.99 (d, *J* = 4.7 Hz, 1H), 8.49 (t, *J* = 7.6 Hz, 1H), 7.94 (s, 1H), 7.63 (dd, *J* = 7.8, 4.7 Hz, 1H), 6.96 (dd, *J* = 19.1, 7.4 Hz, 1H), 4.48 (q, *J* = 6.9 Hz, 2H), 2.58 (s, 3H), 1.50 (t,

J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.95, 172.71, 163.08, 153.97, 149.38, 143.65, 140.31, 135.24, 134.09, 130.84, 128.37, 127.24, 126.82, 122.93, 117.00, 112.69, 103.99, 60.99, 29.70, 14.40. HR-ESI-MS: Calcd for $C_{19}H_{14}N_2O_4S$ $[M+Na]^+$: 389.0572, found: 389.0579.

4.5.4. Ethyl 8-methoxy-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylate (**3c**) and ethyl 9-methoxy-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxylate (**3c'**) mixture (**3c**/**3c'** = 56/44)

Red solid; yield 29%; m.p. = 195-196°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.78 (d, *J* = 7.6 Hz, 0.56H), 9.63 (d, *J* = 7.5 Hz, 0.44H), 9.05–8.87 (m, 1H), 8.52 (d, *J* = 7.7 Hz, 1H), 7.72 (d, *J* = 11.3 Hz, 1H), 7.68–7.54 (m, 1H), 6.96–6.78 (m, 1H), 4.61–4.34 (m, 2H), 3.98 (s, 3H), 1.52 (q, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.20, 178.35, 173.07, 172.59, 164.08, 163.62, 160.25, 160.17, 153.95, 153.40, 149.68, 149.52, 143.27, 143.04, 135.24, 134.06, 130.83, 130.42, 129.88, 129.43, 128.85, 127.03, 126.73, 122.73, 121.40, 112.51, 112.46, 104.49, 103.91, 98.46, 98.35, 61.03, 60.90, 56.01, 55.93, 14.37, 14.21. HR-ESI-MS: Calcd for $C_{19}H_{14}N_2O_5$ $[M+H]^+$: 351.0981, found: 351.0980.

4.5.5. Ethyl 8-methyl-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylate (**3d**) and ethyl 9-methyl-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxylate (**3d'**) mixture (**3d**/**3d'** = 46/54)

Red solid; yield 30%; m.p. = 210-211°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.75 (d, *J* = 6.6 Hz, 0.46H), 9.58 (d, *J* = 5.8 Hz, 0.54H), 8.98 (s, 1H), 8.51 (s, 1H), 8.10 (d, *J* = 8.2 Hz, 1H), 7.64 (s, 1H), 7.16–6.85 (m, 1H), 4.49 (s, 2H), 2.47 (s, 3H), 1.51 (d, *J* = 6.5 Hz, 3H). Lit. [17]

4.5.6. Ethyl 8-iodo-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylate (**3e**) and ethyl 9-iodo-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxylate (**3e'**) mixture (**3e**/**3e'** = 46/53)

Brown solid; yield 27%; m.p. = 268-269°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.64 (d, *J* = 7.3 Hz, 0.46H), 9.50 (d, *J* = 7.3 Hz, 0.53H), 9.03 (s, 1H), 8.82 (d, *J* = 14.6 Hz, 1H), 8.55 (d, *J* = 7.9 Hz, 1H), 7.72–7.65 (m, 1H), 7.48–7.42 (m, 1H), 4.57–4.45 (m, 2H), 1.54–1.49 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.70, 177.90, 173.86, 173.24, 163.11, 162.65, 154.19, 153.86, 149.56, 149.24, 140.40, 140.19, 135.51, 134.40, 130.95, 130.36, 130.12, 130.08, 129.02, 128.60, 128.42, 128.33, 128.00, 127.93, 127.30, 127.17, 126.75, 126.69, 123.08, 121.91, 120.10, 119.39, 105.89, 105.41, 94.35, 94.11, 61.47, 61.39, 14.31, 14.16. HR-ESI-MS: Calcd for $C_{18}H_{11}IN_2O_4$ $[M+H]^+$: 446.9842, found: 446.9836.

4.5.7. 6-Ethyl 8-methyl 5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6,8-dicarboxylate (**3f**) and 11-ethyl 9-methyl 5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-9,11-dicarboxylate (**3f'**) mixture (**3f**/**3f'** = 57/43)

Orange solid; yield 35%; m.p. = 257-258°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.92 (d, *J* = 7.3 Hz,

0.57H), 9.78 (d, *J* = 7.3 Hz, 0.43H), 9.08–8.98 (m, 2H), 8.61–8.56 (m, 1H), 7.78–7.67 (m, 2H), 4.60–4.49 (m, 2H), 4.01 (d, *J* = 4.2 Hz, 3H), 1.58–1.49 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.71, 177.81, 174.25, 173.55, 164.54, 163.00, 162.53, 154.32, 154.08, 149.54, 149.24, 138.72, 138.48, 135.60, 134.57, 130.95, 130.41, 129.19, 129.09, 128.79, 128.39, 128.10, 127.72, 127.33, 123.70, 123.40, 123.34, 116.78, 61.59, 53.04, 14.30. HR-ESI-MS: Calcd for $C_{20}H_{14}N_2O_6$ $[M+Na]^+$: 401.0750, found: 401.0749.

