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Research Article

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Design, synthesis, and biological activity of novel pomalidomide linked with diphenylcarbamide derivatives

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Abstract: Based on 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione as a raw material, a series of novel pomalidomide linked with diphenylcarbamide derivatives were synthesized through several step reactions of substitution, click reaction, and addition reaction. The structures of these compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and MS. We discovered that some of the compounds are capable of suppressing indoleamine pyrrole-2,3-dioxygenase-1 activities in *in vitro* experiments, in which the inhibitory activity of **5b** reached the level of benefits.

Keywords: 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione, pomalidomide, diphenylcarbamide, derivatives, synthesis, IDO1

1 Introduction

In recent years, research on traditional small-molecule inhibitors has encountered difficulties, and scientists have focused on its improvement. The development of small-molecule inhibitors requires a different approach. Targeted protein degradation technology (PROTAC) subverts the treatment of small-molecule inhibitors in the past [1]. It can degrade the target protein through the protein degradation pathway *in vivo*, which has become a hot issue in the field of medicinal chemistry [2]. PROTAC small molecule recognizes E3 ubiquitin ligase and target protein by E3 ubiquitin ligase ligand and target protein ligand, respectively, making the target protein polyubiquitinated, and the proteasome recognizes and degrades the target protein [3]. This method not only improves the efficacy of the drug but also reduces the damage to the normal tissues of the human body, and has received extensive attention in recent years [4,5]. Indoleamine pyrrole-2,3-dioxygenase-1 (IDO1) is a monomeric enzyme containing heme, and its excessive activation has been shown to be closely related to the pathogenesis of cancer and other diseases. IDO1 inhibitors have received much attention as a possible treatment for cancer [6,7].

Pomalidomide is the third generation of IMiD produced by Celgene of USA. It is developed on the basis of the first-generation IMiD thalidomide, which modifies the molecular structure [8]. This improved IMiD could enhance the immune response mediated by T cells and NK cells, inhibit the production of monocyte proinflammatory cytokines, and induce apoptosis of cancer cells [9]. Thus, it is popular in various malignant tumor and immune disease treatments [10]. Recently, the molecular structure of pomalidomide is usually used as the ligand for E3 ligase in PROTAC production and it plays an important role in the development of protease degradation drugs [11,12] (Figure 1).

In this study, the pomalidomide was used as the E3 ubiquitin ligase ligand and diaryl urea structure as the IDO1 ligand, connected by a connecting chain to prepare a

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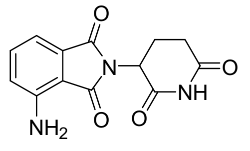


polamidomide

Figure 1: The structure of pomalidomide.

PROTAC small molecule. The process consists of synthesizing ten diphenylcarbamide-linked pomalidomide derivatives via click and other reactions by utilizing 2-(2,6-dioxo-piperidin-3-yl)-4-fluoro-isoindole-1,3-dione as the raw material.

Detailed operation is as follows: the 2-(2,6-dioxo-piperidin-3-yl)-4-fluoro-isoindole-1,3-dione reacted with 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine and N,N-diisopropylethylamine (DIPEA) in the N,N-dimethylformamide (DMF) to form compound **2**. Compound **2** reacted with 3-ethynylaniline or 4-ethynylaniline to form compound **3** or **4**. Compounds **3** and **4** were then reacted with carbonyl derivatives to give compounds **5a–5e** and **6a–6e**, respectively (Figure 2). The molecular structures of these compounds were analyzed using ^{1}H NMR, ^{13}C NMR, and MS. These seven compounds were also further tested for their anti-IDO1 activity.

2 Results and discussion

IDO1 inhibition study: through literature surveys, we employed HeLa cell-based functional assay to study the IDO1 inhibition activities of the designed compounds [13–15].

