Journal Pre-proof

Discovery of the programmed cell death-1/programmed cell death-ligand 1 interaction inhibitors bearing an indoline scaffold

Mingze Qin, Qi Cao, Xia Wu, Chunyang Liu, Shuaishuai Zheng, Hongbo Xie, Ye Tian, Jun Xie, Yanfang Zhao, Yunlei Hou, Xian Zhang, Boxuan Xu, Haotian Zhang, Xiaobo Wang

PII: S0223-5234(19)31008-6

DOI: https://doi.org/10.1016/j.ejmech.2019.111856

Reference: EJMECH 111856

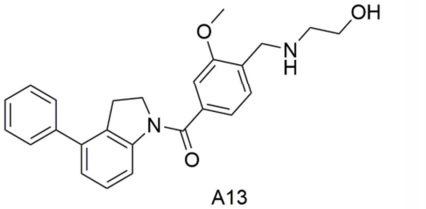
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 30 August 2019

Revised Date: 2 November 2019

Accepted Date: 4 November 2019

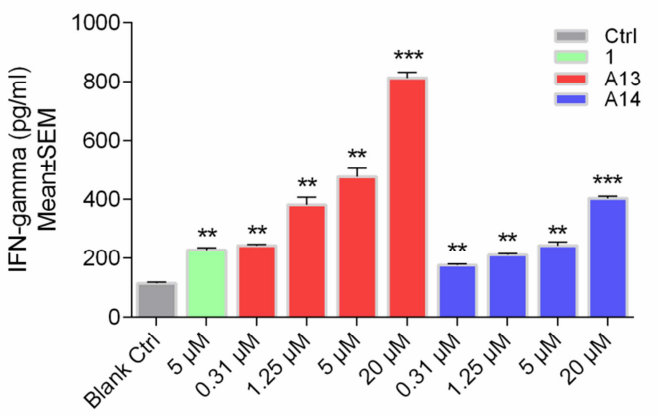
Please cite this article as: M. Qin, Q. Cao, X. Wu, C. Liu, S. Zheng, H. Xie, Y. Tian, J. Xie, Y. Zhao, Y. Hou, X. Zhang, B. Xu, H. Zhang, X. Wang, Discovery of the programmed cell death-1/programmed cell death-ligand 1 interaction inhibitors bearing an indoline scaffold, *European Journal of Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.ejmech.2019.111856.



A13

PD-1/PD-L1 IC_{50} = 132.8 nM

Toxicity to Jurkat T cells EC_{50} = 44.7 μM



Discovery of the Programmed Cell Death-1/Programmed Cell Death-Ligand 1 Interaction Inhibitors Bearing an Indoline Scaffold

Mingze Qin [a, d, *], Qi Cao [a], Xia Wu [a], Chunyang Liu [a], Shuaishuai Zheng [a], Hongbo Xie [c], Ye Tian [a], Jun Xie [b], Yanfang Zhao [a], Yunlei Hou [a], Xian Zhang [a], Boxuan Xu [a], Haotian Zhang [b, ***], Xiaobo Wang [d, **]

[a] *Key Laboratory of Structure-Based Drug Design and Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, PR China*

[b] *Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang 110016, PR China*

[c] *College of Bioinformatics Science and Technology, Harbin Medical University, Harbin 150086, PR China*

[d] *Chinese People's Liberation Army Logistics Support Force No. 967 Hospital, Dalian 116021, PR China*

[*]Corresponding author.

[**]Corresponding author.

[***]Corresponding author.

E-mail address: qinmingze001@126.com (Mingze Qin); wxbbenson0653@sina.com (Xiaobo Wang); zhanghaotian2087@163.com (Haotian Zhang)

ABSTRACT

Inhibiting the programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) pathway is an attractive strategy for tumor immunotherapy. Here, a novel series of indoline-containing compounds were developed, among which, **A13** was identified as the most promising PD-1/PD-L1 pathway inhibitor. At the biochemical level, **A13** demonstrated strong inhibition of the PD-1/PD-L1 interaction, with an IC_{50} of 132.8 nM. Notably, it exhibited outstanding immunoregulatory activity, and significantly elevated interferon-γ secretion in a Hep3B/OS-8/hPD-L1 and CD3 T cell co-culture model, without significant toxic effect. Therefore, **A13** could be employed as a suitable lead compound for further design of non-peptide inhibitors targeting the PD-1/PD-L1 interaction. In addition, the preliminary structure-activity relationships of these new indoline compounds were investigated in this study, providing valuable information for future drug development.

Key words: tumor immunotherapy; PD-1/PD-L1 interaction inhibitor; low toxicity; IFN-γ secretion.

1. Introduction

Immunotherapy is an emerging therapeutic strategy in tumor treatment [1,2]. The process of immune surveillance is modulated by several co-stimulatory molecules, as well as co-inhibitory molecules, whose activation leads to immunosuppressive effects such as T-cell proliferation and cytokine secretion downregulation [3,4]. Under physiological conditions, this negative feedback regulation of immune response plays a critical role in maintaining self-tolerance and protecting against autoimmunity [5].

Programmed cell death-1 (PD-1) is a well-documented immune checkpoint protein expressed on T cells, B cells, natural killer cells, and dendritic cells; its cognate ligand PD-L1 is highly expressed in several types of malignancies, such as non-small cell lung cancer (NSCLC), renal cancer, and melanoma [6–10]. Thus, the abnormal activation of the PD-1/PD-L1 pathway results in tumor-specific immune cells exhaustion [11,12].

Humanized monoclonal antibodies (mAbs) which target the PD-1/PD-L1 axis have exhibited durable antitumor responses in clinical application, and six of them have been approved for tumor treatment by the U.S. Food and Drug Administration (FDA). Additionally, combinations of these anti-PD-1/PD-L1 mAbs with other antitumor agents such as chemotherapeutics, targeted drugs, and antibodies targeting other immune checkpoints, have been extensively investigated [13–16]. The FDA recently approved the combination of pembrolizumab with pemetrexed and carboplatin as first-line therapy for metastatic NSCLC patients, providing a valuable therapeutic regimen [17]. Despite the successful application of anti-PD-1/PD-L1

mAbs, their intrinsic disadvantages such as severe immune-related adverse effects and poor drug diffusion prompted the development of small-molecule inhibitors [18].

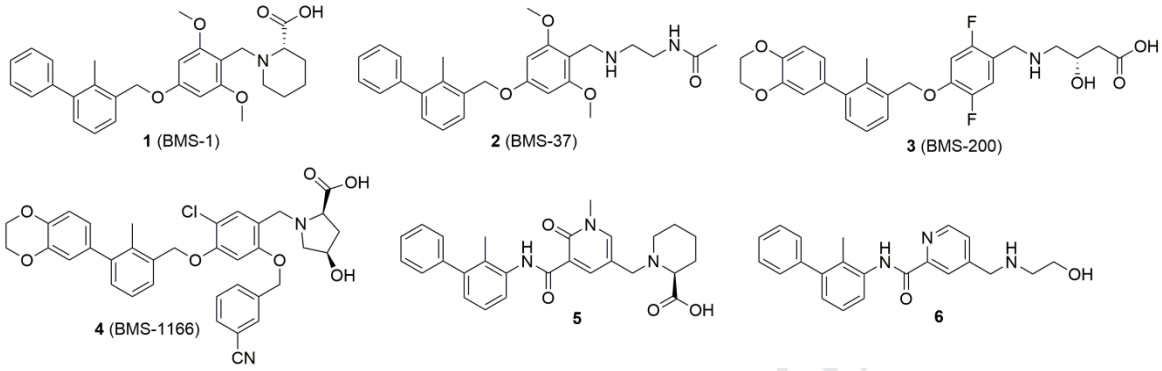


Figure 1. Chemical structures of representative non-peptide inhibitors of the PD-1/PD-L1 interaction.

Recently, Bristol-Myers Squibb (BMS) investigators developed a series of non-peptide inhibitors with a tricyclic scaffold such as compounds **1**, **2**, and **3** (Fig. 1) [19,20]. It has been reported by the Holak group that compound **2** induces PD-L1 dimerization, and thus inhibits the PD-1/PD-L1 interaction [21]. The structural characterization of **3** revealed that its 2,3-dihydro-1,4-benzodioxinyl group induced a conformational change involving the Tyr56 of one PD-L1 protein, forming an enlarged hydrophobic tunnel for inhibitor binding [21]. However, inhibitor **3** failed to antagonize the repression of PD-1/PD-L1 pathway on T cell activation in the cellular assay [22]. Compound **4**, which contained an additional 3-cyanobenzyl moiety, was a more promising inhibitor. It displayed a high level of biochemical and cellular activity, and further investigation on it is awaited. In addition to the BMS inhibitors, Incyte Corporation researchers developed several compounds characterized by an amide linkage group such as **5** and **6**, which potently inhibited the PD-1/PD-L1 interaction at

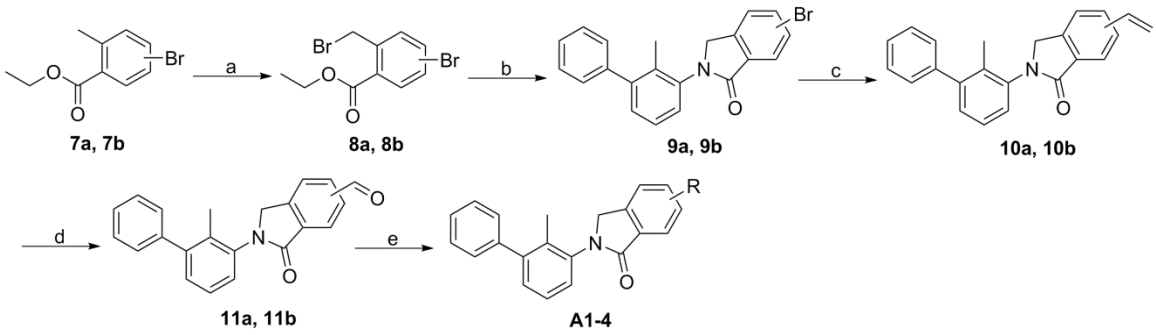
the biochemical level [23,24]. However, their effects on immunity in response to tumor need to be further explored in experimental models. Notably, more comprehensive investigations addressing the toxic, pharmacokinetic, and pharmacodynamic properties of these reported inhibitors are necessary. In addition, the identification of novel inhibitors, especially those with verified activity on immune activation is urgently needed.

In this paper, the discovery of a novel series of indoline-containing inhibitors targeting the PD-1/PD-L1 interaction is reported. Particularly, compound **A13** displayed outstanding inhibition of the PD-1/PD-L1 interaction and restoration of the immune response repression, making it a promising lead compound for future drug development.

2. Results and discussion

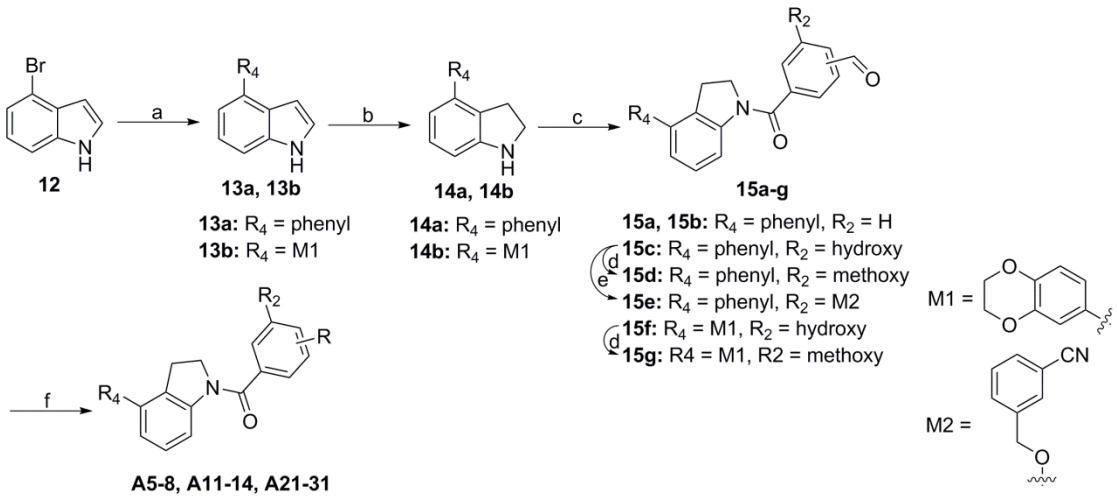
2.1. Chemistry

The synthesis of compounds **A1–4** is shown in Scheme 1. The key intermediates, **9a** and **9b**, which contain an isoindolin-1-one scaffold, were prepared through the free radical-mediated bromination of the starting reagents **7a** and **7b**, followed by cyclization with 2-methyl-[1,1'-biphenyl]-3-amine [25,26]. The Suzuki–Miyaura coupling of **9a** and **9b** with 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane yielded **10a** and **10b**, which were oxidized by $NaIO_4$ to form the aldehydes **11a** and **11b**, respectively [23]. Through the $NaBH_3CN$-mediated reductive amination reaction, **11a** and **11b** were converted to the desired compounds, **A1–4** [27].



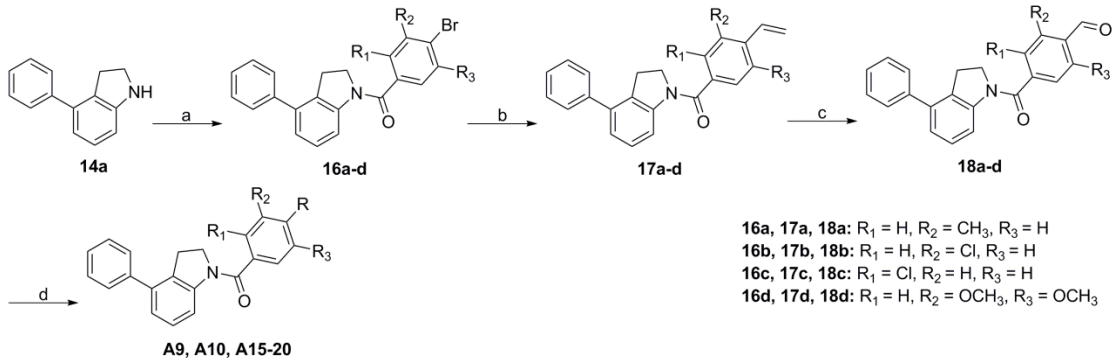
Scheme 1. Reagents and conditions: (a) NBS, BPO, CCl_4, reflux, 10 h, 67–71%; (b) 2-methyl-[1,1'-biphenyl]-3-amine, HOAc, 135 °C, 12 h, 73–75%; (c) 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/H_2O (4/1), 60 °C, 9 h, 54–61%; (d) OsO_4, $NaIO_4$, 1,4-dioxane/H_2O (5/1), rt, 2 h, 90–93%; (e) appropriate amine, HOAc, $NaBH_3CN$, CH_3OH/CH_2Cl_2 (1/1), rt, 7–22 h, 7–16%.