4.5.8. Diethyl 5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6,8-dicarboxylate (**3g**) and diethyl 5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-9,11-dicarboxylate (**3g'**) mixture (**3g**/**3g'** = 59/41)

Orange solid; yield 35%; m.p. = 218-219°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.91 (d, *J* = 7.2 Hz, 0.59H), 9.77 (d, *J* = 7.3 Hz, 0.41H), 9.01 (d, *J* = 23.1 Hz, 2H), 8.57 (d, *J* = 7.8 Hz, 1H), 7.79–7.65 (m, 2H), 4.59–4.51 (m, 2H), 4.51–4.43 (m, 2H), 1.53 (t, *J* = 7.1 Hz, 3H), 1.46 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.70, 177.79, 174.20, 173.52, 164.03, 162.95, 162.45, 154.31, 154.06, 149.54, 149.24, 138.74, 138.50, 135.57, 134.52, 130.93, 130.40, 129.57, 129.48, 128.80, 128.42, 128.05, 127.66, 127.30, 123.68, 123.26, 123.16, 122.51, 116.83, 109.63, 109.12, 62.20, 61.62, 61.54, 14.27. HR-ESI-MS: Calcd for $C_{21}H_{16}N_2O_6$ $[M+Na]^+$: 415.0906, found: 415.0904.

4.5.9. Ethyl 8-amino-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylate (**3h**) and ethyl 9-amino-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxylate (**3h'**) mixture (**3h**/**3h'** = 84/16)

Purple solid; yield 36%; m.p. = 275-276°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (d, *J* = 7.5 Hz, 0.84H), 9.39 (d, *J* = 7.6 Hz, 0.16H), 8.96–8.87 (m, 1H), 8.44–8.33 (m, 1H), 7.80–7.70 (m, 1H), 7.24–7.18 (m, 1H), 6.87 (dd, *J* = 7.5, 2.4 Hz, 1H), 6.82 (s, 2H), 4.32 (q, *J* = 7.1 Hz, 2H), 1.37 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-D6) δ 179.78, 170.73, 163.51, 154.02, 150.90, 150.04, 143.63, 134.79, 130.67, 129.85, 128.97, 127.11, 122.34, 111.24, 100.37, 96.46, 60.27, 14.73. HR-ESI-MS: Calcd for $C_{18}H_{13}N_3O_4$ $[M+H]^+$: 336.0984, found: 336.0974.

4.5.10. Methyl 5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylate (**3i**)

Orange solid; yield 30%; m.p. = 235-236°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.95 (d, *J* = 6.9 Hz, 1H), 9.04 (d, *J* = 3.9 Hz, 1H), 8.56 (d, *J* = 7.7 Hz, 1H), 8.38 (d, *J* = 9.0 Hz, 1H), 7.72–7.59 (m, 1H), 7.52 (t, *J* = 7.9 Hz, 1H), 7.30–7.22 (m, 1H), 4.05 (s, 3H). Lit. [19]

4.5.11. Methyl 5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxylate (**3i'**)

Orange solid; yield 11%; m.p. = 255-256°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.79 (d, *J* = 7.0 Hz, 1H), 9.01 (dd, *J* = 4.5, 1.4 Hz, 1H), 8.56 (dd, *J* = 7.8, 1.4 Hz, 1H), 8.38 (d, *J* = 9.1 Hz, 1H), 7.67 (dd, *J* = 7.8, 4.6 Hz, 1H), 7.54–7.42 (m, 1H), 7.23 (t, *J* = 6.8 Hz, 1H), 4.04 (s, 3H). Lit. [19]

4.5.12. Ethyl 7-fluoro-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylate (**3j**) &

ethyl 10-fluoro-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxylate (**3j'**) mixture (**3j**/**3j'** = 45/55)

Orange solid; yield 28%; m.p. = 220-221°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.64 (d, *J* = 6.4 Hz, 0.44H), 9.50 (d, *J* = 6.2 Hz, 0.55H), 9.04 (d, *J* = 9.2 Hz, 1H), 8.64–8.52 (m, 1H), 7.79–7.64 (m, 1H), 7.20–7.05 (m, 1H), 4.63–4.46 (m, 2H), 1.58–1.42 (m, 3H). Lit. [21]

4.5.13. 6-benzylindolizino[2,3-*g*]quinoline-5,12-dione (**6e**)

Red solid; yield 23%; m.p. = 170-171°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.81 (d, *J* = 7.1 Hz, 1H), 9.00 (dd, *J* = 4.6, 1.5 Hz, 1H), 8.50 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.65 (d, *J* = 9.0 Hz, 1H), 7.58 (dd, *J* = 7.8, 4.7 Hz, 1H), 7.31 (d, *J* = 7.3 Hz, 2H), 7.28–7.21 (m, 3H), 7.17 (t, *J* = 7.2 Hz, 1H), 7.11 (t, *J* = 6.8 Hz, 1H), 4.59 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.13, 171.02, 154.03, 151.25, 139.80, 138.03, 134.87, 130.71, 128.71, 128.60, 128.38, 126.41, 126.27, 125.81, 125.72, 121.31, 119.31, 118.44, 117.77, 29.73. HR-ESI-MS: Calcd for $C_{22}H_{14}N_2O_2$ $[M+H]^+$: 339.1134, found: 339.1127.