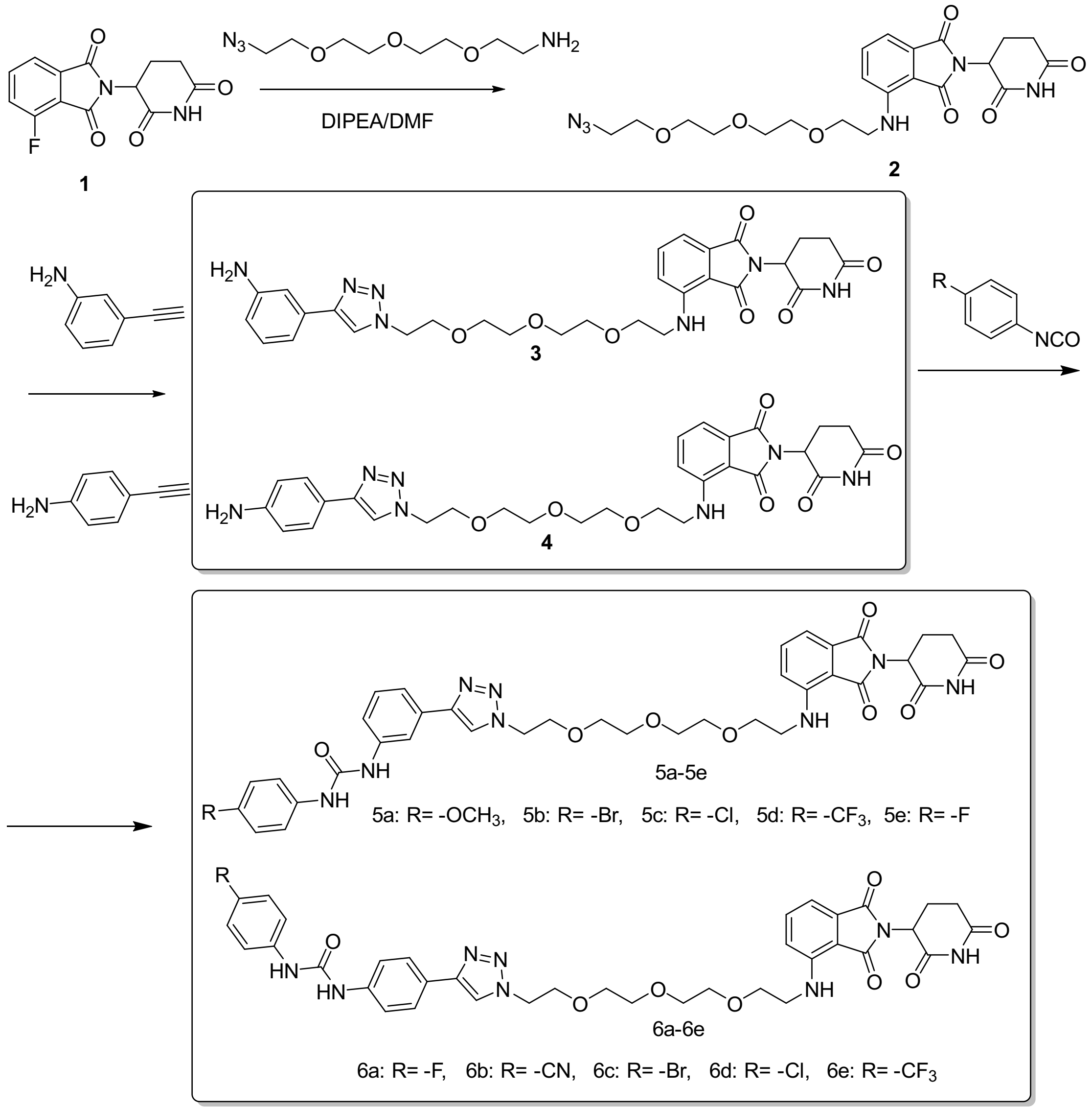


Figure 2: The reaction routes to compounds **5a–5e and 6a–6e**.