The preparation of compounds **A5–8**, **A11–14**, and **A21–31** is shown in Scheme 2. The classical Suzuki–Miyaura coupling of **12** with phenylboronic acid or 2-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane yielded **13a** and **13b**, which were then reduced by $NaBH_3CN$ to generate **14a** and **14b**, respectively. The amidation of **14a** and **14b** with appropriate carboxylic acids readily afforded **15a–c** and **15f**, and the *O*-alkylation of the intermediates **15c** and **15f**, which contain a hydroxyl group, provided good yields of the intermediates **15d**, **15e**, and **15g [**24,28]. The desired compounds **A5–8**, **A11–14**, and **A21–31** were then produced by reacting **15a–g** with appropriate amines in the presence of $NaBH_3CN$.



Scheme 2. Reagents and conditions: (a) phenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane/H_2O (4/1), 60 °C, 10 h, 73%, or 2-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/H_2O (2/1), 100 °C, 2 h, 70%; (b) $NaBH_3CN$, HOAc, rt, 2 h, 73%–80%; (c) appropriate benzoic acids, HATU, DIPEA, DMF, rt, 3 h, 81%–86%, or 4-formyl-3-hydroxybenzoic acid, EDCI, CH_2Cl_2, rt, 3 h, 72%–81%; (d) CH_3I, K_2CO_3, DMF, rt, 2 h, 76%–87%; (e) 3-(bromomethyl)benzonitrile, Cs_2CO_3, DMF, 75 °C, 2 h, 74%; (f) appropriate amine, HOAc, $NaBH_3CN$, CH_3OH/CH_2Cl_2 (1/1) or DMF, rt, 8–20 h, 3–21%.

Scheme 3 shows the synthetic route of compounds **A9**, **A10**, and **A15–20**. The amides **16a–d** were generated via a method similar to that used to synthesize **15a**, by reacting 4-phenylindoline (**14a**) with appropriate carboxylic acids. Subsequently, the intermediates **16a–d** were converted to the desired compounds **A9**, **A10**, and **A15–20**, through a three-step reaction including the Suzuki–Miyaura coupling, olefin oxidization, and reductive amination with appropriate amines.



Scheme 3. Reagents and conditions: (a) appropriate benzoic acids, HATU, DIPEA, DMF, rt, 3 h, 78%–87%; (b) 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane, $PdCl_2$(dppf), K_2CO_3, 1,4-dioxane/H_2O (4/1), 100 °C, 7 h, 36%–45%, or 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane, $Pd_2(dba)_3$, dicyclohexyl(2',6'-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine, K_3PO_4, toluene/EtOH/H_2O (3/3/1), 90 °C, 5 h, 53%. (c) OsO_4, $NaIO_4$, 1,4-dioxane/H_2O (5/1), rt, 2 h, 82–91%; (d) appropriate amine, HOAc, $NaBH_3CN$, CH_3OH/CH_2Cl_2 (1/1), rt, 10–19 h, 5–25%.

2.2. Bioactivity and discussion

*2.2.1. Discovery of the indoline-containing compounds **A5–8***

The Holak group has elucidated the crystal structures of PD-L1 complexed with BMS inhibitors, facilitating the development of inhibitors with a similar mechanism of action [21,22,29]. Accordingly, a pharmacophoric model for non-peptide PD-1/PD-L1 pathway inhibitors was established in our previous study [30]. Regarding this model, hydrophobic interactions such as the π-π interactions formed between the “Core group” and “Aryl group” with PD-L1 residues significantly contributed to inhibitor binding. A tail group with the ability to form hydrogen bonds

with PD-L1 was also required for high activity. Thus, the modification of the linkage group was considered a feasible way to discover novel inhibitors. A ring fusion strategy was utilized by taking compound **1** as the lead compound (Fig. 2). Accordingly, different scaffolds, including isoindolin-1-one (mode a) and indoline (mode b), were developed in the initial study. Based on them, compounds **A1–8**, which have an aminoethanol or *N*-(2-aminoethyl)acetamide tail group incorporated at different positions of the phenyl ring, were synthesized as proof-of-concept compounds.

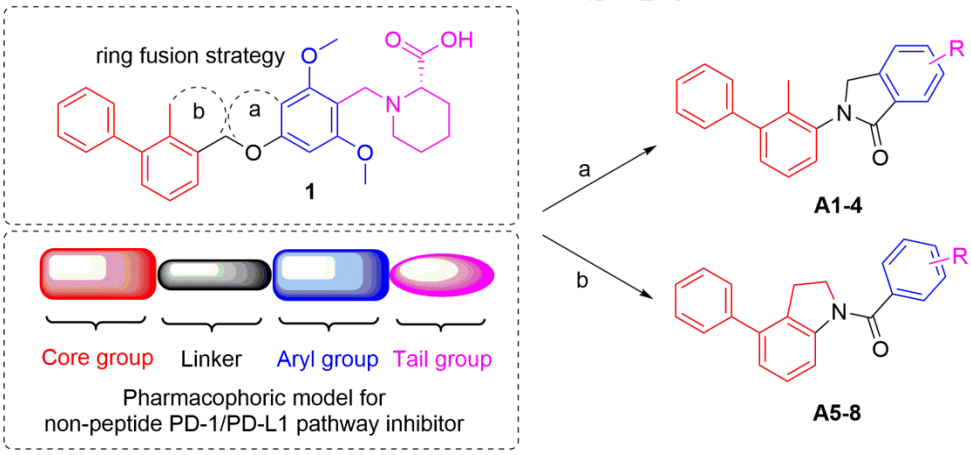
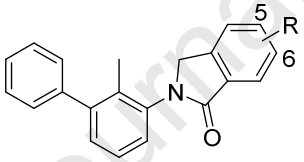


Figure 2. Design strategy of compounds **A1–8**.

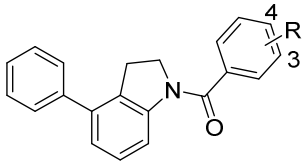
The inhibitory activities of compounds **A1–8** against the PD-1/PD-L1 interaction were evaluated using the well-established homogenous time-resolved fluorescence (HTRF) assay. The biochemical data presented as IC_{50} values are shown in Table 1. In general, the isoindolin-1-one derivatives **A1–4**, displayed unfavorable activity in inhibition of the PD-1/PD-L1 interaction. Among them, compounds **A1** (IC_{50}, 9378 nM) and **A3** (IC_{50}, 7286 nM) exhibited weak activity, and **A2** and **A4** were almost inactive at up to 10 μM concentration. Contrarily, promising activity was exhibited by

all the indoline-based compounds, **A5–8**, and their preliminary structure-activity relationships (SARs) were determined. Compound **A7**, which bears an aminoethanol tail group at the 4-position of the phenyl ring, exhibited strong activity, with an IC_{50} of 191.6 nM. Shifting its tail group from the 4- to 3-position yielded **A5** (IC_{50}, 344 nM), which showed significantly reduced efficacy. Loss of activity could be observed by comparing **A6** (IC_{50}, 744.9 nM) with **A8** (IC_{50}, 622.5 nM). These results clearly indicated that the indoline could serve as a suitable scaffold for developing PD-1/PD-L1 interaction inhibitors, and a hydrophilic tail group incorporated at the 4-position of the phenyl ring was more desirable.

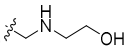
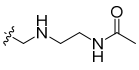
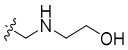
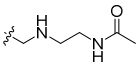
Table 1. Activities of compounds A1–8 in inhibition of the PD-1/PD-L1 interaction.

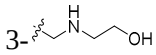
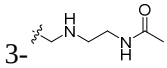
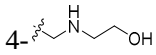
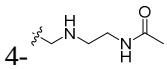


A1-4



A5-8

Compound	R	PD-1/PD-L1 IC_{50} (nM)[a]
A1	6- 	9378 ± 465.7
A2	6- 	>10000
A3	5- 	7286 ± 397.5
A4	5- 	>10000

A5		344 ± 35.8
A6		744.9 ± 56.3
A7		191.6 ± 10.2
A8		622.5 ± 29.5

[a] The data are generated from two independent experiments.

2.2.2. Structural optimization of the indoline precursors

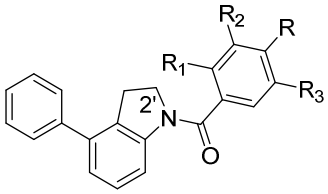
Based on these new indoline precursors, a detailed structural optimization campaign was performed to further explore their SARs and improve their potency. BMS inhibitor **1** was used as the positive control. It exhibited high level of activity in inhibition of the PD-1/PD-L1 interaction, with an IC_{50} of 91.8 nM (Table 2).

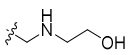
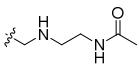
First, compounds **A9–20**, which have a modified phenyl ring, were synthesized and evaluated to explore the SARs of this region. It was observed that the introduction of neither an electron-donating, nor electron-withdrawing group, led to a significant effect on inhibitory activity against the PD-1/PD-L1 interaction. Compound **A13** (IC_{50}, 132.8 nM), which contains a methoxy group, displayed slightly more potent activity as compared with **A7** (IC_{50}, 191.6 nM), while compounds **A9** (IC_{50}, 212.3 nM), **A11** (IC_{50}, 304.4 nM), and **A15** (IC_{50}, 353.1 nM), with a methyl, hydroxyl, and chlorine group, respectively, were slightly less active, indicating that the electron density of the phenyl ring was not a determinant of inhibitory activity at the biochemical level. Shifting the chlorine group from the R_2- (**A15**) to R_1-position (**A17**, IC_{50}, >10000 nM) dramatically reduced the activity. The new R_1 substituent

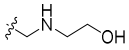
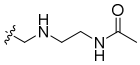
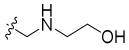
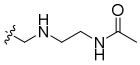
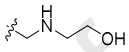
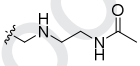
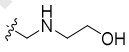
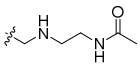
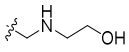
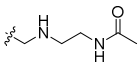
was probably sterically repulsive to the hydrogen atom at the 2'-position of indoline moiety, leading to the formation of an unfavorable molecular conformation. These results suggested that further modification of this region was inappropriate. Among these compounds, **A13**, which has a methoxy group, exhibited the most promising activity. Surprisingly, with an additional methoxy group at the R_3-position, **A19**, showed remarkably decreased activity (IC_{50}, 4766 nM).

Compound **A14** (IC_{50}, 219.7 nM), which contains a *N*-(2-aminoethyl)acetamide group, was less active than **A13**, which has an aminoethanol tail group. Decrease in activity was also observed when **A10** (IC_{50}, 457.1 nM), **A12** (IC_{50}, 601.5 nM), and **A16** (IC_{50}, 589 nM) were compared with **A9** (IC_{50}, 212.3 nM), **A11** (IC_{50}, 304.4 nM), and **A15** (IC_{50}, 353.1 nM), respectively, indicating that the aminoethanol fragment might be one of the preferred moieties for the tail group region.

Table 2. Activities of compounds A9–20 in inhibition of the PD-1/PD-L1 interaction.



Compound	R_1	R_2	R_3	R	PD-1/PD-L1 IC_{50} (nM)[a]
A9	H	CH_3	H		212.3 ± 16.2
A10	H	CH_3	H		457.1 ± 18.9

A11	H	OH	H		304.4 ± 27
A12	H	OH	H		601.5 ± 30.2
A13	H	OCH_3	H		132.8 ± 6.9
A14	H	OCH_3	H		219.7 ± 7.6
A15	H	Cl	H		353.1 ± 39.3
A16	H	Cl	H		589 ± 29.6
A17	Cl	H	H		>10000
A18	Cl	H	H		>10000
A19	H	OCH_3	OCH_3		4766 ± 212.3
A20	H	OCH_3	OCH_3		7258 ± 297.5
1					91.8 ± 8.6

[a] The data are generated from two independent experiments.

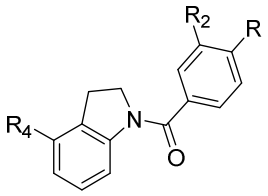
With the preliminary SARs determined, compounds **A21–25** were then prepared to explore the effects of the tail group on their PD-1/PD-L1 interaction inhibitory activity (Table 3). The methylation of the hydroxyl group of **A13** decreased the ability of the compound to inhibit the PD-1/PD-L1 interaction (**A21**, IC_{50}, 349.7 nM). Compound **A22**, which has a hydroxymethyl group attached to the pyrrolidine moiety, showed a 4.67-fold decrease in activity as compared with **A13**, suggesting that the orientation of the tail group played a crucial role on inhibitor activity.

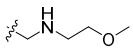
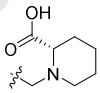
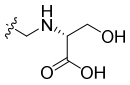
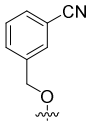
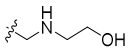
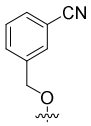
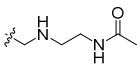
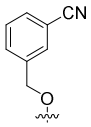
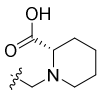
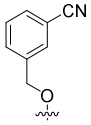
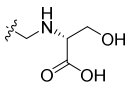
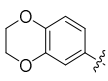
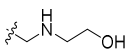
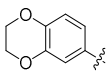
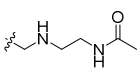
Compound **A25** (IC_{50}, 277.6 nM), which bears a serine tail group, exhibited favorable activity, but was still less potent than **A13**. Disappointingly, compounds **A23** (IC_{50}, 1887 nM) and **A24** (IC_{50}, 1059 nM), which have a proline and pipecolinic acid moiety, respectively, exhibited sharply reduced efficacy.

Based on previous reports on compound **4**, the introduction of the 3-cyanobenzyl moiety resulted in additional π-stacking and hydrogen bond interactions with the dimeric PD-L1, creating an improved ligand binding [22]. Compounds **A26–29** were prepared to examine whether the 3-cyanobenzyl group was also tolerated by the newly synthesized indoline-based compounds. The results revealed that all these compounds displayed significantly decreased activity (IC_{50} values: 572.1–3281 nM) as compared to their parent compounds (**A13**, **A14**, **A24**, and **A25**).

It has been proven that the 2,3-dihydro-1,4-benzodioxinyl group is desirable as terminal moiety in BMS inhibitors and their structural analogues, which induced the conformational change of Tyr56 to form an enlarged hydrophobic tunnel [21]. In this new chemical series, compounds **A30** (IC_{50}, 225.2 nM) and **A31** (IC_{50}, 415.9 nM), which contain a 2,3-dihydro-1,4-benzodioxinyl group, also exhibited attractive activity against the PD-1/PD-L1 interaction, but were less potent than the phenyl compounds **A13** and **A14**, respectively.