4.5.14. 11-benzylindolizino[3,2-*g*]quinoline-5,12-dione (**6e'**)

Red solid; yield 15%; m.p. = 201-202°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.64 (d, *J* = 6.9 Hz, 1H), 8.94 (d, *J* = 3.7 Hz, 1H), 8.55 (d, *J* = 7.4 Hz, 1H), 7.68 (d, *J* = 9.0 Hz, 1H), 7.63 (dd, *J* = 7.7, 4.6 Hz, 1H), 7.39 (d, *J* = 7.5 Hz, 2H), 7.28–7.20 (m, 3H), 7.15 (t, *J* = 7.3 Hz, 1H), 7.08 (t, *J* = 6.8 Hz, 1H), 4.60 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.27, 171.38, 154.91, 152.88, 149.63, 139.95, 138.03, 134.83, 134.45, 132.20, 128.66, 128.54, 128.31, 127.67, 127.19, 126.37, 126.23, 125.59, 120.05, 119.56, 119.46, 117.68, 29.72. HR-ESI-MS: Calcd for $C_{22}H_{14}N_2O_2$ $[M+H]^+$: 339.1134, found: 339.1139.

4.5.15. 5,12-Dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylic acid (**4a**)

Red solid; yield 89%; m.p. = 269.5-271.2°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.81 (s, 1H), 9.07 (s, 1H), 8.55 (d, *J* = 6.8 Hz, 2H), 7.81 (d, *J* = 52.2 Hz, 2H), 7.53 (s, 1H). Lit. [29]

4.5.16. 5,12-Dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxylic acid (**4a'**)

Red solid; yield 89%; m.p. = 269.5-271.2°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.44 (s, 1H), 9.68 (s, 1H), 9.01 (s, 1H), 8.51 (s, 2H), 7.89 (s, 1H), 7.71 (s, 1H), 7.50 (s, 1H). Lit. [29]

4.5.17. 8-(Methylthio)-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylic acid (**4b**)

Blue solid; yield 89%; m.p. = 295-296°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 13.54 (s, 1H), 9.71 (d, *J* = 7.3 Hz, 1H), 9.11 (d, *J* = 4.6 Hz, 1H), 8.61 (d, *J* = 7.7 Hz, 2H), 7.71 (dd, *J* = 7.6, 4.9 Hz, 1H), 7.17 (d, *J* = 8.2 Hz, 1H), 2.67 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 185.45, 171.94, 162.61, 155.72, 150.00, 146.08, 141.97, 135.64, 129.32, 127.04, 125.59, 122.22, 118.80, 113.95, 104.21, 14.59. HR-ESI-MS: Calcd for $C_{17}H_{10}N_2O_4S$ $[M+Na]^+$: 361.0259, found: 361.0264. The structure was confirmed through H-H COSY and HMBC.

4.5.18. 9-(Methylthio)-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxylic acid (**4b'**)

Purple solid; yield 89%; m.p. = 302-303°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 13.63 (s, 1H), 9.60 (d, *J* = 7.3 Hz, 1H), 9.06 (dd, *J* = 4.6, 1.6 Hz, 1H), 8.63 (d, *J* = 1.8 Hz, 1H), 8.59 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.77 (dd, *J* = 7.8, 4.6 Hz, 1H), 7.16 (dd, *J* = 7.3, 2.1 Hz, 1H), 2.67 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 184.40, 172.59, 162.63, 153.88, 148.29, 145.93, 142.06, 134.63, 131.40, 128.70, 126.69, 126.22, 121.21, 118.83, 114.05, 104.84, 14.58. HR-ESI-MS: Calcd for $C_{17}H_{10}N_2O_4S$ $[M+Na]^+$: 361.0259, found: 361.0264. The structure was confirmed through H-H COSY and HMBC.

4.5.19. 8-Methoxy-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylic acid (**4c**)

Red solid; yield 86%; m.p. >320°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 13.53 (s, 1H), 9.77 (d, *J* = 7.6 Hz, 1H), 9.10 (dd, *J* = 4.7, 1.7 Hz, 1H), 8.60 (dd, *J* = 7.9, 1.7 Hz, 1H), 8.28 (d, *J* = 2.8 Hz, 1H), 7.69 (dd, *J* = 7.9, 4.7 Hz, 1H), 7.03 (dd, *J* = 7.6, 2.7 Hz, 1H), 4.04 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 185.46, 171.74, 162.89, 161.29, 155.64, 150.03, 144.64, 135.58, 129.63, 129.23, 126.94, 125.95, 121.95, 114.20, 104.12, 99.80, 56.38. HR-ESI-MS: Calcd for $C_{17}H_{10}N_2O_5$ $[M+H]^+$: 323.0668, found: 323.0652.

4.5.20. 9-Methoxy-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxylic acid (**4c'**)

Red solid; yield 87%; m.p. >320°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 13.62 (s, 1H), 9.66 (d, *J* = 7.6 Hz, 1H), 9.04 (dd, *J* = 4.6, 1.7 Hz, 1H), 8.59 (dd, *J* = 7.9, 1.6 Hz, 1H), 8.29 (d, *J* = 2.7 Hz, 1H), 7.76 (dd, *J* = 8.0, 4.7 Hz, 1H), 7.03–6.99 (m, 1H), 4.04 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 184.42, 172.33, 162.90, 161.17, 153.77, 148.26, 144.72, 134.55, 131.37, 129.26, 128.64, 126.59, 114.23, 104.76, 99.91, 56.40. HR-ESI-MS: Calcd for $C_{17}H_{10}N_2O_5$ $[M+H]^+$: 323.0668, found: 323.0665.