Table 1: IDO1 inhibitory activities of designed derivatives

Compound no.	IDO1/ IC$_{50}$ (μM)	Compound no.	IDO1/ IC$_{50}$ (μM)
5a	>100	**6a**	>100
5b	4.1 + 0.9	**6b**	>100
5c	31.62 + 1.6	**6c**	49.3 + 2.4
5d	>100	**6d**	>100
5e	>100	**6e**	>100

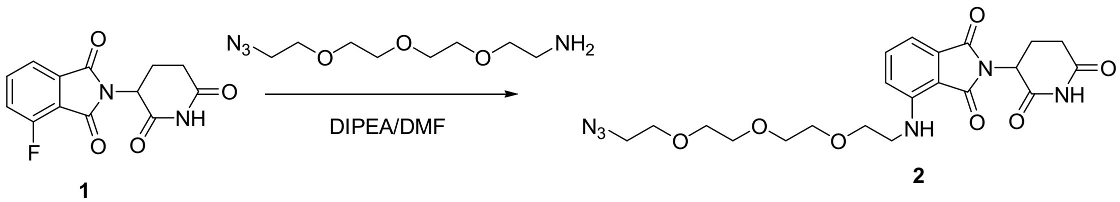
IC$_{50}$ values were fitted from single-point inhibition curves, and two parallel experiments were performed for each compound. These results are reported as average ± SD.

The IC$_{50}$ value of Amg-1 as a positive control was 3.62 μM (data not shown), which is consistent with the results previously reported in the literature (IC$_{50}$ = 3.0 μM) [16]. As shown in Table 1, the activities of compound **5b** against IDO1 were all below 5 μM, which is close to that of the Amg-1.

3 Experimental

1,2,3-Triazole derivatives were synthesized in-house by our research group. All reagents and solvents were obtained from a commercial source and used without further treatment. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 solution with a Bruker 400 spectrometer. Chemical shifts (*d*) were recorded in parts per million. Tetramethylsilane was used as an internal reference, and coupling constants were expressed in hertz. ESI MS was recorded on a Bruker Esquire 3000 instrument.

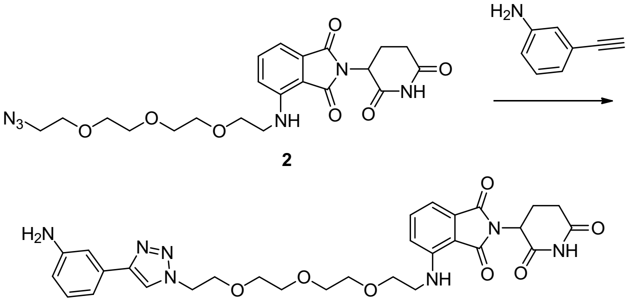
3.1 General procedure for the synthesis of compound 2



1 **2**

To a solution of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (2.8 g, 0.01 mol), dimethylformamide (150 mL) was added with 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine (2.2 g, 0.01 mol) and DIPEA (2.5 g, 0.02 mol) in N$_2$ atmosphere. The reaction mixture was refluxed for 5 h. After completion of the reaction, distilled water (100 mL) was added to the mixture and the aqueous phase was extracted with CH$_2$Cl$_2$ (3 × 70 mL). The combined organic phase was successively washed with water and brine, dried over Na$_2$SO$_4$, and concentrated *in vacuo* to give compound **2** as a pale yellow solid (2.9 g).

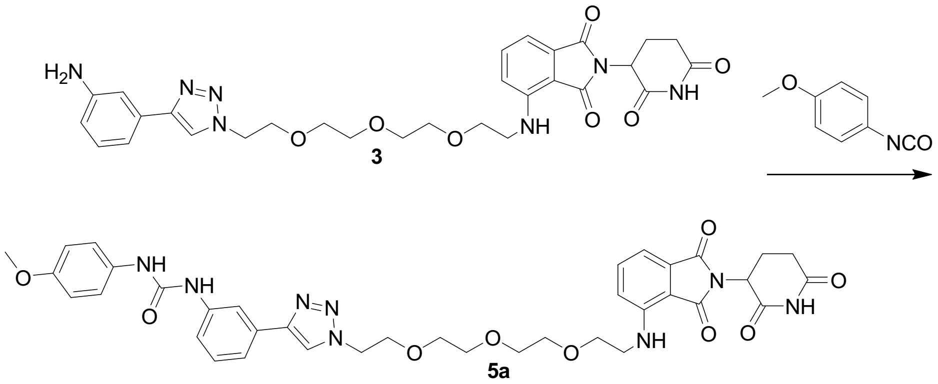
3.2 General procedure for the synthesis of compound 3 (the method is suitable for compound 4)