Table 3. Activities of compounds A21–31 in inhibition of the PD-1/PD-L1 interaction.



Compound	R_4	R_2	R	PD-1/PD-L1 IC_{50} (nM)[a]
A21	Ph	OCH_3		349.7 ± 17.5
A22	Ph	OCH_3		619.9 ± 47
A23	Ph	OCH_3		1887 ± 132.2
A24	Ph	OCH_3		1059 ± 26.5
A25	Ph	OCH_3		277.6 ± 19.7
A26	Ph			572.1 ± 29
A27	Ph			1064 ± 85.5
A28	Ph			1330 ± 36.7
A29	Ph			3281 ± 226.5
A30		OCH_3		225.2 ± 25.6
A31		OCH_3		415.9 ± 11.5

1	91.8 ± 8.6

[a] The data are generated from two independent experiments.

2.2.3. Unspecific toxicity of selected inhibitors

The immunoregulatory activity of the PD-1/PD-L1 interaction inhibitors is strongly affected by their toxicity. Thus, compounds **A7**, **A9**, **A13**, **A14**, and **A30**, which showed promising activity at the biochemical level, were evaluated for toxicity, using the CCK-8 assay. Jurkat T cells which were proven to be favorable surrogates of primary T cells in the analysis of immune toxicity, were treated with the indicated concentrations of the tested compounds for 72 h. The results demonstrated that compounds **A13** and **A14** displayed lower toxicity as compared with **A7**, **A9**, and **A30**, with EC_{50} values of 44.7 and 39.8 μM, respectively (Table S1).

2.2.4. Immunoregulatory activity based on IFN-γ secretion

Combining inhibitor activity and toxicity, **A13** and **A14** were further assessed for ability to restore the immunity repressed by the PD-1/PD-L1 pathway activation. Hep3B cells, which were engineered to stably express OS-8 (anti CD-3 single-chain variable fragment) and human PD-L1, were co-cultured with primary CD3 T cells. The indicated concentrations of **A13** and **A14** were added, and interferon-γ (IFN-γ) levels were determined after 72 h of co-culture. As shown in Figure 3, both compounds **A13** and **A14** promoted IFN-γ secretion in a dose-dependent manner. Particularly, outstanding activity was exhibited by **A13**, which significantly promoted IFN-γ secretion even at the lowest concentration. Upon treatment with 5 μM **A13**, the IFN-γ level was increased by 316.59%, which was 3.21-fold more remarkable than

that induced by **1** (98.69%). At a 20 μM concentration, the IFN-γ level was increased by 609.61%.

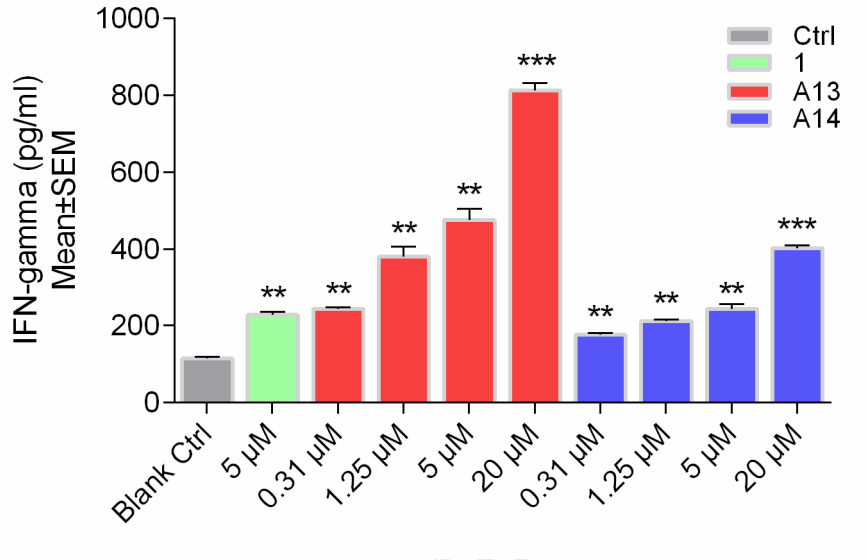


Figure 3. Effects of selected compounds on IFN-γ production in a Hep3B/OS-8/hPD-L1 and CD3 T cell co-culture model. Data are shown as mean ± SEM, *p<0.05, **p<0.01, *** p<0.001 vs control group.

*2.2.5. Docking analysis of **A13** with the dimeric PD-L1*

To elucidate the binding mode of these indoline-based inhibitors, the docking analysis of **A13** with the dimeric PD-L1 was conducted. The **2**/PD-L1 complex (PDB: 5N2D) was employed as the template. As shown in Figure 4A, **A13** matched well to the hydrophobic cleft formed by the PD-L1 dimer. For the distal phenyl and methoxy-phenyl moieties, $\pi-\pi$ interactions with the Tyr56 of each monomer, were formed, which provided strong stabilization for inhibitor binding. Other interactions such as $\pi-$alkyl and $\pi-$anion interactions with Met115 and Asp122 also contributed to this binding mode. The aminoethanol fragment interacted with Asp122 and Lys124

via hydrogen bonds. Notably, the hydrogen bond formed between the hydroxyl group and Asp122 indicated that the hydrogen bond donor at the tail group is necessary for high activity. This observation accounted for the decrease in activity as comparing **A21** with **A13**. The indoline moiety of **A13** created hydrophobic interactions with Met115 and Ala121, similar with those in the methyl-phenyl group of **2**. Additionally, the indoline moiety interacted with the Tyr123 of PD-L1, which might help to stabilize the conformation of the inhibitor. Figure 4B demonstrates that compounds **A13** and **2** were well superimposed.

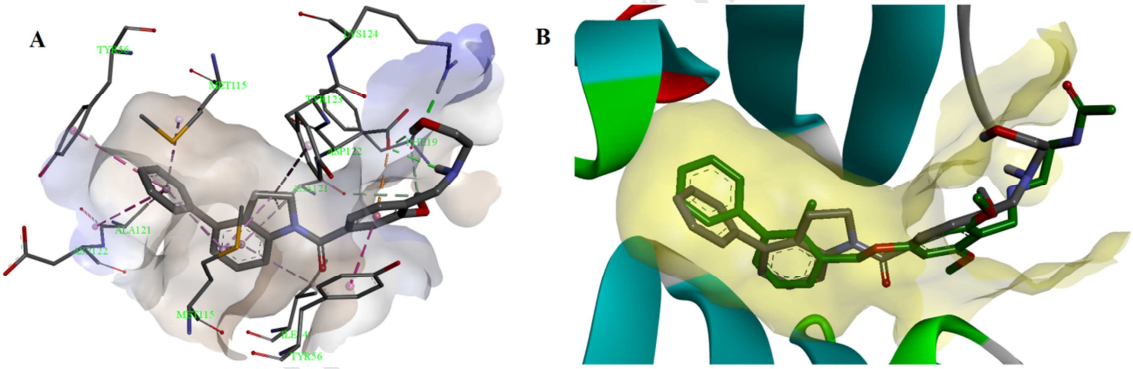


Figure 4. (A) Docking analysis of **A13** in the hydrophobic cleft formed by the dimeric PD-L1. (B) Binding overlap of **A13** and **2** in the binding site. The crystal structure of the dimeric PD-L1 protein was taken from the RCSB Protein Data Bank (PDB: 5N2D).

3. Conclusion

Inhibiting the PD-1/PD-L1 interaction using non-peptide small-molecules is of great potential in tumor treatment, but none of them has entered into clinical trials yet. The inhibitors discovered by BMS investigators provide a favorable starting point for the identification of inhibitors with a similar mode of action. Inhibitors with potent

activity, but containing a novel skeleton are eagerly needed, considering that they may possess distinct pharmacokinetic and pharmacodynamic profiles from the reported compounds. In this study, a series of novel indoline derivatives were developed from **1**, using a ring fusion strategy. Most of them potently inhibited the PD-1/PD-L1 interaction, with IC_{50} values at the nanomolar level, clearly indicating that indoline is a suitable scaffold for designing an inhibitor. Among these compounds, **A13** displayed the strongest activity, with an IC_{50} of 132.8 nM. Furthermore, in a Hep3B/OS-8/hPD-L1 and CD3 T cells co-culture model, it significantly increased IFN-γ secretion in a dose-dependent manner. Additionally, preliminary SAR studies were performed on these indoline-based compounds, paving the way for further development of their structural analogues.

In this study, **A13** was slightly less active than the BMS inhibitor **1** in inhibition of the PD-1/PD-L1 interaction in the HTRF assay, but showed significantly more potent immunoregulatory activity in a T cell-tumor co-culture model, highlighting their potential for further investigations. These observations might be partially ascribed to its low toxicity, which was experimentally addressed using Jurkat T cells.

The future studies should focus on structural optimization of **A13**. Most especially, it is necessary to identify its analogues possessing higher affinity with the dimeric PD-L1, and thus, an improved immunoregulatory activity can be expected. For the tail group, detailed structural modifications should be made when reserving a hydrogen bond donor. Physicochemical properties such as solubility can also be tuned by modifying this fragment. In addition, further investigation on the distal phenyl and

methoxy-phenyl moieties is desirable, diverse heterocyclic fragments may also be well tolerated in each part of the inhibitor.

In conclusion, this study identified the indoline-based compound **A13**, as a promising lead compound in the designing of the PD-1/PD-L1 interaction inhibitors, facilitating future drug design.

4. Experimental procedures

4.1. Chemistry

Reagents and solvents were obtained from commercial sources and used without further purification. All the reactions were monitored by TLC using silica gel GF/UV 254. Flash chromatography was performed using silica gel (300–400 mesh). Melting points were determined on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer, with TMS as an internal standard. The low resolution of ESI-MS was recorded on an Agilent 1100 LC-MS spectrometer, and high-resolution mass spectrometry was performed on an Agilent Accurate-Mass Q-TOF 6530 in ESI mode. The purities of **A1–31** were determined by HPLC, which were determined to be higher than 95%. The HPLC analysis was performed on an Agilent 1260 machine with a C18 reverse-phase column (4.6 mm × 250 mm, 5 μm), the column temperature was 25 °C. Mobile phase A (100% methanol) and mobile phase B (NaH_2PO_4 and H_3PO_4 buffer solution, pH = 2.5) were used in a gradient elution program (0 min, phase A: 5%, phase B: 95%; 25 min, phase A: 95%, phase B: 5%) with a flow rate of 1.0 mL/min. UV Detection was conducted at 230 nm.

*4.1.1. General procedure for preparation of intermediates **8a** and **8b***

*4.1.1.1. Ethyl 5-bromo-2-(bromomethyl)benzoate (**8a**).* To a solution of ethyl 5-bromo-2-methylbenzoate (**7a**, 10.53 g, 43.51 mmol) and BPO (0.21 g, 0.87 mmol) in CCl_4 (80 mL) was added NBS (8.08 g, 45.67 mmol) in portions. The mixture was stirred under reflux for 10 h. When TLC showed the completion of the reaction, the precipitate was filtered off and the filtrate was evaporated to give a residue, which was purified by column chromatography (petroleum ether) to afford compound **8a** (9.87 g, 70.9%). It was obtained as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.98 (d, J = 2.2 Hz, 1H), 7.82 (dd, J = 8.2, 2.2 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 4.98 (s, 2H), 4.34 (t, J = 7.1 Hz, 2H), 1.35 (m, 3H). ESI-MS m/z: 320.9 $[M+H]^+$.

*4.1.1.2. Ethyl 4-bromo-2-(bromomethyl)benzoate (**8b**).* Compound **8b** was prepared from intermediate ethyl 4-bromo-2-methylbenzoate (**7b**) in a similar manner as described for compound **8a**. It was obtained as a white solid. Yield, 67%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.88 (s, 1H), 7.81 (d, J = 8.3 Hz, 1H), 7.69 (d, J = 8.3 Hz, 1H), 4.99 (s, 2H), 4.34 (q, J = 6.7 Hz, 2H), 1.34 (t, J = 6.7 Hz, 3H). ESI-MS m/z: 320.9 $[M+H]^+$.

*4.1.2. General procedure for preparation of intermediates **9a** and **9b***

*4.1.2.1. 6-Bromo-2-(2-methyl-[1,1'-biphenyl]-3-yl)isoindolin-1-one (**9a**).* A mixture of intermediate **8a** (6.39 g, 19.97 mmol) and 2-methyl-[1,1'-biphenyl]-3-amine (2.81 g, 15.35 mmol) in acetic acid (30 mL) was stirred at 135 °C for 12 h. When TLC showed the completion of the reaction, the reaction mixture was cooled to room temperature and poured into water. The precipitate was filtered off to give a residue,

which was triturated with ether to afford compound **9a** (4.24 g, 73.3%). It was obtained as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.93 (d, *J* = 1.7 Hz, 1H), 7.87 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.66 (d, *J* = 8.1 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 2H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.42–7.38 (m, 2H), 7.37–7.35 (m, 2H), 7.28 (d, *J* = 7.5 Hz, 1H), 4.88 (s, 2H), 2.02 (s, 3H). ESI-MS m/z: 378.0 $[M+H]^+$.

*4.1.2.2. 5-Bromo-2-(2-methyl-[1,1'-biphenyl]-3-yl)isoindolin-1-one (**9b**).* Compound **9b** was prepared from intermediate **8b** in a similar manner as described for compound **9a**. It was obtained as a white solid. Yield, 75%. ^{1}H NMR (600 MHz, DMSO-***d*$_6$) δ 7.95 (s, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 8.1 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 2H), 7.44 (d, *J* = 7.8 Hz, 1H), 7.42–7.35 (m, 4H), 7.28 (d, *J* = 7.5 Hz, 1H), 4.90 (s, 2H), 2.02 (s, 3H). ESI-MS m/z: 378.1 $[M+H]^+$.

*4.1.3. General procedure for preparation of intermediates **10a** and **10b***

*4.1.3.1. 2-(2-Methyl-[1,1'-biphenyl]-3-yl)-6-vinylisoindolin-1-one (**10a**).* A mixture of intermediate **9a** (3.26 g, 8.65 mmol), 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (2.67 g, 17.32 mmol), $PdCl_2$(dppf) (0.63 g, 0.86 mmol) and K_2CO_3 (2.38 g, 17.26 mmol) in 1,4-dioxane (24 mL) and water (6 mL) was stirred at 60 °C for 9 h under the N_2 atmosphere. The reaction mixture was cooled to room temperature, the catalyst was filtered off. The filtrate was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with brine, and concentrated to give a residue, which was purified by column chromatography (ethyl acetate/petroleum ether, 1/35 to 1/20) to yield **10a** (1.53 g, 54.5%). It was obtained as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.88 (s, 1H), 7.80 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.65 (d, *J* = 7.9 Hz, 1H),

7.51–7.43 (m, 3H), 7.42–7.34 (m, 4H), 7.27 (dd, *J* = 7.6, 1.0 Hz, 1H), 6.90 (dd, *J* = 17.7, 11.0 Hz, 1H), 6.00 (d, *J* = 17.7 Hz, 1H), 5.36 (d, *J* = 11.1 Hz, 1H), 4.89 (s, 2H), 2.03 (s, 3H). ESI-MS m/z: 326.2 $[M+H]^+$.