4.5.21. 8-Methyl-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylic acid (**4d**)

Red solid; yield 89%; m.p. >320°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 13.58 (s, 1H), 9.81 (d, *J* = 6.9 Hz, 1H), 9.11 (d, *J* = 4.1 Hz, 1H), 8.77 (s, 1H), 8.62 (d, *J* = 7.5 Hz, 1H), 7.71 (dd, *J* = 7.6, 4.6 Hz, 1H), 7.21 (d, *J* = 7.0 Hz, 1H), 2.57 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 185.51, 171.97, 162.53, 155.75, 150.06, 142.48, 142.13, 135.70, 129.33, 127.80, 127.05, 125.48, 122.30, 122.01, 121.24, 105.19, 21.97. HR-ESI-MS: Calcd for $C_{17}H_{10}N_2O_4$ $[M+H]^+$: 307.0719, found: 307.0726.

4.5.22. 9-Methyl-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxylic acid (**4d'**)

Red solid; yield 89%; m.p. >320°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 13.67 (s, 1H), 9.71 (d, *J* = 7.4 Hz, 1H), 9.06 (d, *J* = 3.2 Hz, 1H), 8.79 (s, 1H), 8.61 (d, *J* = 7.0 Hz, 1H), 7.77 (dd, *J* = 7.5, 5.1 Hz, 1H), 7.20 (d, *J* = 7.6 Hz, 1H), 2.56 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 184.47, 172.62, 162.55, 153.89, 148.31, 142.30, 134.68, 131.48, 128.70, 127.47, 126.12, 122.25, 121.42, 121.00, 105.85, 21.94. HR-ESI-MS: Calcd for $C_{17}H_{10}N_2O_4$ $[M+H]^+$: 307.0719, found: 307.0723.

4.5.23. 8-Iodo-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxylic acid (**4e**)

Red solid; yield 89%; m.p. >320°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 13.53 (s, 1H), 9.64 (dd, *J* = 7.2,

0.7 Hz, 1H), 9.46 (d, *J* = 1.0 Hz, 1H), 9.14 (dd, *J* = 4.7, 1.6 Hz, 1H), 8.64 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.74 (dd, *J* = 7.9, 4.7 Hz, 1H), 7.61 (dd, *J* = 7.3, 1.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 185.22, 172.38, 161.96, 155.95, 149.82, 141.71, 135.84, 131.55, 129.40, 128.64, 128.03, 127.33, 125.21, 122.38, 121.59, 121.20, 105.39, 96.51. HR-ESI-MS: Calcd for $C_{16}H_7N_2O_4I$ $[M+H]^+$: 418.9529, found: 418.9529.

4.5.24. 9-Iodo-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxylic acid (**4e'**)

Red solid; yield 89%; m.p. >320°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.42 (d, *J* = 7.2 Hz, 1H), 9.05 (dd, *J* = 4.6, 1.6 Hz, 1H), 8.87 (s, 1H), 8.53 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.92 (dd, *J* = 7.8, 4.7 Hz, 1H), 7.76 (d, *J* = 6.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.61, 173.75, 163.20, 154.06, 149.12, 140.38, 134.60, 131.10, 129.55, 128.94, 128.54, 127.69, 127.42, 122.02, 97.32. HR-ESI-MS: Calcd for $C_{16}H_7N_2O_4I$ $[M+H]^+$: 418.9529, found: 418.9530.

4.5.25. 5,12-Dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6,8-dicarboxylic acid (**4f**) & 5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-9,11-dicarboxylic acid (**4f'**)

Red solid; yield 78%; m.p. >320°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.69 (d, *J* = 7.5 Hz, 0.24H), 9.63 (d, *J* = 7.2 Hz, 0.76H), 9.07–8.99 (m, 1H), 8.89 (s, 1H), 8.53 (d, *J* = 7.7 Hz, 1H), 7.93–7.79 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 181.99, 173.54, 163.30, 155.03, 153.90, 149.94, 149.18, 140.28, 135.50, 134.61, 131.36, 128.88, 127.88, 127.72, 127.34, 121.87, 121.26, 119.94. HR-ESI-MS: Calcd for $C_{17}H_8N_2O_6$ $[M-H]^-$: 335.0304, found: 335.0298.