2 **3**

Compound 2 (4.8 g, 0.01 mol) and 3-ethynylaniline (1.4 g, 0.012 mol) were added to a mixed solvent (water:CH$_2$Cl$_2$:*tert*-butanol:THF = 1:1:1:1, 40 mL). Cuprous chloride (0.2 g, 0.002 mol) was added to the mixture, and the reaction was stirred at 65°C. After completion of the reaction, the mixture was extracted with CH$_2$Cl$_2$ (15 mL × 4). The combined organic phase was washed successively with water and brine, then dried with MgSO$_4$ and desolventized. The residue was purified by column chromatography (CH$_2$Cl$_2$/MeOH = 20:1) to obtain the desired compound **3** as a crystalline powder: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 8.33 (s, 1H), 7.11–7.03 (m, 4H), 6.92 (d, J = 8.0 Hz, 1H), 6.57 (t, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 2H), 6.52 (d, J = 8.0 Hz, 1H), 5.16–5.03 (m, 3H), 4.53 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H), 3.84 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 3.58–3.50 (m, 7H), 3.44–3.40 (m, 2H), 2.93–2.84 (m, 1H), 2.61–2.53 (m, 1H), 2.04–2.01 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.29, 170.56, 169.41, 167.76, 149.50, 147.34, 146.86, 136.70, 132.55, 131.76, 129.78, 121.74, 117.91, 113.97, 113.45, 111.14, 110.89, 109.70, 70.23, 70.21, 70.15, 70.09, 69.30, 69.13, 49.94, 49.02, 42.13, 40.54, 31.45, 22.61.

3.3 General procedure for the preparation of compound 5a (the method is suitable for 5b–5e, 6a–6e)



To a solution of compound **3** (6.0 g, 0.01 mol), CH_2Cl_2 (100 mL) was added with 1-isocyanato-4-methoxybenzene (1.5 g, 0.01 mol) in one portion. After stirring at room temperature for 2.5 h, the mixture was concentrated, and the residue was purified by column chromatography on silica gel (eluent:petroleum ether:ethyl acetate = 2:1) to give compound **5a** as a pale yellow solid (3.9 g).

3.3.1 Compound 5a

Yield: 72.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (s, 1H), 8.58 (s, 1H), 8.35 (d, J = 8.0 Hz, 1H), 7.42 (t, J_1 = 4.0 Hz, J_2 = 8.0 Hz, 1H), 7.27–7.18 (m, 5H), 6.96–6.89 (m, 2H), 6.75 (d, J = 12.0 Hz, 2H), 6.46–6.42 (m, 1H), 5.62 (s, 1H), 4.94 (dd, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 1H), 4.45–4.40 (m, 2H), 3.74 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 3.60 (s, 3H), 3.45–3.41 (m, 7H), 3.29–3.26 (m, 4H), 2.82–2.72 (m, 1H), 2.46–2.38 (m, 2H), 1.91 (d, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.29, 170.57, 169.42, 167.77, 155.00, 153.21, 146.82, 146.75, 140.92, 136.62, 133.13, 132.53, 131.80, 129.78, 122.19, 121.73, 120.57, 120.40, 119.14, 118.01, 117.80, 115.14, 114.44, 111.13, 110.93, 109.71, 70.23, 70.16, 70.11, 69.28, 69.12, 55.62, 55.36, 50.04, 49.04, 42.12, 31.47, 22.63. MS (ESI) m/z: 741 $[M + H]^+$.