*4.1.3.2. 2-(2-Methyl-[1,1'-biphenyl]-3-yl)-5-vinylisoindolin-1-one (**10b**).* Compound **10b** was prepared from intermediate **9b** in a similar manner as described for compound **10a**. It was obtained as a white solid. Yield, 61%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.80–7.73 (m, 2H), 7.67 (d, *J* = 7.9 Hz, 1H), 7.51–7.43 (m, 3H), 7.42–7.34 (m, 4H), 7.27 (d, *J* = 7.5 Hz, 1H), 6.90 (dd, *J* = 17.6, 11.0 Hz, 1H), 6.03 (d, *J* = 17.7 Hz, 1H), 5.43 (d, *J* = 11.0 Hz, 1H), 4.89 (s, 2H), 2.03 (s, 3H). ESI-MS m/z: 326.2 $[M+H]^+$.

*4.1.4. General procedure for preparation of intermediates **11a** and **11b***

*4.1.4.1. 2-(2-Methyl-[1,1'-biphenyl]-3-yl)-3-oxoisoindoline-5-carbaldehyde (**11a**).* To a solution of intermediate **10a** (0.6 g, 1.85mmol) in 1,4-dioxane (25 mL) and water (5 mL) was added aqueous solution of osmium tetraoxide (2% w/w in water, 3.5 mL, 0.28 mmol). Stirring was continued at room temperature for 10 min, then sodium periodate (1.58 g, 7.38 mmol) was added. The mixture was stirred at room temperature for another 2 h. Upon the completion of the reaction, the reaction mixture was poured into water. The precipitate was filtered off to give compound **11a** (0.55 g, 90.9%). It was obtained as a gray solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.16 (s, 1H), 8.32 (s, 1H), 8.20 (d, *J* = 9.0 Hz, 1H), 7.90 (d, *J* = 7.9 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 3H), 7.42–7.35 (m, 4H), 7.29 (d, *J* = 7.6 Hz, 1H), 5.03 (s, 2H), 2.04 (s, 3H). ESI-MS m/z: 328.1 $[M+H]^+$.

*4.1.4.2. 2-(2-Methyl-[1,1'-biphenyl]-3-yl)-1-oxoisoindoline-5-carbaldehyde (**11b**).* Compound **11b** was prepared from intermediate **10b** in a similar manner as described for compound **11a**. It was obtained as an off-white solid. Yield, 93%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.20 (s, 1H), 8.22 (s, 1H), 8.11 (d, *J* = 7.7 Hz, 1H), 8.01 (d, *J* = 7.8 Hz, 1H), 7.49 (t, *J* = 7.3 Hz, 3H), 7.39 (m, 4H), 7.30 (d, *J* = 7.5 Hz, 1H), 5.02 (s, 2H), 2.05 (s, 3H). ESI-MS m/z: 328.1 $[M+H]^+$.

*4.1.5. Procedure for preparation of intermediates **13a** and **13b***

*4.1.5.1. 4-Phenyl-1H-indole (**13a**).* A mixture of intermediate **12** (10 g, 51.29 mmol), phenylboronic acid (6.26 g, 51.29 mmol), $Pd(PPh_3)_4$ (1.78g, 1.54 mmol) and K_2CO_3 (21.23 g, 153.94 mmol) in 1,4-dioxane (80 mL) and water (20 mL) was stirred at 60 °C for 10 h under the N_2 atmosphere. After that time, the reaction mixture was cooled to room temperature, the catalyst was filtered off. The filtrate was concentrated, and extracted with CH_2Cl_2. The organic layer was washed with brine, and evaporated to give a residue, which was purified by column chromatography (ethyl acetate/petroleum ether, 1/50 to 1/20) to afford intermediate **13a** (7.25 g, 73.2%). It was obtained as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.27 (s, 1H), 7.67 (d, *J* = 7.3 Hz, 2H), 7.49 (t, *J* = 7.7 Hz, 2H), 7.42 (t, *J* = 5.9 Hz, 2H), 7.37 (t, *J* = 7.4 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.08 (d, *J* = 7.1 Hz, 1H), 6.55 (s, 1H). ESI-MS m/z: 194.1 $[M+H]^+$.

*4.1.5.2. 4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-1H-indole (**13b**).* A mixture of intermediate **12** (1 g, 5.13 mmol), 2-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborol

ane (2.00 g, 7.63 mmol), $PdCl_2$(dppf) (0.37 g, 0.51 mmol) and K_2CO_3 (2.1 g, 15.23 mmol) in 1,4-dioxane (10 mL) and water (5 mL) was stirred at 100 °C for 2 h under the N_2 atmosphere. The catalyst was filtered off, the filtrate was evaporated to give a residue. The residue was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried and evaporated to provide the crude product, which was purified by column chromatography (ethyl acetate/petroleum ether, 1/100 to 1/15) to give intermediate **13b** (0.91 g, 70.7%). It was obtained as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.22 (s, 1H), 7.40–7.33 (m, 2H), 7.12 (dt, *J* = 6.1, 4.6 Hz, 3H), 7.00 (d, *J* = 7.1 Hz, 1H), 6.96 (d, *J* = 8.2 Hz, 1H), 6.51 (s, 1H), 4.29 (s, 4H). ESI-MS m/z: 252.1 $[M+H]^+$.

*4.1.6. General procedure for preparation of intermediates **14a** and **14b***

*4.1.6.1. 4-Phenylindoline (**14a**).* To a solution of intermediate **13a** (1.5 g, 7.77 mmol) in acetic acid (9 mL) was added $NaBH_3CN$ (1.47 g, 23.32 mmol) slowly in an ice bath. The mixture was stirred at room temperature for 2 h. After that time, the reaction mixture was alkalified with aqueous NaOH and extracted with ethyl acetate. The organic layer was washed with brine and concentrated to give a residue, which was purified by column chromatography (ethyl acetate/petroleum ether, 1/60 to 1/15) to give compound **14a** (1.21 g, 79.8%). It was obtained as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.47–7.40 (m, 4H), 7.36–7.29 (m, 1H), 7.00 (t, *J* = 7.7 Hz, 1H), 6.59 (d, *J* = 7.1 Hz, 1H), 6.51 (d, *J* = 7.7 Hz, 1H), 5.59 (s, 1H), 3.39 (t, *J* = 8.4 Hz, 2H), 2.97 (t, *J* = 8.4 Hz, 2H). ESI-MS m/z: 196.1 $[M+H]^+$.

*4.1.6.2. 4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)indoline (**14b**).* Compound **14b** was prepared from intermediate **13b** in a similar manner as described for compound **14a**. It was obtained as a white solid. Yield, 73%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.96 (t, *J* = 7.6 Hz, 1H), 6.91 (s, 1H), 6.89 (t, *J* = 6.4 Hz, 2H), 6.54 (d, *J* = 7.6 Hz, 1H), 6.48 (d, *J* = 7.7 Hz, 1H), 5.68 (s, 1H), 4.26 (s, 4H), 3.37 (t, *J* = 8.3 Hz, 2H), 2.96 (t, *J* = 8.3 Hz, 2H). ESI-MS m/z: 254.1 [M+H]+.

*4.1.7. General procedure for preparation of intermediates **15a–g***

*4.1.7.1. 3-(4-Phenylindoline-1-carbonyl)benzaldehyde (**15a**).* To a solution of intermediate **14a** (1 g, 5.13 mmol), 3-formylbenzoic acid (0.85 g, 5.64 mmol), and HATU (2.92 g, 7.69 mmol) in DMF (15 mL) was added DIPEA (3.31 g, 25.63 mmol). The mixture was stirred at room temperature for 3 h. After that time, the solution was poured into water. The precipitate was filtered off to give a residue, which was triturated with ether to give compound **15a** (1.37 g, 81.7%). It was obtained as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.13 (s, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 7.95 (d, *J* = 7.8 Hz, 1H), 7.75 (t, *J* = 7.6 Hz, 1H), 7.47 (dd, *J* = 7.9, 5.9 Hz, 5H), 7.41–7.36 (m, 1H), 7.32 (s, 1H), 7.13 (s, 1H), 4.05–3.98 (m, 2H), 3.15 (t, *J* = 8.1 Hz, 2H). ESI-MS m/z: 328.1 $[M+H]^+$.

*4.1.7.2. 4-(4-Phenylindoline-1-carbonyl)benzaldehyde (**15b**).* Compound **15b** was prepared from intermediate **14a** in a similar manner as described for compound **15a**, with 4-formylbenzoic acid replace 3-formylbenzoic acid. It was obtained as a white solid. Yield, 86%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.10 (s, 1H), 8.04 (d, *J* = 8.1

Hz, 2H), 7.81 (d, *J* = 7.5 Hz, 2H), 7.52–7.32 (m, 7H), 7.13 (s, 1H), 3.35 (s, 2H), 3.14 (t, *J* = 8.1 Hz, 2H). ESI-MS m/z: 328.1 $[M+H]^+$.

*4.1.7.3. 2-Hydroxy-4-(4-phenylindoline-1-carbonyl)benzaldehyde (**15c**).* To a solution of intermediate **14a** (1.5 g, 7.69 mmol) and 4-formyl-3-hydroxybenzoic acid (1.34 g, 8.07 mmol) in CH_2Cl_2 (20 mL) was added EDCI (1.92 g, 10.00 mmol). The mixture was stirred at room temperature for 3 h when TLC showed the completion of the reaction. The reaction mixture was washed with water and brine. The organic layer was dried and concentrated to give compound **15c** (2.13 g, 80.7%). It was obtained as a yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.04 (s, 1H), 10.34 (s, 1H), 8.14 (s, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.52–7.30 (m, 6H), 7.19–7.03 (m, 3H), 3.97 (s, 2H), 3.15 (t, *J* = 8.0 Hz, 2H). ESI-MS m/z: 344.1 $[M+H]^+$.

*4.1.7.4. 2-Methoxy-4-(4-phenylindoline-1-carbonyl)benzaldehyde (**15d**).* To a solution of intermediate **15c** (0.5 g, 1.46 mmol) and K_2CO_3 (0.6 g, 4.35 mmol) in DMF (10 mL) was added CH_3I (0.62 g, 4.37 mmol) dropwise. The mixture was stirred at room temperature for 2 h. After that time, the mixture was poured into water. The precipitate was filtered off and dried to give compound **15d** (0.45 g, 86.6%). It was obtained as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.41 (s, 1H), 8.17 (s, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.48 (d, *J* = 4.2 Hz, 4H), 7.45 (s, 1H), 7.40 (dt, *J* = 8.6, 4.3 Hz, 2H), 7.27 (d, *J* = 7.3 Hz, 1H), 7.15 (s, 1H), 3.98 (s, 5H), 3.16 (t, *J* = 8.1 Hz, 2H). ESI-MS m/z: 358.1 $[M+H]^+$.

*4.1.7.5. 3-((2-Formyl-5-(4-phenylindoline-1-carbonyl)phenoxy)methyl)benzonitrile (**15e**).* A mixture of intermediate **15c** (0.6 g, 1.75 mmol),

3-(bromomethyl)benzonitrile (0.41 g, 2.1 mmol) and Cs_2CO_3 (1.14 g, 3.5 mmol) in DMF (15 mL) was stirred at 75 °C for 2 h. After that time, the solution was cooled to room temperature and was poured into water. The precipitate was filtered off and dried to give compound **15e** (0.59 g, 73.6%). It was obtained as a yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.48 (s, 1H), 8.15 (s, 1H), 8.04 (s, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.83 (t, *J* = 7.2 Hz, 2H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.52 (s, 1H), 7.47 (d, *J* = 4.3 Hz, 5H), 7.39 (dt, *J* = 8.7, 4.3 Hz, 1H), 7.29 (d, *J* = 6.6 Hz, 1H), 7.14 (s, 1H), 5.42 (s, 2H), 3.87 (s, 2H), 3.13 (t, *J* = 7.9 Hz, 2H). ESI-MS m/z: 459.2 $[M+H]^+$.

4.1.7.6.

*4-(4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)indoline-1-carbonyl)-2-hydroxybenzaldehyde (**15f**).* Compound **15f** was prepared from intermediate **14b** in a similar manner as described for compound **15c**. It was obtained as a yellow solid. Yield, 72%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.03 (s, 1H), 10.33 (s, 1H), 8.07 (s, 1H), 7.75 (d, *J* = 7.9 Hz, 1H), 7.29 (s, 1H), 7.16–7.02 (m, 3H), 6.94 (t, *J* = 2.1 Hz, 1H), 6.92 (d, *J* = 2.0 Hz, 2H), 4.27 (s, 4H), 3.93 (s, 2H), 3.13 (t, *J* = 8.1 Hz, 2H). ESI-MS m/z: 402.1 $[M+H]^+$.

4.1.7.7.

*4-(4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)indoline-1-carbonyl)-2-methoxybenzaldehyde (**15g**).* Compound **15g** was prepared from intermediate **15f** in a similar manner as described for compound **15d**. It was obtained as a white solid. Yield, 76%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.39 (s, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.43 (s, 1H), 7.32 (s, 1H), 7.25 (d, *J* = 6.3 Hz, 1H), 7.19 (s, 1H), 7.07 (s, 1H), 6.94 (d, *J* = 9.3 Hz, 3H), 4.27 (s, 4H), 3.97 (s, 5H), 3.13 (t, *J* = 8.2 Hz, 2H). ESI-MS m/z: 416.1 $[M+H]^+$.

*4.1.8. General procedure for preparation of intermediates **16a–d***

Compounds **16a–d** were prepared in a similar manner as described for compound **15a** by reacting **14a** with appropriate carboxylic acids.

*4.1.8.1. (4-Bromo-3-methylphenyl)(4-phenylindolin-1-yl)methanone (**16a**).* It was obtained as a white solid. Yield, 87%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.71 (d, J = 8.2 Hz, 1H), 7.60 (s, 1H), 7.52–7.18 (m, 8H), 7.11 (d, J = 7.2 Hz, 1H), 3.99 (t, J = 8.0 Hz, 2H), 3.13 (t, J = 8.1 Hz, 2H), 2.41 (s, 3H). ESI-MS m/z: 392.1 $[M+H]^+$.