4.5.26. *N*-(2-(dimethylamino)ethyl)-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxamide (**5a**)

Red solid; yield 86%; m.p. = 169-170°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.89 (t, *J* = 5.0 Hz, 1H), 9.77 (d, *J* = 6.9 Hz, 1H), 9.01 (d, *J* = 4.3 Hz, 1H), 8.76 (d, *J* = 9.1 Hz, 1H), 8.43 (d, *J* = 7.7 Hz, 1H), 7.80 (dd, *J* = 7.7, 4.7 Hz, 1H), 7.68–7.52 (m, 1H), 7.44 (t, *J* = 6.8 Hz, 1H), 3.55 (dd, *J* = 11.8, 5.9 Hz, 2H), 2.73 (s, 2H), 2.42 (s, 6H). Lit. [29]

4.5.27. *N*-(2-(dimethylamino)ethyl)-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxamide (**5a'**)

Red solid; yield 86%; m.p. = 169-170°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.35 (s, 1H), 9.86 (d, *J* = 7.0 Hz, 1H), 9.10 (d, *J* = 9.2 Hz, 1H), 9.01 (d, *J* = 4.5 Hz, 1H), 8.57 (d, *J* = 7.8 Hz, 1H), 7.72 (dd, *J* = 7.7, 4.7 Hz, 1H), 7.59–7.45 (m, 1H), 7.25 (d, *J* = 6.8 Hz, 1H), 3.73 (dd, *J* = 12.4, 6.3 Hz, 2H), 2.81 (t, *J* = 6.7 Hz, 2H), 2.45 (s, 6H). Lit. [29]

4.5.28. *N*-(2-(dimethylamino)ethyl)-8-(methylthio)-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxamide (**5b**)

Purple solid; yield 88%; m.p. = 241-242°C; ^{1}H NMR (400 MHz, MeOD): δ = 9.39 (d, *J* = 6.4 Hz, 1H), 8.95 (s, 1H), 8.21 (s, 1H), 7.93 (d, *J* = 7.9 Hz, 1H), 7.59–7.50 (m, 1H), 7.25 (d, *J* = 4.4 Hz, 1H),

3.94 (s, 2H), 3.68–3.62 (m, 2H), 3.22 (s, 6H), 2.65 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ = 181.00, 170.67, 162.70, 154.67, 148.41, 146.00, 140.60, 134.54, 128.71, 127.61, 126.62, 124.54, 120.71, 118.42, 114.15, 107.48, 58.01, 43.12, 34.25, 13.04. HR-ESI-MS: Calcd for $C_{21}H_{20}N_4O_3S$ $[M+H]^+$: 409.1334, found: 409.1328.

4.5.29. *N*-(2-(dimethylamino)ethyl)-9-(methylthio)-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxamide (**5b'**)

Purple solid; yield 88%; m.p. = 247-248°C; ^{1}H NMR (400 MHz, MeOD): δ = 9.06 (d, *J* = 7.3 Hz, 1H), 8.90 (d, *J* = 3.3 Hz, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 7.81 (s, 1H), 7.76–7.67 (m, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 3.89 (s, 2H), 3.67–3.61 (m, 2H), 3.25 (s, 6H), 2.50 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ = 181.25, 170.82, 163.16, 153.38, 147.20, 145.02, 140.18, 133.90, 129.65, 128.82, 125.93, 123.77, 120.14, 118.08, 113.31, 105.87, 58.60, 43.04, 34.19, 13.00. HR-ESI-MS: Calcd for $C_{21}H_{20}N_4O_3S$ $[M+H]^+$: 409.1334, found: 409.1336.

4.6. Synthesis of compound 6a,a'-6c,c''

4a or **4a'** (1.0 mmol) was dissolved in DCM and HBTU (1.5 mmol), DIPEA (1.5 mmol) were added to the solution. The mixture was stirred at room temperature for 1h. Then different anilines (1.5 mmol) were added and the reaction mixture was stirred for 5h. The solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography to give the target compounds **6a-c** and **6a'-c'**.

4.6.1. 5,12-Dioxo-*N*-phenyl-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxamide (**6a**)

Red solid; yield 85%; m.p. >320°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.35 (s, 1H), 10.06 (d, *J* = 7.7 Hz, 1H), 9.25 (d, *J* = 8.7 Hz, 1H), 9.09 (d, *J* = 5.0 Hz, 1H), 8.66 (d, *J* = 7.7 Hz, 1H), 7.93 (d, *J* = 8.5 Hz, 2H), 7.73–7.68 (m, 1H), 7.56 (t, *J* = 8.4 Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 2H), 7.33 (t, *J* = 6.7 Hz, 1H), 7.17 (t, *J* = 7.5 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 184.07, 172.55, 160.82, 155.16, 149.80, 141.66, 138.80, 135.75, 130.17, 129.05, 128.99, 128.28, 126.98, 124.39, 124.11, 123.70, 122.50, 120.51, 119.30, 110.96. HR-ESI-MS: Calcd for $C_{22}H_{13}N_3O_3$ $[M+Na]^+$: 390.0855, found: 390.0854.

4.6.2. 5,12-Dioxo-*N*-phenyl-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxamide (**6a'**)

Red solid; yield 86%; m.p. >320°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.43 (s, 1H), 9.93 (d, *J* = 5.9 Hz, 1H), 9.25 (d, *J* = 8.9 Hz, 1H), 9.06 (d, *J* = 4.5 Hz, 1H), 8.61 (d, *J* = 8.7 Hz, 1H), 7.98 (d, *J* = 8.0 Hz, 2H), 7.77–7.71 (m, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.39 (t, *J* = 7.1 Hz, 2H), 7.33–7.27 (m, 1H), 7.15 (t, *J* = 7.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 183.13, 173.08, 160.70, 153.82, 148.93, 141.74, 138.83, 134.56, 131.00, 128.96, 128.84, 128.20, 127.94, 124.93, 124.04, 123.92, 120.48, 119.25, 111.67. HR-ESI-MS: Calcd for $C_{22}H_{13}N_3O_3$ $[M+Na]^+$: 390.0855, found: 390.0848.