3.3.2 Compound 5b

Yield: 79.7%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1H), 8.86 (s, 2H), 8.50 (s, 1H), 8.07 (s, 1H), 7.57 (t, J_1 = 8.0 Hz, J_2 = 8.0 Hz, 1H), 7.49–7.45 (m, 5H), 7.42–7.35 (m, 2H), 7.11–7.05 (m, 2H), 6.59 (s, 1H), 5.09 (dd, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H), 4.59 (s, 2H), 3.89 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 3.60–3.53 (m, 10H), 3.44 (s, 2H), 2.96–2.87 (m, 1H), 2.62–2.52 (m, 2H), 2.07–2.02 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.29, 170.56, 169.40, 167.76, 152.88, 146.82, 146.66, 140.51, 139.56, 139.45, 136.64, 132.53, 131.98, 131.84, 129.83, 122.24, 120.65, 119.50, 118.23, 117.81, 115.36, 113.75, 111.13, 109.70, 70.22, 70.15, 70.10, 69.28, 69.10, 60.24, 55.36, 50.04, 49.03, 42.12, 31.46, 22.63, 21.22. MS (ESI) m/z: 789 $[M + H]^+$.

3.3.3 Compound 5c

Yield: 78.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 8.46 (s, 1H), 8.04 (s, 1H), 7.57–7.53 (m, 4H), 7.41–7.36 (m, 2H), 7.32–7.30 (m, 4H), 7.08 (d, J = 12.0 Hz, 1H), 7.02 (d, J = 8.0 Hz, 1H), 6.55 (s, 1H), 5.04 (dd, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H), 4.55 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 4.04–4.00 (m, 3H), 3.85 (t, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 3H), 3.56–3.52 (m, 9H), 2.89–2.83 (m, 1H), 2.60–2.56 (m, 2H), 2.03–2.00 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.28, 170.56, 167.76, 153.17, 146.84, 146.76, 144.67, 139.55, 136.66, 132.54, 129.71, 128.98, 122.20, 120.19, 120.18, 118.22, 117.86, 115.37, 111.12, 109.69, 73.61, 70.66, 70.21, 70.15, 70.10, 69.28, 69.10, 63.82, 50.01, 49.02, 42.11, 31.78, 31.45, 22.60. MS (ESI) m/z: 745 $[M + H]^+$.

3.3.4 Compound 5d

^{1}H NMR (400 MHz, DMSO-d_6): 11.11 (s, 1H), 9.12 (s, 1H), 8.93 (s, 1H), 8.49 (s, 1H), 8.05 (s, 1H), 7.66 (dd, J_1 = 8.0 Hz, J_2 = 8.0 Hz, 4H), 7.55 (t, J_1 = 4.0 Hz, J_2 = 8.0 Hz, 1H), 7.44 (d, J = 4.0 Hz, 1H), 7.39–7.33 (m, 2H), 7.08 (d, J = 8.0 Hz, 1H), 7.02 (d, J = 8.0 Hz, 1H), 6.56 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H), 5.05 (dd, J_1 = 4.0 Hz, J_2 = 8.0 Hz, 1H), 4.56 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 3.86 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H), 3.57–3.53 (m, 5H), 3.52–3.48 (m, 5H), 3.45–3.38 (m, 3H), 2.91–2.84 (m, 1H), 2.58 (d, J = 16.0 Hz, 1H), 1.99 (s, 1H). MS (ESI) m/z: 779 [M + H]$^+$.

3.3.5 Compound 5e

Yield: 69.9%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 8.78 (s, 1H), 8.71 (s, 1H), 8.46 (s 1H), 8.01 (s, 1H), 7.57–7.53 (m, 1H), 7.50–7.46 (m, 2H), 7.41–7.30 (m, 3H), 7.14–7.07 (m, 3H), 7.02 (d, J = 8.0 Hz, 1H), 6.56 (t, J_1 = 4.0 Hz, J_2 = 8.0 Hz, 1H), 5.08–5.02 (m, 1H), 4.55 (t, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 2H), 3.85 (t, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 2H), 3.57–3.47 (m, 11H), 3.42–3.38 (m, 2H), 2.92–2.83 (m, 1H), 2.58 (d, J = 16.0 Hz, 1H), 2.04–1.98 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.28, 170.56, 169.40, 167.76, 158.63, 157.05, 153.10, 146.84, 146.68, 140.69, 136.66, 136.46, 132.54, 131.82, 129.81, 122.23, 120.52, 120.47, 119.33, 118.14, 117.86, 115.82, 115.67, 115.27, 111.13, 109.70, 70.22, 70.15, 70.10, 69.29, 69.10, 50.03, 49.02, 42.12, 40.53, 31.45, 22.61. MS (ESI) m/z: 729 [M + H]$^+$.