*4.1.8.2. (4-Bromo-3-chlorophenyl)(4-phenylindolin-1-yl)methanone (**16b**).* It was obtained as a white solid. Yield, 80%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.91 (d, J = 8.2 Hz, 1H), 7.88 (d, J = 1.2 Hz, 1H), 7.51 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 4.4 Hz, 4H), 7.39 (dt, J = 8.7, 4.2 Hz, 1H), 7.33 (s, 1H), 7.13 (d, J = 6.7 Hz, 1H), 4.00 (t, J = 7.6 Hz, 2H), 3.13 (t, J = 8.1 Hz, 2H). ESI-MS m/z: 412.0 $[M+H]^+$.

*4.1.8.3. (4-Bromo-2-chlorophenyl)(4-phenylindolin-1-yl)methanone (**16c**).* It was obtained as a white solid. Yield, 86%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.20 (d, J = 8.0 Hz, 1H), 7.93 (s, 1H), 7.72 (d, J = 8.2 Hz, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.47 (d, J = 6.9 Hz, 4H), 7.38 (m, 2H), 7.16 (d, J = 7.6 Hz, 1H), 3.74 (t, J = 7.8 Hz, 2H), 3.15 (t, J = 8.2 Hz, 2H). ESI-MS m/z: 412.0 $[M+H]^+$.

*4.1.8.4. (4-Bromo-3,5-dimethoxyphenyl)(4-phenylindolin-1-yl)methanone (**16d**).* It was obtained as a white solid. Yield, 78%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.47 (t, J = 6.6 Hz, 4H), 7.41–7.28 (m, 2H), 7.11 (d, J = 7.2 Hz, 1H), 6.94 (s, 2H), 4.02 (t, J = 7.9 Hz, 2H), 3.87 (s, 6H), 3.13 (t, J = 8.2 Hz, 2H). ESI-MS m/z: 438.1 $[M+H]^+$.

*4.1.9. General procedure for preparation of intermediates **17a–d***

*4.1.9.1. (3-Methyl-4-vinylphenyl)(4-phenylindolin-1-yl)methanone (**17a**).* To a solution of intermediate **16a** (0.76 g, 1.94 mmol), 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (0.39 g, 2.53 mmol), and K_2CO_3 (0.54 g, 3.92 mmol) in 1,4-dioxane (12 mL) and water (3 mL) was added $PdCl_2$(dppf) (0.14 g, 0.19 mmol). The reaction mixture was stirred at 100 °C for 7 h under the N_2 atmosphere. After that time, the solution was cooled down and the catalyst was filtered off. The filtrate was evaporated to give a residue. The residue was diluted with CH_2Cl_2 and washed with water. The organic layer was concentrated to give the crude product, which was purified by column chromatography (ethyl acetate/petroleum ether, 1/50 to 1/10) to give compound **17a** (0.28 g, 42.6%). It was obtained as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.46 (dd, *J* = 7.5, 6.0 Hz, 4H), 7.44–7.36 (m, 3H), 7.29 (s, 1H), 7.09 (d, *J* = 7.4 Hz, 1H), 7.00 (dd, *J* = 17.5, 11.0 Hz, 1H), 5.87–5.79 (m, 1H), 5.45–5.39 (m, 1H), 4.01 (t, *J* = 8.1 Hz, 2H), 3.12 (t, *J* = 8.2 Hz, 2H), 2.37 (s, 3H). ESI-MS m/z: 340.2 $[M+H]^+$.

*4.1.9.2. (3-Chloro-4-vinylphenyl)(4-phenylindolin-1-yl)methanone (**17b**).* Compound **17b** was prepared from **16b** in a similar manner as described for compound **17a**. It was obtained as a white solid. Yield, 36%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.86 (d, *J* = 8.1 Hz, 1H), 7.70 (s, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.47 (d, *J* = 4.3 Hz, 4H), 7.39 (dd, *J* = 8.5, 4.3 Hz, 1H), 7.31 (s, 1H), 7.20 (s, 1H), 7.12 (d, *J* = 6.7 Hz, 1H), 7.07 (dd, *J* = 17.5, 11.0 Hz, 1H), 6.02 (d, *J* = 17.5 Hz, 1H), 5.57 (d, *J* = 11.4 Hz, 1H), 4.02 (t, *J* = 8.1 Hz, 2H), 3.14 (t, *J* = 8.1 Hz, 2H). ESI-MS m/z: 360.1 $[M+H]^+$.

*4.1.9.3. (2-Chloro-4-vinylphenyl)(4-phenylindolin-1-yl)methanone (**17c**).* Compound **17c** was prepared from **16c** in a similar manner as described for compound **17a**. It was obtained as a white solid. Yield, 45%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.21 (d, *J* = 7.9 Hz, 1H), 7.73 (s, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 1.8 Hz, 3H), 7.46 (s, 1H), 7.38 (m, 2H), 7.15 (d, *J* = 7.6 Hz, 1H), 6.78 (dd, *J* = 17.7, 11.0 Hz, 1H), 6.02 (d, *J* = 17.7 Hz, 1H), 5.42 (d, *J* = 11.0 Hz, 1H), 3.75 (t, *J* = 8.2 Hz, 2H), 3.15 (t, *J* = 8.2 Hz, 2H). ESI-MS m/z: 360.1 $[M+H]^+$.

*4.1.9.4. (3,5-Dimethoxy-4-vinylphenyl)(4-phenylindolin-1-yl)methanone (**17d**).* A mixture of intermediate **16d** (1 g, 2.29 mmol), 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (0.42 g, 2.73 mmol), $Pd_2(dba)_3$ (0.027 g, 0.03 mmol), dicyclohexyl(2',6'-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine (0.06 g, 0.14 mmol) and K_3PO_4 (1.45 g, 6.84 mmol) in toluene (3 mL), EtOH (3 mL) and H_2O (1 mL) was stirred at 90 °C for 5 h under the N_2 atmosphere. Upon the completion of the reaction, the catalyst was filtered off. The filtrate was concentrated and extracted with CH_2Cl_2. The organic layer was washed with brine, and evaporated to give a residue, which was purified by column chromatography (ethyl acetate/petroleum ether, 1/35 to 1/10) to afford compound **17d** (0.47 g, 53.2%). It was obtained as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.47 (d, *J* = 3.8 Hz, 4H), 7.41–7.36 (m, 1H), 7.31 (s, 1H), 7.10 (d, *J* = 7.0 Hz, 1H), 6.93–6.82 (m, 3H), 6.10 (dd, *J* = 18.0, 2.6 Hz, 1H), 5.44 (dd, *J* = 12.2, 2.7 Hz, 1H), 4.04 (t, *J* = 8.1 Hz, 2H), 3.84 (s, 6H), 3.13 (t, *J* = 8.1 Hz, 2H). ESI-MS m/z: 386.2 $[M+H]^+$.

*4.1.10. General procedure for preparation of intermediates **18a–d***

Compounds **18a–d** were prepared from **17a–d**, respectively, in a similar manner as described for compound **11a**.

*4.1.10.1 2-Methyl-4-(4-phenylindoline-1-carbonyl)benzaldehyde (**18a**).* It was obtained as a gray solid. Yield, 91%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.30 (s, 1H), 8.14 (s, 1H), 7.94 (d, *J* = 7.9 Hz, 1H), 7.65–7.53 (m, 2H), 7.47 (d, *J* = 4.0 Hz, 4H), 7.39 (m, 2H), 7.13 (s, 1H), 3.97 (s, 2H), 3.14 (t, *J* = 7.9 Hz, 2H), 2.68 (s, 3H). ESI-MS m/z: 342.1 $[M+H]^+$.

*4.1.10.2. 2-Chloro-4-(4-phenylindoline-1-carbonyl)benzaldehyde (**18b**).* It was obtained as an off-white solid. Yield, 87%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.38 (s, 1H), 8.15 (s, 1H), 7.98 (d, *J* = 7.9 Hz, 1H), 7.88 (s, 1H), 7.73 (d, *J* = 7.4 Hz, 1H), 7.48 (s, 2H), 7.47 (s, 2H), 7.41–7.36 (m, 2H), 7.15 (s, 1H), 3.99 (d, *J* = 7.9 Hz, 2H), 3.14 (d, *J* = 8.5 Hz, 2H). ESI-MS m/z: 362.1 $[M+H]^+$.

*4.1.10.3. 3-Chloro-4-(4-phenylindoline-1-carbonyl)benzaldehyde (**18c**).* It was obtained as a gray solid. Yield, 82%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.24 (d, *J* = 7.9 Hz, 1H), 8.12 (d, *J* = 1.1 Hz, 1H), 8.02 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.48 (t, *J* = 4.6 Hz, 4H), 7.39 (m, 2H), 7.18 (d, *J* = 7.7 Hz, 1H), 3.75 (s, 2H), 3.19 (m, 2H). ESI-MS m/z: 362.1 $[M+H]^+$.

*4.1.10.4. 2,6-Dimethoxy-4-(4-phenylindoline-1-carbonyl)benzaldehyde (**18d**).* It was obtained as an off-white solid. Yield, 89%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.38 (s, 1H), 8.16 (s, 1H), 7.47 (m, 4H), 7.39 (dt, *J* = 8.6, 4.3 Hz, 2H), 7.14 (s, 1H), 6.94 (s, 2H), 3.99 (s, 2H), 3.87 (s, 6H), 3.15 (t, *J* = 8.1 Hz, 2H). ESI-MS m/z: 388.2 $[M+H]^+$.

*4.1.11. General procedure for preparation of compounds **A1–31***

4.1.11.1.

*6-(((2-Hydroxyethyl)amino)methyl)-2-(2-methyl-[1,1'-biphenyl]-3-yl)isoindolin-1-one (**A1**).* A mixture of intermediate **11a** (0.08 g, 0.24 mmol), 2-aminoethan-1-ol (0.073 g, 1.2 mmol), and two drops of acetic acid in CH_3OH (4 mL) and CH_2Cl_2 (4 mL) was stirred at room temperature for 6 h. At that time, $NaBH_3CN$ (0.076 g, 1.21 mmol) was added. Stirring was continued for 10 h, when TLC indicated the completion of the reaction. The solvent was concentrated and diluted with CH_2Cl_2. The organic layer was washed with brine, and concentrated to give a residue, which was purified by column chromatography (CH_3OH/CH_2Cl_2, 1/100 to 1/15) to afford compound **A1** (0.0073 g, 8.2%). It was obtained as a light-yellow solid. mp 78.8–80.8 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.77 (s, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.60 (d, J = 7.7 Hz, 1H), 7.48 (t, J = 7.5 Hz, 2H), 7.44 (d, J = 7.7 Hz, 1H), 7.42–7.34 (m, 4H), 7.27 (d, J = 7.5 Hz, 1H), 4.87 (s, 2H), 4.53 (s, 1H), 3.86 (s, 2H), 3.49 (m, 2H), 2.59 (t, J = 5.7 Hz, 2H), 2.02 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 143.2, 141.3, 138.5, 133.9, 132.2, 132.2, 129.5 (2C), 128.7 (2C), 127.6, 127.2, 126.7, 123.6, 123.0, 60.5, 52.9, 52.6, 51.0, 16.0. HRMS (ESI) for $C_{24}H_{24}N_2O_2$ $[M + H]^+$, calcd: 373.1911, found: 373.1907. Purity, 97.2%.

Compounds **A2–31** were prepared in a similar manner as described for compound **A1** through the reaction of **11a**, **11b**, **15a–g**, or **18a–d** with appropriate amines. The preparation of compounds **A25** and **A29** were conducted using DMF as the solvent.

4.1.11.2.

*N-(2-(((2-(2-Methyl-[1,1'-biphenyl]-3-yl)-3-oxoisoindolin-5-yl)methyl)amino)ethyl)acetamide (**A2**).* It was obtained as a light-yellow solid. Yield, 7%. mp 190.1–193.0 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.93 (s, 1H), 7.83 (s, 1H), 7.68 (d, *J* = 7.7 Hz, 1H), 7.64 (d, *J* = 7.7 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 2H), 7.44 (d, *J* = 7.7 Hz, 1H), 7.42–7.35 (m, 4H), 7.28 (d, *J* = 7.4 Hz, 1H), 4.89 (s, 2H), 3.95 (s, 2H), 3.21 (dd, *J* = 12.0, 6.0 Hz, 2H), 2.66 (t, *J* = 6.2 Hz, 2H), 2.03 (s, 3H), 1.81 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.1, 167.0, 143.2, 141.7, 141.3, 138.3, 133.8, 132.6, 132.2, 129.6, 129.4 (2C), 128.7 (2C), 127.6, 127.2, 126.8, 123.7, 123.5, 53.0, 52.0, 47.9, 38.2, 23.0, 16.0. HRMS (ESI) for $C_{26}H_{27}N_3O_2$ $[M + H]^+$, calcd: 414.2176, found: 414.2172. Purity, 98.0%.

4.1.11.3.

*5-(((2-Hydroxyethyl)amino)methyl)-2-(2-methyl-[1,1'-biphenyl]-3-yl)isoindolin-1-one (**A3**).* It was obtained as a yellow solid. Yield, 12%. mp 79.9–83.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.73 (d, *J* = 7.8 Hz, 1H), 7.64 (s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.43 (d, *J* = 7.1 Hz, 1H), 7.41–7.34 (m, 4H), 7.26 (d, *J* = 7.5 Hz, 1H), 4.87 (s, 2H), 4.55 (s, 1H), 3.88 (s, 2H), 3.50 (t, *J* = 5.7 Hz, 2H), 2.62 (t, *J* = 5.8 Hz, 2H), 2.03 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 145.3, 143.2, 142.9, 141.3, 138.4, 133.9, 130.7, 129.5, 129.4 (2C), 128.7 (2C), 128.4, 127.6, 127.2, 126.8, 123.4, 123.2, 60.5, 53.0, 53.0, 51.2, 16.0. HRMS (ESI) for $C_{24}H_{24}N_2O_2$ $[M + H]^+$, calcd: 373.1911, found: 373.1913. Purity, 98.3%.

4.1.11.4.

*N-(2-(((2-(2-Methyl-[1,1'-biphenyl]-3-yl)-1-oxoisoindolin-5-yl)methyl)amino)ethyl)acetamide (**A4**).* It was obtained as a white solid. Yield, 16%. mp 159.6–162.3 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.86 (t, *J* = 5.1 Hz, 1H), 7.74 (d, *J* = 7.8 Hz, 1H), 7.64 (s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.43 (s, 1H), 7.42–7.35 (m, 4H), 7.27 (d, *J* = 7.5 Hz, 1H), 4.88 (s, 2H), 3.86 (s, 2H), 3.17 (q, *J* = 6.3 Hz, 2H), 2.59 (t, *J* = 6.5 Hz, 2H), 2.03 (s, 3H), 1.81 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.8, 167.0, 145.5, 143.2, 142.9, 141.3, 138.4, 133.9, 130.7, 129.5, 129.4 (2C), 128.7 (2C), 128.4, 127.6, 127.2, 126.8, 123.4, 123.2, 53.0, 52.9, 48.4, 39.0, 23.0, 16.0. HRMS (ESI) for $C_{26}H_{27}N_3O_2$ $[M + H]^+$, calcd: 414.2176, found: 414.2182. Purity, 97.9%.