4.6.3. 5,12-Dioxo-*N*-(o-tolyl)-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxamide (**6b**)

Red solid; yield 90%; m.p. = 250-251°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.73 (s, 1H), 10.00 (d, *J*

= 7.1 Hz, 1H), 9.17 (d, *J* = 8.9 Hz, 1H), 9.05 (d, *J* = 3.3 Hz, 1H), 8.58 (d, *J* = 7.8 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 1H), 7.65 (dd, *J* = 7.5, 4.5 Hz, 1H), 7.51 (t, *J* = 7.4 Hz, 1H), 7.31–7.22 (m, 3H), 7.13 (t, *J* = 7.4 Hz, 1H), 2.52 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 183.53, 172.46, 161.09, 155.02, 149.76, 141.76, 136.30, 135.66, 130.64, 130.08, 128.91, 128.15, 126.88, 126.39, 125.18, 124.52, 124.37, 123.78, 122.37, 119.20, 110.54, 18.67. HR-ESI-MS: Calcd for $C_{23}H_{15}N_3O_3$ $[M+Na]^+$: 404.1011, found: 404.1015.

4.6.4. 5,12-Dioxo-*N*-(o-tolyl)-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxamide (**6b'**)

Red solid; yield 86%; m.p. = 242-243°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.75 (s, 1H), 9.88 (d, *J* = 6.9 Hz, 1H), 9.18 (d, *J* = 9.2 Hz, 1H), 9.02 (d, *J* = 4.8 Hz, 1H), 8.57 (d, *J* = 7.8 Hz, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 7.69 (dd, *J* = 7.8, 4.7 Hz, 1H), 7.49 (t, *J* = 7.1 Hz, 1H), 7.30–7.22 (m, 3H), 7.12 (t, *J* = 7.4 Hz, 1H), 2.58 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.55, 172.99, 161.07, 153.78, 148.89, 141.85, 136.23, 134.47, 130.97, 130.82, 130.62, 128.76, 128.04, 127.84, 126.23, 125.15, 124.36, 123.99, 121.28, 119.13, 111.21, 18.90. HR-ESI-MS: Calcd for $C_{23}H_{15}N_3O_3$ $[M+Na]^+$: 404.1011, found: 404.1011.

4.6.5. *N*-(3-chlorophenyl)-5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxamide (**6c**)

Red solid; yield 83%; m.p. >320°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.45 (s, 1H), 10.07 (d, *J* = 7.4 Hz, 1H), 9.23 (d, *J* = 9.1 Hz, 1H), 9.11 (dd, *J* = 4.9, 1.6 Hz, 1H), 8.67 (dd, *J* = 7.7, 1.9 Hz, 1H), 8.06 (t, *J* = 1.8 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.72 (dd, *J* = 8.1, 4.6 Hz, 1H), 7.61–7.55 (m, 1H), 7.37–7.31 (m, 2H), 7.14 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 184.21, 172.59, 160.91, 155.26, 149.74, 141.68, 140.03, 135.78, 134.68, 130.11, 129.99, 129.22, 128.33, 127.05, 124.36, 124.05, 123.56, 122.58, 120.43, 119.38, 118.36, 110.44. HR-ESI-MS: Calcd for $C_{22}H_{12}N_3O_3Cl$ $[M+Na]^+$: 424.0465, found: 424.0464.

4.6.6. *N*-(3-chlorophenyl)-5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxamide (**6c'**)

Red solid; yield 81%; m.p. >320°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.56 (s, 1H), 9.95 (d, *J* = 7.0 Hz, 1H), 9.24 (d, *J* = 9.5 Hz, 1H), 9.08 (dd, *J* = 4.5, 1.7 Hz, 1H), 8.63 (dd, *J* = 7.8, 1.7 Hz, 1H), 8.13 (t, *J* = 2.1 Hz, 1H), 7.86 (d, *J* = 8.1 Hz, 1H), 7.77 (dd, *J* = 7.8, 4.6 Hz, 1H), 7.60–7.54 (m, 1H), 7.35–7.28 (m, 2H), 7.12 (d, *J* = 7.4 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 183.29, 173.14, 160.81, 153.90, 148.85, 141.77, 140.02, 134.68, 134.61, 130.97, 129.92, 129.10, 128.33, 128.01, 124.91, 124.01, 123.78, 121.51, 120.36, 119.36, 118.33, 111.16. HR-ESI-MS: Calcd for $C_{22}H_{12}N_3O_3Cl$ $[M+Na]^+$: 424.0465, found: 424.0464.

4.7. Synthesis of compound 6d and 6d'

4a or **4a'** (1.0 mmol) was dissolved in DCM and HBTU (1.5 mmol), DIPEA (1.5 mmol) were added to the solution. The mixture was stirred at room temperature for 1h. Then ethyl-m-aminobenzoate (1.5 mmol) was added and the reaction mixture was stirred for 5h. The

solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography to give the target compound **8b** and **8b'**. The ethyl ester group of **8b** and **8b'** was hydrolyzed using KOH in MeOH/H_2O. When the hydrolysis was complete, the reaction mixture was filtrated and washed with CH_2Cl_2 and H_2O, followed by recrystallization in methanol to obtain **6d** and **6d'**.

4.7.1. Ethyl 3-(5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxamido)benzoate (**8b**)

Red solid; yield 90%; m.p. >320°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.44 (s, 1H), 10.00 (d, *J* = 7.0 Hz, 1H), 9.18 (d, *J* = 9.2 Hz, 1H), 9.08 (dd, *J* = 4.5, 1.3 Hz, 1H), 8.64 (dd, *J* = 7.9, 1.3 Hz, 1H), 8.43 (s, 1H), 8.19 (d, *J* = 8.5 Hz, 1H), 7.83 (d, *J* = 7.7 Hz, 1H), 7.69 (dd, *J* = 7.9, 4.7 Hz, 1H), 7.54 (t, *J* = 7.6 Hz, 1H), 7.47 (t, *J* = 7.9 Hz, 1H), 7.31 (t, *J* = 6.6 Hz, 1H), 4.44 (q, *J* = 7.1 Hz, 2H), 1.45 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 184.02, 172.55, 166.48, 160.91, 155.18, 149.70, 141.61, 139.01, 135.81, 131.38, 130.11, 129.12, 129.06, 128.26, 127.04, 125.09, 124.76, 124.33, 123.57, 122.52, 121.29, 119.33, 110.44, 61.14, 14.44. HR-ESI-MS: Calcd for $C_{25}H_{17}N_3O_5$ $[M+Na]^+$: 462.4319, found: 462.4322.

4.7.2. Ethyl 3-(5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxamido)benzoate (**8b'**)

Red solid; yield 91%; m.p. >320°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.46 (s, 1H), 9.90 (d, *J* = 7.0 Hz, 1H), 9.21 (d, *J* = 9.2 Hz, 1H), 9.05 (dd, *J* = 4.6, 1.6 Hz, 1H), 8.60 (dd, *J* = 7.8, 1.6 Hz, 1H), 8.53 (s, 1H), 8.19 (d, *J* = 8.2 Hz, 1H), 7.83 (d, *J* = 7.7 Hz, 1H), 7.74 (dd, *J* = 7.8, 4.6 Hz, 1H), 7.53 (t, *J* = 7.4 Hz, 1H), 7.45 (t, *J* = 7.9 Hz, 1H), 7.30 (d, *J* = 6.9 Hz, 1H), 4.44 (q, *J* = 7.1 Hz, 2H), 1.45 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 183.16, 173.11, 166.46, 160.94, 153.84, 148.89, 141.80, 138.91, 134.63, 131.46, 131.00, 129.04, 128.92, 128.30, 127.99, 125.31, 124.94, 123.88, 121.47, 121.31, 119.33, 111.19, 61.08, 14.48. HR-ESI-MS: Calcd for $C_{25}H_{17}N_3O_5$ $[M+Na]^+$: 462.4319, found: 462.4324.

4.7.3. Potassium 3-(5,12-dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carboxamido)benzoate (**6d**)

Red solid; yield 82%; m.p. >320°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.87 (s, 1H), 9.88 (d, *J* = 6.8 Hz, 1H), 9.06 (d, *J* = 4.1 Hz, 1H), 8.87 (d, *J* = 7.6 Hz, 1H), 8.70 (d, *J* = 7.9 Hz, 1H), 8.07 (s, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 7.89–7.82 (m, 1H), 7.69 (t, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.51 (t, *J* = 6.8 Hz, 1H), 7.28 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 184.03, 172.89, 168.24, 160.74, 155.05, 149.78, 143.27, 140.40, 138.21, 135.96, 130.78, 129.59, 128.22, 127.93, 125.10, 122.77, 122.48, 121.00, 119.96, 119.78, 115.05, 110.11. HR-ESI-MS: Calcd for $C_{23}H_{12}N_3O_5K$ $[M-K]^-$: 410.0777, found: 410.0785.

4.7.4. Potassium 3-(5,12-dioxo-5,12-dihydroindolizino[3,2-*g*]quinoline-11-carboxamido)benzoate (**6d'**)

Red solid; yield 80%; m.p. >320°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.86 (s, 1H), 9.89 (d, *J* =

7.1 Hz, 1H), 9.06 (d, *J* = 5.2 Hz, 1H), 8.88 (d, *J* = 8.9 Hz, 1H), 8.70 (d, *J* = 7.7 Hz, 1H), 8.06 (s, 1H), 8.02 (d, *J* = 8.1 Hz, 1H), 7.86 (dd, *J* = 7.9, 4.7 Hz, 1H), 7.69 (t, *J* = 8.3 Hz, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.51 (t, *J* = 6.8 Hz, 1H), 7.27 (t, *J* = 7.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 184.01, 172.87, 168.22, 160.71, 155.03, 149.76, 143.29, 140.37, 138.18, 135.93, 130.76, 127.90, 125.07, 122.75, 122.45, 120.98, 119.91, 119.77, 110.09. HR-ESI-MS: Calcd for $C_{23}H_{12}N_3O_5K$ $[M-K]^-$: 410.0777, found: 410.0774.

4.8. Synthesis of compound 5c

A mixture of **4a** (1.0 mmol) and *N*,*N*'-carbonyldiimidazole (2.0 mmol) in DMF was stirred at room temperature for 1h. The reaction mixture was heated at 70°C for 40 minutes. Thiosemicarbazide (3.0 mmol) was added and the reaction mixture was stirred at 70°C for another 30 minutes. The solvent was evaporated under reduced pressure. 1M HCl was added and the solid was filtrated and washed with water to give the target compound **5c**. Lit. [18]

4.8.1. 2-(5,12-Dioxo-5,12-dihydroindolizino[2,3-*g*]quinoline-6-carbonyl)hydrazine-1-carbothioamide (**5c**)

Red solid; yield 55%; m.p. = 270-271°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.19 (s, 1H), 9.86 (d, *J* = 6.9 Hz, 1H), 9.65 (s, 1H), 9.10 (d, *J* = 4.6 Hz, 1H), 8.58 (d, *J* = 7.9 Hz, 1H), 8.51 (s, 1H), 8.22 (s, 1H), 7.89 (dd, *J* = 7.8, 4.6 Hz, 1H), 7.83 (s, 1H), 7.72 (t, *J* = 7.3 Hz, 1H), 7.53 (t, *J* = 6.9 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 183.14, 182.85, 172.66, 162.08, 155.10, 150.19, 149.80, 139.76, 135.66, 130.58, 129.64, 128.29, 127.98, 125.68, 122.46, 121.48, 119.68, 107.11. HR-ESI-MS: Calcd for $C_{17}H_{11}N_5O_3S$ $[M-H]^-$: 364.0504, found: 364.0496.

4.9. IDO1 inhibition assay

The assays were performed at room temperature as described in the literature using 20 nM IDO 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'-formlylkynurenine. The IC_{50} values were calculated using nonlinear regression with normalized dose-response fit using Prism GraphPad [20].

4.10. TDO inhibition assay

The assay was performed by UV absorption using recombinant TDO and L-Tryptophan as substrate. The UV absorption signal at 321 nm is correlated with the amount of N-formylkynurenine reaction product of TDO. All reactions were conducted at room temperature. The 100 µL reaction mixture in TDO Assay Buffer contained 50 mM TDO, the indicated amount of the inhibitor, 200 µM tryptophan. The reaction mixture was incubated for 75 min prior to reading the UV absorption signal. For the negative control (blank), 5 µL of the assay buffer was added instead of the TDO. The initial reaction rates were recorded by continuously following the

absorbance increase at 321 nm due to the formation of N'-formylkynurenine. The IC_{50} values were calculated using nonlinear regression with normalized dose−response fit using Prism GraphPad [30].

4.11. MTT assay of cell viability

Human cancer cells [human cervical carcinoma cell (HeLa), breast carcinoma cell (MCF-7), and embryonic kidney cells (HEK293T)] were seeded in each well of 96-well culture plate ($3\text{-}4\times10^3$ per well). After overnight incubation, solution of compounds in DMSO (10 mM) was added to the cells. Cells treated with DMSO 1% were used as negative control. After further incubation for 48 h, MTT solution (5 mg/mL in PBS) was added to each well. After incubation for 4 h at 37 °C, MTT solution was removed and 150 μL of DMSO was added to dissolve the crystals formed. Then, absorbance at 570 nm was read using a microplate reader. The IC_{50} values were calculated using Prism 5.0.

4.12. Cellular inhibition assay

HeLa Cells were seeded on 96-well plates (40,000 cells per well) and allowed to adhere for 24 hours. A final concentration of 100 ng/mL of human recombinant IFN-γ was added to the cells to stimulate IDO1 expression. After 24 hours, cells were then treated with IDO inhibitor in DMSO at a top dose of 10 μM (3-fold dilution all the way down to 0.1μM). Treated cells were then incubated for 24 hours. At the end of the incubation, reactions were terminated by the addition of 30% TCA to each well. Plate were incubated for 30 minutes at 50°C. Cells were centrifuged 10 minutes at 4200 rpm. 100 μL of supernatants were transferred to new 96-well plates and mixed with Ehrlich reagent. The resulting solution was incubated 10 minutes at room temperature. Absorbance at 490 nm was read and the results were calculated using Prism GraphPad.

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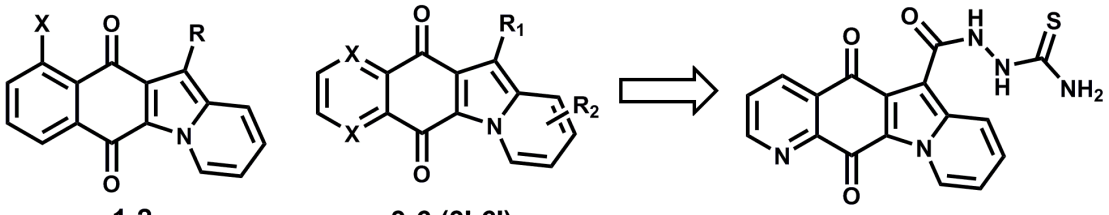
Highlights

- 48 naphthoindolizine and indolizinoquinoline-5,12-dione derivatives were synthesized.
- These compounds showed significant IDO1 inhibition and high selectivity over TDO.
- The most potent **5c** (IDO1 IC_{50} 23 nM) is a low cytotoxic, promising lead compound.



48 naphthoquinone derivatives

5c IDO1 IC_{50} = 23 nM
TDO IC_{50}=1200 nM
CC_{50}>20 μM