3.3.6 Compound 6a

Yield: 69.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 8.78 (s, 1H), 8.74 (s, 1H), 8.41 (s 1H), 7.75 (d, J = 4.0 Hz, 1H), 7.56 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H), 7.53 (d, J = 4.0 Hz, 2H), 7.49–7.46 (m, 2H), 7.15–7.09 (m, 3H), 7.03 (d, J = 8.0 Hz, 1H), 6.57 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H), 5.05 (dd, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H), 4.54 (t, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 2H), 3.85 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 3.58–3.50 (m,11H), 3.42 (dd, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 2.91–2.85 (m, 1H), 2.58 (d, J = 8.0 Hz, 1H), 2.06–1.98 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.30, 170.56, 169.40, 167.76, 158.64, 157.06, 157.03, 153.03, 146.84, 146.66, 143.13, 139.80, 136.67, 136.43, 132.54, 126.16, 124.99, 121.36, 120.51, 120.46, 118.91, 117.86, 115.83, 115.69, 111.14, 109.70, 70.22, 70.16, 70.11, 69.30, 69.14, 50.02, 49.02, 42.13, 31.45, 22.60. MS (ESI) m/z: 729 [M + H]$^+$.

3.3.7 Compound 6b

Yield: 55.8%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 9.28 (s, 1H), 9.01 (s, 1H), 8.42 (s 1H), 7.76 (dd, J_1 = 4.0 Hz, J_2 = 8.0 Hz, 1H), 7.66 (d, J = 4.0 Hz, 2H), 7.57–7.54 (m, 3H), 7.10 (d, J = 4.0 Hz, 1H), 7.03 (d, J = 4.0 Hz, 1H), 6.57 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H), 5.07–5.04 (m, 1H), 4.54 (t, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 2H), 3.85 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 3.55–3.50 (m, 8H), 3.42 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 2.91–2.85 (m, 1H), 2.58 (d, J = 12.0 Hz, 2H), 2.03–2.01 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.30, 170.56, 169.40, 167.76, 152.51, 146.84, 146.57, 144.65, 139.25, 136.67, 133.77, 132.53, 126.19, 125.49, 121.48, 119.80, 119.45, 119.23, 119.05, 118.54, 117.86, 111.14, 109.70, 103.74, 70.22, 70.15, 70.11, 69.30, 69.14, 50.04, 49.02, 42.13, 40.50, 31.45, 22.60. MS (ESI) m/z: 736 [M + H]$^+$.

3.3.8 Compound 6c

Yield: 74.9%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 8.84 (s, 1H), 8.81 (s, 1H), 8.41 (s 1H), 7.75 (d, J = 8.0 Hz, 2H), 7.57–7.52 (m, 3H), 7.47–7.43 (m, 5H), 7.11 (d, J = 4.0 Hz, 1H), 7.04 (d, J = 4.0 Hz, 1H), 6.58 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H), 5.07–5.04 (m, 1H), 4.54 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 3.85 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 3.58–3.51 (m, 10H), 3.43–3.41 (m, 2H), 2.91–2.85 (m, 1H), 2.60–2.55 (m, 1H), 2.04–2.01 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.29, 170.95, 170.56, 169.41, 167.76, 165.81, 152.80, 146.85, 146.63, 139.61, 139.56, 136.67, 132.55, 132.00, 126.17, 125.15, 121.40, 120.72, 120.64, 119.00, 117.87, 113.73, 111.14, 109.71, 70.23, 70.16, 70.11, 69.31, 69.14, 50.02, 49.02, 42.13, 31.45, 22.60. MS (ESI) m/z: 789 [M + H]$^+$.

3.3.9 Compound 6d

Yield: 70.04%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 8.83 (s, 1H), 8.80 (s, 1H), 8.41 (s 1H), 7.75 (d, J = 8.0 Hz, 2H), 7.57–7.49 (m, 5H), 7.34 (d, J = 4.0 Hz, 2H), 7.11 (d, J = 4.0 Hz, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.57 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H), 5.07–5.04 (m, 1H), 4.54 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 3.85 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 3.58–3.50 (m, 10H), 3.42 (dd, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 2.91–2.85 (m, 1H), 2.60–2.57 (m, 1H), 2.03–2.01 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.30, 173.26, 170.57, 167.77, 152.86, 146.85, 146.66, 139.71, 139.18, 136.68, 132.53, 129.09, 126.17, 125.85, 125.06, 121.42, 120.32, 120.25, 119.00, 111.15, 105.91, 91.79, 88.37, 75.29, 73.74, 70.22, 70.16, 70.11, 69.30, 69.14, 50.05, 49.02, 42.13, 31.76, 31.45, 22.61. MS (ESI) m/z: 745 [M + H]$^+$.

3.3.10 Compound 6e

Yield: 71.92%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 9.86–9.70 (m, 1H), 8.42 (s, 1H), 7.76 (d, J = 4.0 Hz, 2H), 7.69 (d, J = 4.0 Hz, 2H), 7.64 (d, J = 4.0 Hz, 2H), 7.58–7.54 (m, 3H), 7.11 (d, J = 4.0 Hz, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.57 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H), 5.07–5.04 (m, 1H), 4.54 (t, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 2H), 3.85 (t, J_1 =

4.0 Hz, J_2 = 4.0 Hz, 2H), 3.58–3.50 (m, 12H), 2.90–2.85 (m, 1H), 2.63–2.57 (m, 1H), 2.04–1.98 (m, 1H), 1.88–1.80 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.30, 172.56, 169.30, 168.76, 152.31, 147.84, 145.57, 144.25, 139.55, 137.67, 134.77, 132.53, 128.87, 126.19, 125.49, 121.48, 119.80, 119.45, 119.23, 119.05, 118.84, 118.54, 117.86, 117.05, 111.14, 109.70, 103.74, 70.22, 70.15, 70.11, 69.30, 69.14, 50.04, 49.02, 42.13, 40.50, 31.45, 21.60. MS (ESI) *m*/*z*: 779 $[M + H]^+$.

3.4 IDO1 enzymatic inhibition assay

To demonstrate the inhibitory effect of the designed compounds against IDO1, HeLa cells were seeded at 50,000–60,000 cells per well into a 96-well plate in 100 μL of Dulbecco's modified Eagle's medium, which is a complete growth medium for 12–18 h. On the second day, 100 μL per well of diluted inhibitor was added at a final concentration of 100 ng/mL human IFN-γ and then incubated at 37°C, 5% CO_2 for 18 h. On the third day, 140 μL of the medium was removed into a new 96-well plate and the protein was precipitated with 10 μL of 6.1 N TCA (CCl_3COOH) at 50°C for 30 min. The plate was centrifuged at 2,500 rpm for 10 min. Then, 100 μL of supernatant per well was transferred to another 96-well plate and mixed with 100 μL of *p*-dimethylaminobenzaldehyde in CH_3COOH [2% (w/v)]. The plate was incubated at 25°C for 10 min, and the yellow color derived from kynurenine was recorded by measuring absorbance at 480 nm using a microplate reader (PE, USA). Graphs of inhibition curves with IC_{50} values were generated using Prism 6.0.

4 Conclusions

In summary, a series of novel pomalidomide linked with diphenylcarbamide derivatives were synthesized and their inhibiting activities against IDO1 were evaluated. The inhibition assay results showed that some of the compounds exhibited moderate inhibitory activity. In this study, we displayed a new kind of novel anti-IDO1 agent, which could be further modified.

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Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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