*4.1.11.5. (3-(((2-Hydroxyethyl)amino)methyl)phenyl)(4-phenylindolin-1-yl)methanone (**A5**).* It was obtained as an off-white solid. Yield, 15%. mp 75.0–77.3 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.72–7.28 (m, 11H), 7.10 (s, 1H), 4.55 (s, 1H), 3.99 (s, 2H), 3.80 (s, 2H), 3.49 (s, 2H), 3.13 (m, 2H), 2.60 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.7, 143.5, 141.5, 140.0, 138.5, 137.2, 130.8, 130.2, 129.0 (2C), 128.7, 128.5 (2C), 128.0, 127.8, 126.7, 125.5, 124.4, 60.6, 52.7, 51.3. HRMS (ESI) for $C_{24}H_{24}N_2O_2$ $[M + H]^+$, calcd: 373.1911, found: 373.1915. Purity, 96.8%.

*4.1.11.6. N-(2-((3-(4-Phenylindoline-1-carbonyl)benzyl)amino)ethyl)acetamide (**A6**).* It was obtained as a yellow solid. Yield, 6%. mp 80.4–83.4. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.82 (s, 1H), 7.56 (s, 1H), 7.46 (m, 7H), 7.39 (m, 1H), 7.30 (s, 1H), 7.10 (d, *J* = 6.3 Hz, 1H), 4.00 (t, *J* = 7.6 Hz, 2H), 3.77 (s, 2H), 3.19–3.10 (m,

4H), 2.56 (t, *J* = 6.4 Hz, 2H), 1.79 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.6, 168.7, 143.5, 141.6, 140.0, 138.5, 137.2, 130.8, 130.2, 129.0 (2C), 128.7, 128.5 (2C), 128.0, 127.8, 126.7, 125.5, 124.4, 52.6, 48.4, 39.0, 23.0. HRMS (ESI) for $C_{26}H_{27}N_3O_2$ $[M + H]^+$, calcd: 414.2176, found: 414.2169. Purity, 98.9%.

4.1.11.7. (4-(((2-Hydroxyethyl)amino)methyl)phenyl)(4-phenylindolin-1-yl)methanone ***(A7)****.* It was obtained as a light-yellow solid. Yield, 10%. mp 118.1–120.3 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.54 (d, *J* = 7.6 Hz, 2H), 7.46 (t, *J* = 6.0 Hz, 7H), 7.40–7.34 (m, 1H), 7.28 (s, 1H), 7.09 (d, *J* = 6.8 Hz, 1H), 4.53 (s, 1H), 4.00 (t, *J* = 7.8 Hz, 2H), 3.79 (s, 2H), 3.49 (t, *J* = 5.5 Hz, 2H), 3.12 (t, *J* = 8.0 Hz, 2H), 2.60 (t, *J* = 5.6 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.5, 143.6, 143.4, 140.0, 138.5, 135.6, 130.8, 129.0 (2C), 128.5 (2C), 128.3, 128.0, 127.8, 127.3, 124.4, 116.0, 60.6, 52.8, 51.4. HRMS (ESI) for $C_{24}H_{24}N_2O_2$ $[M + H]^+$, calcd: 373.1911, found: 373.1915. Purity, 96.6%.

4.1.11.8. N-(2-((4-(4-Phenylindoline-1-carbonyl)benzyl)amino)ethyl)acetamide ***(A8)****.* It was obtained as a yellow solid. Yield, 13%. mp 141.8–143.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.81 (s, 1H), 7.54 (d, *J* = 7.8 Hz, 2H), 7.46 (m, 6H), 7.41–7.35 (m, 1H), 7.29 (s, 1H), 7.09 (d, *J* = 7.0 Hz, 1H), 4.00 (t, *J* = 8.0 Hz, 2H), 3.76 (s, 2H), 3.18–3.09 (m, 4H), 2.56 (t, *J* = 6.4 Hz, 2H), 1.79 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.5, 168.5, 143.6, 140.0, 138.5, 135.6, 130.8, 129.0 (2C), 128.5 (2C), 128.3, 128.0, 127.8, 127.3, 124.4, 52.7, 48.5, 39.1, 23.0. HRMS (ESI) for $C_{26}H_{27}N_3O_2$ $[M + H]^+$, calcd: 414.2176, found: 414.2181. Purity, 99.1%.

4.1.11.9.

*(4-(((2-Hydroxyethyl)amino)methyl)-3-methylphenyl)(4-phenylindolin-1-yl)methanone (**A9**).* It was obtained as a white solid. Yield, 11%. mp 223.2–225.9 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.56–7.44 (m, 5H), 7.40 (s, 3H), 7.28 (s, 1H), 7.09 (s, 1H), 3.99 (m, 2H), 3.87 (s, 2H), 3.58 (s, 2H), 3.12 (m, 2H), 2.76 (s, 2H), 2.37 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.5, 168.5, 143.5, 140.02, 139.3, 138.5, 137.2, 136.1, 130.7, 129.0 (2C), 128.9, 128.8, 128.5 (2C), 128.0, 127.8, 124.6, 124.4, 59.7, 51.2, 49.8, 28.1, 21.6, 19.0. HRMS (ESI) for $C_{25}H_{26}N_2O_2$ $[M + H]^+$, calcd: 387.2067, found: 387.2064. Purity, 96.1%.

4.1.11.10.

*N-(2-((2-Methyl-4-(4-phenylindoline-1-carbonyl)benzyl)amino)ethyl)acetamide (**A10**).* It was obtained as an off-white solid. Yield, 7%. mp 218.3–220.9 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.87 (s, 2H), 7.47 (m, 5H), 7.39 (m, 3H), 7.29 (s, 1H), 7.10 (s, 1H), 4.00 (s, 2H), 3.75 (s, 2H), 3.20 (s, 2H), 3.12 (s, 2H), 2.65 (s, 2H), 2.35 (s, 3H), 1.82 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.6, 168.6, 162.7, 143.6, 141.0, 140.0, 138.5, 136.8, 135.6, 130.7, 129.0 (2C), 128.7, 128.5 (2C), 128.4, 128.0, 127.7, 124.6, 124.3, 50.4, 48.9, 38.9, 36.2, 31.1, 23.0, 18.9. HRMS (ESI) for $C_{27}H_{29}N_3O_2$ $[M + H]^+$, calcd: 428.2333, found: 428.2329. Purity, 95.8%.

4.1.11.11.

*(3-Hydroxy-4-(((2-hydroxyethyl)amino)methyl)phenyl)(4-phenylindolin-1-yl)methanone (**A11**).* It was obtained as an off-white solid. Yield, 3%. mp 138.0–140.2 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.48 (d, J = 4.1 Hz, 4H), 7.42–7.36 (m, 1H), 7.29 (s,

1H), 7.23 (d, *J* = 7.6 Hz, 1H), 7.16 (t, *J* = 7.8 Hz, 1H), 7.09 (d, *J* = 7.1 Hz, 1H), 6.96 (d, *J* = 7.4 Hz, 1H), 6.90 (s, 1H), 6.77 (t, *J* = 8.4 Hz, 1H), 4.00 (t, *J* = 8.0 Hz, 2H), 3.92 (s, 2H), 3.53 (t, *J* = 5.6 Hz, 2H), 3.13 (t, *J* = 8.1 Hz, 2H), 2.65 (t, *J* = 5.5 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.4, 162.7, 157.6, 143.5, 140.0, 138.5, 130.8, 129.7, 129.2, 129.0 (2C), 128.5 (2C), 127.9, 127.8, 119.2, 117.4, 115.6, 114.1, 60.0, 50.8, 50.0, 36.2, 31.2. HRMS (ESI) for $C_{24}H_{24}N_2O_3$ $[M + H]^+$, calcd: 389.1860, found: 389.1856. Purity, 97.1%.

4.1.11.12.

N-(2-((2-Hydroxy-4-(4-phenylindoline-1-carbonyl)benzyl)amino)ethyl)acetamide ***(A12).*** It was obtained as an off-white solid. Yield, 10%. mp 136.3–139.2 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.86 (s, 1H), 7.48 (d, *J* = 4.2 Hz, 4H), 7.39 (dt, *J* = 8.6, 4.4 Hz, 1H), 7.29 (s, 1H), 7.22 (d, *J* = 7.7 Hz, 1H), 7.16 (t, *J* = 7.8 Hz, 1H), 7.09 (d, *J* = 7.4 Hz, 1H), 6.96 (d, *J* = 7.4 Hz, 1H), 6.90 (s, 1H), 6.76 (d, *J* = 8.3 Hz, 1H), 4.00 (t, *J* = 8.1 Hz, 2H), 3.86 (s, 2H), 3.18 (dd, *J* = 12.2, 6.2 Hz, 2H), 3.13 (t, *J* = 8.2 Hz, 2H), 2.60 (t, *J* = 6.4 Hz, 2H), 1.81 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.6, 162.7, 157.7, 157.4, 140.0, 138.5, 138.5, 130.8, 129.7, 129.0, 129.0 (2C), 128.5 (2C), 127.9, 127.8, 119.2, 117.4, 115.6, 114.1, 49.9, 48.1, 38.6, 36.2, 31.2, 23.0. HRMS (ESI) for $C_{26}H_{27}N_3O_3$ $[M + H]^+$, calcd: 430.2125, found: 430.2121. Purity, 98.3%.

4.1.11.13.

(4-(((2-Hydroxyethyl)amino)methyl)-3-methoxyphenyl)(4-phenylindolin-1-yl)methanone ***(A13).*** It was obtained as a white solid. Yield, 6%. mp 240.5–242.3 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.08 (s, 1H), 7.48 (d, *J* = 2.7 Hz, 4H), 7.45 (d, *J* = 7.4 Hz,

1H), 7.40 (s, 1H), 7.30 (s, 1H), 7.23–7.03 (m, 3H), 4.58 (s, 1H), 4.12–3.96 (m, 2H), 3.84 (s, 3H), 3.79 (s, 2H), 3.51 (s, 2H), 3.14 (t, *J* = 7.4 Hz, 2H), 2.65 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.4, 162.7, 157.3, 143.5, 140.0, 138.5, 137.1, 130.7, 129.2, 129.0 (2C), 128.5 (2C), 128.0, 127.8, 124.4, 124.4, 119.0, 109.5, 60.4, 56.0, 51.4, 47.5, 36.2, 31.2. HRMS (ESI) for $C_{25}H_{26}N_2O_3$ $[M + H]^+$, calcd: 403.2016, found: 403.2013. Purity, 96.5%.

4.1.11.14.

N-(2-((2-Methoxy-4-(4-phenylindoline-1-carbonyl)benzyl)amino)ethyl)acetamide ***(A14)****.* It was obtained as a white solid. Yield, 11%. mp 184.7–186.8 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.87 (s, 1H), 7.47 (m, 5H), 7.39 (dt, *J* = 8.4, 4.3 Hz, 1H), 7.30 (s, 1H), 7.19–7.14 (m, 2H), 7.11 (d, *J* = 7.0 Hz, 1H), 6.77 (t, *J* = 8.0 Hz, 1H), 4.02 (t, *J* = 8.0 Hz, 2H), 3.84 (s, 3H), 3.78 (s, 2H), 3.18 (dd, *J* = 12.1, 6.1 Hz, 2H), 3.14 (t, *J* = 8.1 Hz, 2H), 2.63 (t, *J* = 6.2 Hz, 2H), 1.81 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.7, 168.4, 162.7, 157.7, 157.3, 140.0, 138.5, 130.8, 129.7, 129.2, 129.0 (2C), 128.5 (2C), 128.0, 127.8, 119.2, 119.0, 115.6, 109.5, 56.0, 48.5, 47.2, 38.7, 36.2, 31.2, 23.0. HRMS (ESI) for $C_{27}H_{29}N_3O_3$ $[M + H]^+$, calcd: 444.2282, found: 444.2280. Purity, 97.7%.

4.1.11.15.

(3-Chloro-4-(((2-hydroxyethyl)amino)methyl)phenyl)(4-phenylindolin-1-yl)methanone ***(A15)****.* It was obtained as a white solid. Yield, 8%. mp 118.8–121.4 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.08 (s, 1H), 7.65 (s, 2H), 7.56 (d, *J* = 6.6 Hz, 1H), 7.47 (d, *J* = 2.6 Hz, 4H), 7.42–7.24 (m, 2H), 7.11 (d, *J* = 5.0 Hz, 1H), 4.54 (s, 1H), 4.01 (m, 2H),

3.86 (s, 2H), 3.50 (d, *J* = 4.4 Hz, 2H), 3.13 (t, *J* = 7.3 Hz, 2H), 2.63 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.9, 162.7, 143.4, 140.5, 140.0, 138.5, 137.3, 133.0, 130.8, 130.1, 129.0 (2C), 128.5 (2C), 128.0, 127.9, 127.8, 126.0, 124.6, 60.7, 51.5, 50.2, 36.2, 31.2. HRMS (ESI) for $C_{24}H_{23}ClN_2O_2$ $[M + H]^+$, calcd: 407.1521, found: 407.1523. Purity, 98.9%.

4.1.11.16.

N-(2-((2-Chloro-4-(4-phenylindoline-1-carbonyl)benzyl)amino)ethyl)acetamide ***(A16).*** It was obtained as a white solid. Yield, 12%. mp 130.6–133.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.84 (s, 1H), 7.66 (s, 2H), 7.58 (s, 1H), 7.48 (s, 4H), 7.40 (s, 1H), 7.34 (s, 1H), 7.13 (s, 1H), 4.01 (s, 2H), 3.84 (s, 2H), 3.16 (m, 4H), 2.61 (s, 2H), 1.81 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.9, 162.7, 143.4, 140.5, 140.0, 138.5, 137.3, 133.0, 130.8, 130.1, 129.0 (2C), 128.5 (2C), 128.0, 127.9, 127.8, 126.0, 124.6, 60.7, 51.5, 50.2, 36.2, 31.2, 28.1. HRMS (ESI) for $C_{26}H_{26}ClN_3O_2$ $[M + H]^+$, calcd: 448.1786, found: 448.1783. Purity, 96.9%.

4.1.11.17.

(2-Chloro-4-(((2-hydroxyethyl)amino)methyl)phenyl)(4-phenylindolin-1-yl)methanone ***(A17).*** It was obtained as a white solid. Yield, 25%. mp 148.9–150.1 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.23 (d, *J* = 7.2 Hz, 1H), 7.57 (s, 1H), 7.54–7.42 (m, 6H), 7.38 (d, *J* = 5.9 Hz, 2H), 7.15 (d, *J* = 6.7 Hz, 1H), 4.54 (s, 1H), 3.79 (m, 4H), 3.50 (s, 2H), 3.16 (s, 2H), 2.59 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.6, 144.6, 142.9, 139.9, 138.7, 135.4, 130.6, 129.1, 129.0 (2C), 128.5 (2C), 128.3, 128.0, 127.8,

127.8, 127.7, 125.0, 115.8, 60.6, 52.1, 51.2, 49.7, 27.8. HRMS (ESI) for $C_{24}H_{23}ClN_2O_2$ $[M + H]^+$, calcd: 407.1521, found: 407.1523. Purity, 98.2%.

4.1.11.18.

N-(2-((3-Chloro-4-(4-phenylindoline-1-carbonyl)benzyl)amino)ethyl)acetamide ***(A18)***. It was obtained as a white solid. Yield, 16%. mp 170.9–173.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.23 (d, J = 7.3 Hz, 1H), 7.83 (s, 1H), 7.57 (s, 1H), 7.41 (m, 8H), 7.15 (d, J = 6.7 Hz, 1H), 3.77 (m, 4H), 3.16 (m, 4H), 2.56 (s, 2H), 1.81 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.5, 165.6, 144.7, 142.9, 139.9, 138.7, 135.4, 130.6, 129.0, 129.0 (2C), 128.5 (2C), 128.3, 127.8, 127.8, 127.6, 125.0, 115.8, 112.1, 51.9, 49.7, 48.4, 39.0, 27.8, 23.0. HRMS (ESI) for $C_{26}H_{26}ClN_3O_2$ $[M + H]^+$, calcd: 448.1786, found: 448.1790. Purity, 96.1%.

4.1.11.19.

(4-(((2-Hydroxyethyl)amino)methyl)-3,5-dimethoxyphenyl)(4-phenylindolin-1-yl)methanone ***(A19)***. It was obtained as a white solid. Yield, 5%. mp 224.2–226.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.47 (s, 4H), 7.39 (s, 1H), 7.31 (s, 1H), 7.10 (s, 1H), 6.89 (s, 2H), 4.80 (s, 1H), 4.01 (s, 2H), 3.92 (s, 2H), 3.84 (s, 6H), 3.55 (s, 2H), 3.14 (s, 2H), 2.72 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.1, 158.7 (2C), 143.4, 140.0, 138.9, 138.6, 130.7, 129.0 (2C), 128.5 (2C), 128.1, 127.8, 124.6, 102.9 (2C), 58.7, 56.6, 50.3. HRMS (ESI) for $C_{26}H_{28}N_2O_4$ $[M + H]^+$, calcd: 433.2122, found: 433.2119. Purity, 96.1%.

4.1.11.20.

N-(2-((2,6-Dimethoxy-4-(4-phenylindoline-1-carbonyl)benzyl)amino)ethyl)acetamide

(A20). It was obtained as a white solid. Yield, 6%. mp 186.9–188.3 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.99 (s, 1H), 7.47 (d, *J* = 4.3 Hz, 4H), 7.43–7.26 (m, 2H), 7.11 (d, *J* = 6.4 Hz, 1H), 6.89 (s, 2H), 4.01 (t, *J* = 7.9 Hz, 2H), 3.92 (s, 2H), 3.84 (s, 6H), 3.23 (d, *J* = 5.9 Hz, 2H), 3.14 (t, *J* = 8.2 Hz, 2H), 2.72 (t, *J* = 5.7 Hz, 2H), 1.81 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.1, 168.0, 158.7 (2C), 143.4, 140.0, 139.1, 138.6, 130.7, 129.0 (2C), 128.5 (2C), 128.1, 127.8, 124.6, 109.9, 102.9 (2C), 63.5, 56.6, 50.9, 47.6, 37.3, 28.0, 23.0. HRMS (ESI) for $C_{28}H_{31}N_3O_4$ $[M + H]^+$, calcd: 474.2387, found: 474.2391. Purity, 97.9%.

4.1.11.21.

*(3-Methoxy-4-(((2-methoxyethyl)amino)methyl)phenyl)(4-phenylindolin-1-yl)methanone (**A21**)*. It was obtained as an off-white solid. Yield, 12%. mp 247.1–250.1 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.40 (s, 1H), 8.10 (s, 1H), 7.56 (d, *J* = 5.6 Hz, 1H), 7.52–7.30 (m, 5H), 7.25 (s, 1H), 7.19 (s, 1H), 7.11 (s, 1H), 4.02 (d, *J* = 13.1 Hz, 4H), 3.87 (s, 3H), 3.59 (s, 2H), 3.29 (s, 3H), 3.14 (m, 2H), 2.96 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.4, 168.0, 157.7, 143.4, 140.0, 138.8, 138.5, 131.0, 130.8, 129.0 (2C), 128.5 (2C), 128.0, 127.8, 124.9, 124.5, 119.0, 109.8, 68.9, 58.5, 56.2, 46.9, 45.9, 21.5. HRMS (ESI) for $C_{26}H_{28}N_2O_3$ $[M + H]^+$, calcd: 417.2173, found: 417.2169. Purity, 98.2%.

4.1.11.22.

*(S)-(4-((2-(Hydroxymethyl)pyrrolidin-1-yl)methyl)-3-methoxyphenyl)(4-phenylindolin-1-yl)methanone (**A22**)*. It was obtained as a white solid. Yield, 16%. mp 271.5–273.1 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.48 (s, 5H), 7.39 (s, 1H), 7.30 (s,

1H), 7.22–7.07 (m, 3H), 4.36 (s, 1H), 4.02 (s, 3H), 3.84 (s, 3H), 3.51 (s, 2H), 3.14 (s, 2H), 2.94 (s, 1H), 2.62 (s, 1H), 2.22 (s, 1H), 1.95 (m, 2H), 1.66 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.3, 143.5, 140.0, 138.5, 130.8, 130.0, 129.0 (2C), 128.5 (2C), 128.0, 127.8, 124.4, 119.0, 109.7, 56.4, 56.1, 54.6, 52.6, 51.0, 28.1, 22.9, 18.9, 14.3. HRMS (ESI) for $C_{28}H_{30}N_2O_3$ $[M + H]^+$, calcd: 443.2329, found: 443.2335. Purity, 98.2%.

*4.1.11.23. (2-Methoxy-4-(4-phenylindoline-1-carbonyl)benzyl)-L-proline (**A23**).* It was obtained as a white solid. Yield, 13%. mp 289.3–293.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.48 (s, 5H), 7.35 (m, 2H), 7.25–7.08 (m, 3H), 4.10 (d, J = 11.4 Hz, 1H), 3.99 (m, 3H), 3.86 (s, 3H), 3.44 (s, 1H), 3.14 (s, 3H), 2.67 (s, 1H), 2.13 (s, 1H), 1.93 (s, 1H), 1.82 (s, 1H), 1.71 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.5, 168.1, 157.7, 143.4, 140.0, 138.5, 138.4, 131.2, 130.8, 130.0, 129.0 (2C), 128.5 (2C), 128.1, 127.8, 125.7, 124.5, 119.1, 109.9, 66.8, 56.4, 56.2, 53.4, 52.1, 29.0, 23.6, 18.9. HRMS (ESI) for $C_{28}H_{28}N_2O_4$ $[M + H]^+$, calcd: 457.2122, found: 457.2119. Purity, 97.9%.

4.1.11.24.

*(S)-1-(2-Methoxy-4-(4-phenylindoline-1-carbonyl)benzyl)piperidine-2-carboxylic acid (**A24**).* It was obtained as a white solid. Yield, 5%. mp 232.8–235.9 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.96 (s, 1H), 7.61–7.25 (m, 7H), 7.17 (s, 2H), 7.10 (s, 1H), 4.02 (s, 2H), 3.81 (s, 3H), 3.70 (d, J = 14.5 Hz, 1H), 3.19 (s, 1H), 3.12 (s, 2H), 2.94 (s, 1H), 2.89 (s, 1H), 2.73 (s, 1H), 2.29 (s, 1H), 1.81 (s, 1H), 1.77 (s, 1H), 1.52 (s, 2H), 1.40 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.3, 168.4, 162.7, 157.5,

143.5, 140.0, 138.5, 137.3, 130.7, 129.9, 129.0 (2C), 128.5 (2C), 128.1, 128.0, 127.8, 124.4, 119.0, 109.6, 64.1, 56.0, 53.1, 49.6, 36.2, 31.2, 29.0, 24.9, 22.1. HRMS (ESI) for $C_{29}H_{30}N_2O_4$ $[M + H]^+$, calcd: 471.2278, found: 471.2283. Purity, 96.7%.

*4.1.11.25. (2-Methoxy-4-(4-phenylindoline-1-carbonyl)benzyl)-D-serine (**A25**).* It was obtained as a white solid. Yield, 7%. mp 212.9–215.6 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.47 (s, 5H), 7.39 (s, 1H), 7.30 (s, 1H), 7.25–7.05 (m, 3H), 4.02 (s, 3H), 3.86 (s, 3H), 3.71 (s, 1H), 3.64 (s, 1H), 3.20 (s, 2H), 3.14 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 157.6, 140.0, 138.5, 130.8, 130.3, 130.0, 129.0 (2C), 128.5 (2C), 128.0, 127.8, 124.5, 119.4, 119.1, 109.8, 63.1, 61.5, 56.2, 45.8. HRMS (ESI) for $C_{26}H_{26}N_2O_5$ $[M + H]^+$, calcd: 447.1914, found: 447.1915. Purity, 97.2%.

4.1.11.26.

*3-((2-(((2-Hydroxyethyl)amino)methyl)-5-(4-phenylindoline-1-carbonyl)phenoxy)methyl)benzonitrile (**A26**).* It was obtained as a light-yellow solid. Yield, 10%. mp 138.7–142.3 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.08 (s, 1H), δ 7.95 (s, 1H), 7.83 (m, 2H), 7.63 (t, J = 7.7 Hz, 1H), 7.48 (d, J = 4.1 Hz, 5H), 7.40 (dd, J = 8.5, 4.2 Hz, 1H), 7.26 (s, 2H), 7.17 (d, J = 7.4 Hz, 1H), 7.10 (d, J = 6.8 Hz, 1H), 5.28 (s, 2H), 4.53 (s, 1H), 3.92 (s, 2H), 3.83 (s, 2H), 3.51 (s, 2H), 3.11 (t, J = 8.0 Hz, 2H), 2.63 (t, J = 5.4 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.2, 155.8, 143.5, 140.0, 139.2, 138.5, 136.9, 132.5, 132.0, 131.5, 131.2, 130.7, 130.2, 129.5, 129.0 (2C), 128.5 (2C), 128.0, 127.8, 124.4, 119.6, 119.1, 111.8, 111.0, 68.6, 60.7, 51.6, 47.8. HRMS (ESI) for $C_{32}H_{29}N_3O_3$ $[M + H]^+$, calcd: 504.2282, found: 504.2279. Purity, 98.3%.

4.1.11.27.

*N-(2-((2-((3-Cyanobenzyl)oxy)-4-(4-phenylindoline-1-carbonyl)benzyl)amino)ethyl)acetamide (**A27**).* It was obtained as a light-yellow solid. Yield, 21%. mp 195.3–198.1 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.95 (s, 2H), 7.82 (t, *J* = 8.4 Hz, 3H), 7.63 (t, *J* = 7.7 Hz, 1H), 7.48 (d, *J* = 4.4 Hz, 5H), 7.40 (dd, *J* = 8.2, 4.0 Hz, 1H), 7.25 (s, 2H), 7.17 (d, *J* = 7.4 Hz, 1H), 7.11 (d, *J* = 6.8 Hz, 1H), 5.28 (s, 2H), 3.92 (s, 2H), 3.81 (s, 2H), 3.17 (dd, *J* = 11.9, 5.9 Hz, 2H), 3.11 (t, *J* = 7.9 Hz, 2H), 2.60 (t, *J* = 6.2 Hz, 2H), 1.79 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.5, 168.2, 155.7, 143.5, 140.0, 139.2, 138.5, 136.8, 132.5, 132.0, 131.5, 131.2, 130.7, 130.2, 129.3, 129.0 (2C), 128.5 (2C), 128.0, 127.8, 124.4, 119.6, 119.1, 111.8, 111.0, 68.6, 56.4, 48.7, 47.4, 39.1, 23.0, 18.9. HRMS (ESI) for $C_{34}H_{32}N_4O_3$ $[M + H]^+$, calcd: 545.2547, found: 545.2550. Purity, 99.1%.

4.1.11.28.

*(S)-1-(2-((3-Cyanobenzyl)oxy)-4-(4-phenylindoline-1-carbonyl)benzyl)piperidine-2-carboxylic acid (**A28**).* It was obtained as a white solid. Yield, 12%. mp 248.8–251.2 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.10 (s, 1H), δ 7.95 (s, 1H), 7.82 (m, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.50 (m, 5H), 7.40 (d, *J* = 3.6 Hz, 1H), 7.30 (s, 1H), 7.25 (s, 1H), 7.19 (d, *J* = 7.1 Hz, 1H), 7.11 (d, *J* = 4.8 Hz, 1H), 5.28 (s, 2H), 3.94–3.83 (m, 3H), 3.73 (d, *J* = 14.4 Hz, 1H), 3.24 (s, 1H), 3.13–3.08 (m, 2H), 2.95 (s, 1H), 2.32 (s, 1H), 1.81 (s, 2H), 1.52 (s, 3H), 1.42 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.4, 168.2, 156.0, 143.5, 140.0, 139.2, 138.5, 137.2, 132.5, 132.0, 131.2, 130.7, 130.5, 130.1, 129.0(2C), 128.5 (2C), 128.0, 127.8, 124.5, 119.6, 119.1, 111.8, 111.2, 68.5,

63.8, 56.4, 53.6, 49.5, 29.1, 25.1, 22.0, 18.9. HRMS (ESI) for $C_{36}H_{33}N_3O_4$ $[M + H]^+$, calcd: 572.2544, found: 572.2548. Purity, 95.9%.

*4.1.11.29. (2-((3-Cyanobenzyl)oxy)-4-(4-phenylindoline-1-carbonyl)benzyl)-D-serine (**A29**).* It was obtained as a white solid. Yield, 3%. mp 221.9–224.8 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.89 (d, J = 8.1 Hz, 1H), 7.81 (d, J = 6.9 Hz, 1H), 7.62 (t, J = 7.5 Hz, 1H), 7.49 (m, 6H), 7.40 (dd, J = 8.5, 4.1 Hz, 2H), 7.27 (s, 1H), 7.18 (d, J = 6.2 Hz, 1H), 7.11 (s, 1H), 5.29 (s, 2H), 3.98 (s, 1H), 3.92 (s, 2H), 3.59 (d, J = 3.7 Hz, 2H), 3.18 (s, 1H), 3.11 (t, J = 7.4 Hz, 2H), 3.04 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 140.0, 139.0, 132.7, 132.1, 131.3, 130.2, 130.0, 129.0 (2C), 128.5 (2C), 128.0, 127.8, 119.6, 119.1, 111.8, 68.7, 31.7, 29.4, 26.9, 22.5. HRMS (ESI) for $C_{33}H_{29}N_3O_5$ $[M + H]^+$, calcd: 548.2180, found: 548.2175. Purity, 97.2%.

4.1.11.30.

*(4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)indolin-1-yl)(4-(((2-hydroxyethyl)amino)methyl)-3-methoxyphenyl)methanone (**A30**).* It was obtained as a light-yellow solid. Yield, 5%. mp 268.2–270.7 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.01 (s, 1H), 7.44 (d, J = 6.7 Hz, 1H), 7.25 (s, 1H), 7.20–7.11 (m, 2H), 7.04 (d, J = 4.7 Hz, 1H), 6.94 (s, 1H), 6.93 (s, 2H), 4.65 (s, 1H), 4.27 (s, 4H), 4.05–3.91 (m, 2H), 3.84 (br, 5H), 3.53 (s, 2H), 3.11 (t, J = 7.6 Hz, 2H), 2.68 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.3, 162.7, 157.4, 143.7, 143.3, 138.0, 137.5, 133.2, 130.6, 129.6, 127.9, 124.2, 121.5, 119.0, 117.5, 117.0, 109.6, 64.5, 59.9, 56.0, 51.1, 47.2, 36.2, 31.2, 31.1. HRMS (ESI) for $C_{27}H_{28}N_2O_5$ $[M + H]^+$, calcd: 461.2071, found: 461.2069. Purity, 98.0%.

4.1.11.31.

*N-(2-((4-(4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)indoline-1-carbonyl)-2-methoxybenzyl)amino)ethyl)acetamide (**A31**).* It was obtained as a light-yellow solid. Yield, 9%. mp 190.1–193.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.02 (s, 1H), 7.87 (s, 1H), 7.46 (m, 1H), 7.25 (s, 1H), 7.20–7.11 (m, 2H), 7.04 (d, J = 7.3 Hz, 1H), 6.94 (s, 1H), 6.93 (s , 2H), 4.27 (s, 4H), 3.99 (t, J = 7.8 Hz, 2H), 3.83 (s, 3H), 3.79 (s, 2H), 3.18 (dd, J = 11.4, 5.4 Hz, 2H), 3.11 (t, J = 8.1 Hz, 2H), 2.64 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.7, 168.3, 162.7, 157.3, 143.6, 143.3, 138.0, 133.2, 130.6, 127.9, 121.5, 119.0, 117.5, 117.0, 109.5, 64.5, 56.0, 48.4, 47.0, 36.2, 31.2, 31.1, 23.0. HRMS (ESI) for $C_{29}H_{31}N_3O_5$ $[M + H]^+$, calcd: 502.2336, found: 502.2341. Purity, 97.2%.

4.2. Pharmacology

4.2.1. PD-1/PD-L1 binding assay

This biochemical assay was performed using the PD-1/PD-L1 binding assay kit (Cisbio, Cat. no. 64ICP01PEG), following the manufacturer's guidelines.

4.2.2. Cytotoxicity assay

Jurkat cells were seeded onto a 96-well plate at a density of 4 × 10^4/mL in RMPI 1640 with 10% FBS. The indicated concentrations of the test compounds (50, 16.67, 5.56, 1.85, 0.62, 0.21, and 0.069 μM) were added, and the cells were cultured for 72 h. Thereafter, one-tenth of the culture volume of CCK-8 solution (Dojindo, Japan) was added to the plate, and incubated at 37 °C for 3 h. Absorbance was detected at 450 nm using a microplate reader (Molecular Device,

USA). Survival Rate = (OD (drug treatment group)/OD (con)) × 100%. The EC_{50} values were calculated accordingly, using GraphPad Prism 6.

4.2.3. Determination of IFN-γ secretion

The effects of the test compounds on IFN-γ secretion were evaluated using the T cell-tumor co-culture assay, as previously described.[30]

4.3. Molecular docking

Molecular graphic manipulations and visualizations were performed using Discovery Studio Visualizer 4.0. Autodock 4.2 was employed to determine molecular docking. The identification of the torsion angles in the ligands, the addition of the solvent model, and the determination of protein and ligand atomic charges were performed using Autodock Tools. The grid-enclosing box was placed on the centroid of the co-crystallized ligand. The crystal structure of PD-L1 was obtained from Protein Data Bank (PDB ID: 5N2D). Water molecules and original ligand were manually removed before molecular docking. Hydrogen atoms and Kollman charges were added to each protein atom. Each docking experiment was conducted 200 times, giving 200 docked conformations. All the other parameters used in the docking process were set to default values. Finally, the model with the lowest energy was used for further analysis.

Acknowledgments

This work was supported by grants from the Natural Science Foundation of Liaoning Province (20180550522) and Chinese People's Liberation Army Logistics Support Force No. 967 Hospital.

References

[1] J. Couzin-Frankel, Cancer immunotherapy, Science 342 (2013) 1432−1433.

[2] A. Ribas, J.D. Wolchok, Cancer immunotherapy using checkpoint blockade, Science 359 (2018) 1350−1355.

[3] M.A. Postow, M.K. Callahan, J.D. Wolchok, Immune checkpoint blockade in cancer therapy, J. Clin. Oncol. 33 (2015) 1974−1982.

[4] R.C. Acurcio, A. Scomparin, J. Conniot, J.A.R. Salvador, R. Satchi-Fainaro, H.F. Florindo, R.C. Guedes, Structure-function analysis of immune checkpoint receptors to guide emerging anticancer immunotherapy, J. Med. Chem. 61 (2018) 10957–10975.

[5] L. Chen, D.B. Flies, Molecular mechanisms of T cell co-stimulation and co-inhibition, Nat. Rev. Immunol. 13 (2013) 227−242.

[6] A. Constantinidou, C. Alifieris, D.T. Trafalis, Targeting programmed cell death-1 (PD-1) and ligand (PD-L1): A new era in cancer active immunotherapy, Pharmacol. Therapeut. 194 (2019) 84−106.

[7] C. Sun, R. Mezzadra,, T.N. Schumacher, Regulation and function of the PD-L1 checkpoint, Immunity 48 (2018) 434−452.

[8] X. Meng, Y. Liu, J. Zhang, F. Teng, L. Xing, J. Yu, PD-1/PD-L1 checkpoint blockades in non-small cell lung cancer: New development and challenges, Cancer Lett. 405 (2017) 29–37.

[9] K.E. Beckermann, D.B. Johnson, J.A. Sosman, PD-1/PD-L1 blockade in renal cell cancer, Expert Rev. Clin. Immu. 13 (2017) 77–84.

[10] D.B. Johnson, C. Peng, J.A. Sosman, Nivolumab in melanoma: latest evidence and clinical potential, Ther. Adv. Med. Oncol. 7 (2015) 97–106.

[11] M.J. Butte, M.E. Keir, T.B. Phamduy, A.H. Sharpe, G.J. Freeman, Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses, Immunity 27 (2007) 111–122.

[12] M.E. Keir, S.C. Liang, I. Guleria, Y.E. Latchman, A. Qipo, L.A. Albacker, M. Koulmanda, G.J. Freeman, M.H. Sayegh, A.H. Sharpe, Tissue expression of PD-L1 mediates peripheral T cell tolerance, J. Exp. Med. 203 (2006) 883–895.

[13] H.O. Alsaab, S. Sau, R. Alzhrani, K. Tatiparti, K. Bhise, S.K. Kashaw, A.K. Iyer, PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome, Front. Pharmacol. 8 (2017) 1−15.

[14] K. Azijli, E. Stelloo, G.J. Peters, A.J. Van Den Eertwegh, New developments in the treatment of metastatic melanoma: immune checkpoint inhibitors and targeted therapies, Anticancer Res. 34 (2014) 1493–1506.

[15] J. Larkin, V. Chiarion-Sileni, R. Gonzalez, J.J. Grob, C.L. Cowey, C.D. Lao, D. Schadendorf, R. Dummer, M. Smylie, P. Rutkowski, P.F. Ferrucci, A. Hill, J. Wagstaff, M.S. Carlino, J.B. Haanen, M. Maio, I. Marquez-Rodas, G.A. McArthur, P.A. Ascierto, G.V. Long, M.K. Callahan, M.A. Postow, K. Grossmann, M. Sznol, B. Dreno, L. Bastholt, A. Yang, L.M. Rollin, C. Horak, F.S. Hodi, J.D. Wolchok, Combined nivolumab and ipilimumab or monotherapy in untreated melanoma, New Engl. J. Med. 373 (2015) 23–34.

[16] A.M. Seifert, S. Zeng, J.Q. Zhang, T.S. Kim, N.A. Cohen, M.J. Beckman, B.D. Medina, J.H. Maltbaek, J.K. Loo, M.H. Crawley, F. Rossi, P. Besmer, C.R. Antonescu, R.P. DeMatteo, PD-1/PD-L1 blockade enhances T-cell activity and antitumor efficacy of imatinib in gastrointestinal stromal tumors, Clin. Cancer Res. 23 (2017) 454−465.

[17] C.J. Langer, S.M. Gadgeel, H. Borghaei, V.A. Papadimitrakopoulou, A. Patnaik, S.F. Powell, R.D. Gentzler, R.G. Martins, J.P. Stevenson, S.I. Jalal, A. Panwalkar, J.C-H. Yang, M. Gubens, L.V. Sequist, M.M. Awad, J. Fiore, Y. Ge, H. Raftopoulos, L. Gandhi, Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-celllungcancer: A randomised, phase 2 cohort of the open-label KEYNOTE-021 study, Lancet Oncol. 17 (2016) 1497–1508.

[18] S. Shaabani, H.P.S. Huizinga, R. Butera, A. Kouchi, K. Guzik, K. Magiera-Mularz, T.A. Holak, A. Domling, A patent review on PD-1/PD-L1 antagonists: small molecules, peptides and macrocycles (2015-2018), Expert Opin. Ther. Pat. 28 (2018) 665−678.

[19] L.S. Chupak, X. Zheng, Compounds Useful as Immunomodulators, WO2015034820, March 12, 2015.

[20] L.S. Chupak, M. Ding, S.W. Marin, X. Zheng, P. Hewawasam, T.P. Connolly, N. Xu, K-S. Yeung, J. Zhu, D.R. Langley, D.J. Tenney, P.M. Scola, P.A. Mingo, Compounds Useful as Immunomodulators, WO2015160641, October 22, 2015.

[21] K. Guzik, K.M. Zak, P. Grudnik, K. Magiera, B. Musielak, R. Torner, L. Skalniak, A. Domling, G. Dubin, T.A. Holak, Small-molecule inhibitors of the

programmed cell death-1/programmed death-ligand 1 (PD-1/PD-L1) interaction via transiently induced protein states and dimerization of PD-L1, J. Med. Chem. 60 (2017) 5857−5867.

[22] L. Skalniak, K.M. Zak, K. Guzik, K. Magiera, B. Musielak, M. Pachota, B. Szelazek, J. Kocik, P. Grudnik, M. Tomala, S. Krzanik, K. Pyrc, A. Dömling, G. Dubin, T.A. Holak, Small-molecule inhibitors of PD-1/PD-L1 immune checkpoint alleviate the PD-L1-induced exhaustion of T-cells, Oncotarget 8 (2017) 72167−72181.

[23] Z. Yu, L. Wu, W. Yao, Heterocyclic Compounds as Immunomodulators, US20180016260, January 18, 2018.

[24] L. Wu, Z. Yu, F. Zhang, W. Yao, N-Phenyl-pyridine-2-carboxamide Derivatives and Their Use as PD-1/PD-L1 Protein/Protein Interaction Modulators, WO2017106634, June 22, 2017.

[25] H. Shinkai, T. Ito, T. Iida, Y. Kitao, H. Yamada, I. Uchida, 4-Aminoquinolines: Novel nociceptin antagonists with analgesic activity, J. Med. Chem. 43 (2000) 4667−4677.

[26] J.H. Come, P.N. Collier, J.A. Henderson, A.C. Pierce, R.J. Davies, A.L. Tiran, H. O'Dowd, U.K. Bandarage, J. Cao, D. Deininger, R. Grey, E.B. Krueger, D.B. Lowe, J. Liang, Y. Liao, D. Messersmith, S. Nanthakumar, E. Sizensky, J. Wang, J. Xu, E.Y. Chin, V. Damagnez, J.D. Doran, W. Dworakowski, J.P. Griffith, M.D. Jacobs, S. Khare-Pandit, S. Mahajan, C.S. Moody, A.M. Aronov, Design and synthesis of a novel series of orally bioavailable, CNS-penetrant, isoform selective phosphoinositide

3-kinase γ (PI3Kγ) inhibitors with potential for the treatment of multiple sclerosis (MS), J. Med. Chem. 61 (2018) 5245−5256.

[27] Y. Wang, Z. Xu, T. Wu, M. He, N. Zhang, Aromatic Acetylene or Aromatic Ethylene Compound, Intermediate, Preparation Method, Pharmaceutical Composition and Use Thereof, WO2018006795, January 11, 2018.

[28] S. Basu, J. Yang, B. Xu, K. Magiera-Mularz, L. Skalniak, B. Musielak, V. Kholodovych, T.A. Holak, L. Hu, Design, synthesis, evaluation and structural studies of C2-symmetric small molecule inhibitors of the programmed cell death-1/programmed death-ligand 1 (PD-1/PD-L1) protein-protein interaction, J. Med. Chem. DOI: 10.1021/acs.jmedchem.9b00795.

[29] K.M. Zak, P. Grudnik, K. Guzik, B.J. Zieba, B. Musielak, A. Dömling, G. Dubin, T.A. Holak, Structural basis for small molecule targeting of the programmed death ligand 1 (PD-L1), Oncotarget 7 (2016) 30323−30335.

[30] M. Qin, Q. Cao, S. Zheng, Y. Tian, H. Zhang, J. Xie, H. Xie, Y. Liu, Y. Zhao, P. Gong, Discovery of [1,2,4]triazolo[4,3-*a*]pyridines as potent inhibitors targeting the programmed cell death-1/programmed cell death-ligand 1 interaction, J. Med. Chem. 62 (2019) 4703−4715.

Highlights

1. Several indoline compounds targeting the PD-1/PD-L1 pathway were developed.
2. **A13** potently inhibited of the PD-1/PD-L1 interaction, with an IC_{50} of 132.8 nM.
3. **A13** exhibited low toxicity to Jurkat T cells.
4. **A13** restored the immune response in a T cell-tumor co-culture model.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: