ABSTRACT
In the current study, we designed and synthesised a novel series of 2-(2,6-dioxopiperidin-3-yl)isoquinoline-1,3(2H,4H)-dione derivatives as cereblon (CRBN) modulators. The results of the CCK8 assay revealed potent antiproliferative activity for the selected compound 10a against NCI-H929 (IC\textsubscript{50}=2.25 \mu M) and U239 (IC\textsubscript{50}=5.86 \mu M) cell lines. Compound 10a also inhibited the TNF-\alpha level (IC\textsubscript{50}=0.76 \mu M) in LPS stimulated PMBC and showed nearly no toxicity to this normal human cell line. The TR-FRET assay showed compound 10a having potent inhibitory activity against CRBN (IC\textsubscript{50}=4.83 \mu M), and the docking study confirmed a nice fitting of 10a into the active sites of CRBN. Further biology studies revealed compound 10a can increase the apoptotic events, arrest the NCI-H929 cells at G0/G1 cell cycle, and induce the ubiquitination degradation of IKZF1 and IKZF3 proteins by CRL4\textsubscript{CRBN}. These preliminary results suggested that compound 10a could serve as a potential antitumor drug and worthy of further investigation.

1. Introduction
Multiple myeloma (MM) is a malignant blood neoplasm characterised by an abnormal intramedullary proliferation of bone marrow cells and hypersecretion of monoclonal immunoglobulins\textsuperscript{1–12}. It accounts for 10% of all haematologic malignancies and generally occurs between 40 and 70 years of life\textsuperscript{3–5}. The immunomodulatory drugs (IMiDs), such as lenalidomide, a new class of anticancer agents with the glutarimide group are clinically effective in the treatment of MM\textsuperscript{6–8}. These drugs can inhibit the production of many inflammatory mediators such as tumour necrosis factor-alpha (TNF-\alpha), IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, and interferon-\gamma (IFN-\gamma), inhibiting the secretion of beta fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF)\textsuperscript{9,10}, showing pleiotropic effects on MM cells and their microenvironment, promoting cell apoptosis, interfering with the production of cell adhesion factors, regulating the production of cytokines and inhibiting the production of tumour related angiogenesis\textsuperscript{11–13}.

Cereblon (CRBN), the molecular target of these IMiDs, is a substrate receptor for the CRL4 (CUL4-RBX1-DDB1) ubiquitin ligase complex\textsuperscript{14–17}. CRBN ligand binding confers neomorphic activity, altering the substrate specificity of the ubiquitin ligase by promoting the recruitment of substrate proteins\textsuperscript{18–21}. Once binding to CRBN, IMiDs promote the degradation of IKZF1 and IKZF3 through the ubiquitination dependent proteasome pathway, thereby driving the clinical activity in MM\textsuperscript{22–26}. Thalidomide is the first IMID approved for the treatment of MM. As thalidomide functioned successfully as an IMID\textsuperscript{27,28}, next generation IMiDs, such as lenalidomide\textsuperscript{29,30}, pomalidomide\textsuperscript{31,32}, CC-122\textsuperscript{33,34}, and TD-106\textsuperscript{35}, which have a good effect on MM, were developed (Figure 1).

The crystal structure of CRBN-DDB1 binding to lenalidomide shows mechanistic insight into how IMiDs act on CRL4\textsubscript{CRBN}. The IMiD compounds bind CRBN through their shared glutarimide ring, leaving portions of their variable phthaloyl ring solvent-exposed\textsuperscript{14}. In this study, we describe the discovery of a series of isoquinoline-1,3(2H,4H)-dione derivatives as a type of novel CRBN modulator, which retain the glutarimide group and enlarge the five membered ring in the middle of the compound to six membered ring (Figure 2). The SAR of all the newly synthesised compounds were studied by the proliferation assay. The TNF-\alpha inhibition ability and toxicity to normal human cells were also investigated. The most potent compound 10a was selected to be further studied through the TR-FRET assay and molecular docking to identify its CRBN binding activity. Furthermore, the effect of 10a on the induction of apoptosis and cell cycle on NCI-H929 cell line were investigated using flow cytometry. The IKZF1 and IKZF3 proteins degradation ability of 10a was also investigated by immunoblot.

2. Results and discussion
2.1. Chemistry
The synthetic route for 2-(2,6-dioxopiperidin-3-yl)isoquinoline-1,3(2H,4H)-dione derivatives is depicted in Scheme 1. Briefly, compounds 3a–c were synthesised from the commercial homophthalic anhydride derivatives and reacted with 3-aminopiperidine-2,6-
were prepared from the reaction of compound selected cancer cell lines with IC50 values (Table 1). The ability of the new compounds to inhibit the growth of the contrasted with lenalidomide using CCK8 assay. The results revealed All the new compounds were evaluated for their antiproliferative

2.2.1. Antiproliferative activity

The IMiDs were reported to induce apoptosis through activation of caspase 8 in MM cells. Accordingly, the ability of the selected compound 10a to induce apoptosis in NCI-H929 cells was investigated using annexin V fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining assay. NCI-H929 cells were treated with compound 10a and lenalidomide for 72 h. The results are presented in Figure 4.

The results revealed a significant dose dependent increase of the apoptotic events by compound 10a from 6.0% in the control, to 29.5% and 34.6% at 0, 1, and 5 μM concentration, and be equivalent to lenalidomide from 5.9% in the control, to 31.2% and 43.9%. We can see the significant increase in the percentages of early apoptosis from 3.4% to 13.3% and late apoptosis from 2.6% to 21.3%. Confirmed compound 10a can induce NCI-H929 cells apoptosis.

2.2.4. Annexin V-FITC/PI apoptosis assay

To better understand the mechanism by which compound 10a inhibits MM cell growth, cell cycle analysis was performed with the selected compound 10a and contrasted with lenalidomide. The NCI-H929 cells were treated with each of the compounds at concentrations.

The results of the cell cycle analysis revealed that compound 10a and lenalidomide can induce G0/G1 cell cycle arrest. The cell cycle arrest ability of 10a showed a dose-dependent manner in the G0/G1 cell cycle from 34.0% to 42.9% at 0 and 5.0 μM concentrations.
2.2.6. Immunoblot analysis

The earlier studies have revealed that the CRBN modulators thalidomide, lenalidomide, and pomalidomide can induce the ubiquitination of IKZF1 and IKZF3 by CRL4CRBN. Subsequent proteasome degradation of these transcription factors kills MM cells. Accordingly, we used the immunoblot assay to measure the IKZF1 and IKZF3 proteins degradation in the current study. The results revealed that compound 10a induced the degradation of IKZF1 and IKZF3 as lenalidomide (Figure 6(A)).

**Table 1.** Antiproliferative activity and TNF-α inhibition in LPS stimulated human PBMC of the compounds.

| Comp. no | IC₅₀ (µM ± SD)ᵃ | PBMC cell viability (%)ᵇ |
|----------|-----------------|-------------------------|
| 3a       | 32.24 ± 1.42    | >50                     |
| 3b       | 28.22 ± 0.92    | 36.38 ± 1.22            | 43.84 ± 1.88 |
| 3c       | >50             | >50                     | 100         |
| 9a       | 9.26 ± 0.56     | 12.24 ± 0.58            | 5.48 ± 1.04 |
| 10a      | 2.25 ± 0.09     | 5.86 ± 0.12             | 0.76 ± 0.08 |
| 10b      | 16.28 ± 0.56    | 20.56 ± 0.82            | 21.28 ± 1.26 |
| 10c      | 18.65 ± 0.83    | 26.32 ± 0.76            | 38.46 ± 1.38 |
| 10d      | >50             | >50                     | >50         |
| 12a      | >50             | >50                     | 100         |
| 12b      | >50             | >50                     | 100         |
| Lenalidomide | 1.12 ± 0.06   | 3.24 ± 0.11            | 0.13 ± 0.02 |

ᵃIC₅₀: the half maximal inhibitory concentration.
ᵇCell viability measured by the CCK-8. The viable cell number was expressed as a percentage relative to control cells.

**Scheme 1.** Reagents and conditions: (a) CH₃COONa, CH₃COOH, reflux, 24 h; (b) CH₃ONa, CuBr, 80°C, 24 h; (c) TBTU, DIPEA, DCM, rt, overnight; (d) DM5O, 10% NaOH, rt, 6 h; (e) CH₃COOH, reflux, 12 h; (f) SnCl₂·2H₂O, CH₃OH; (g) K₂CO₃, DMF, alkyl halide, rt, 4 h; (h) SnCl₂·2H₂O, CH₃OH.

**Figure 3.** Determination of the relative cereblon binding affinities for compound 10a and lenalidomide by TR-FRET.
significant dose dependent, and can completely degrade IKZF1 and IKZF3 at 10 μM concentration for 72 h in NCI-H929 cells.

To further explore this phenomenon, we treated the NCI-H929 cells with compound 10a (10 μM) alone or in combination with the proteasome inhibitor bortezomib (100 nm)\(^{37,38}\). The Western blot results (Figure 6(B)) showed that beginning 1 h at 100 nM bortezomib treatment can block the degradation of IKZF1/3, confirmed the proteins degradation was mediated by the proteasome.
2.3. Docking study into CRBN

To elucidate whether compound 10a targeted the CRBN protein, we carried out a molecular docking study to predict the possible binding mode of compound 10a with the CRBN. The outcomes of the molecular docking study showed that the binding mode of compound 10a within the binding pocket of CRBN (binding energy of $-7.5$ kcal/mol). The glutarimide group is held in a buried cavity between CRBN sheets $\beta_{10}$ and $\beta_{13}$, which has the similar binding pose with lenalidomide as the literature report (Figure 7)\textsuperscript{14}. Furthermore, the 8-aminoisoquinoline-1,3(2H,4H)-dione carbonyl (C1), the glutarimide carbonyl (C6) and the intervening amide (N1) are in hydrogen-bonding distance to CRBN residues Trp402, Trp382, and His380, respectively. By comparison to lenalidomide, the glutarimide carbonyls (C2 and C6) are in hydrogen-bonding distance to CRBN residues His380 and Trp382, respectively. The molecular docking analyses indicated that compound 10a binds snugly into the active sites of CRBN.

3. Conclusions

In summary, we designed and synthesised a novel series of 2-(2,6-dioxopiperidin-3-yl)isoquinoline-1,3(2H,4H)-dione derivatives as new kinds of CRBN modulators. Study on the SAR of the derivatives based on cell proliferation assay, which resulted in the discovery of compound 10a with considerably antiproliferative potency against MM cell lines NCI-H929 ($IC_{50}=2.25$ mM) and U239 ($IC_{50}=5.86$ mM), and nearly no toxicity to the normal human cell line PMBC at 20 mM concentration. Compound 10a also can reduce the TNF-$\alpha$ level ($IC_{50}=0.76$ $\mu$M) in LPS stimulated PMBC. The TR-FRET analysis and molecular docking study results of compound 10a agreed with its ability of inhibiting CRBN and predicted binding mode. Further biology studies revealed compound 10a can increase the apoptotic events, arrest the NCI-H929 cells at G0/G1 cell cycle, and induce the degradation of IKZF1 and IKZF3 proteins degradation by CRL4$^{CRBN}$. Our findings suggested that compound 10a could be considered as a potential anti-MM drug candidate or as a novel CRBN modulator which can be used for targeted protein degradation for further development.

4. Experimental

4.1. Chemistry

4.1.1. Materials and methods

Unless otherwise noted, all reagents and solvents were obtained from commercially available sources and were used without purification. $^1$H NMR spectra were tested in CDCl$_3$ or DMSO-$d_6$ with TMS as the internal reference on a Bruker AVANCE 400 (Billerica, MA). Mass spectra (MS) were obtained from Agilent 1100 mass spectrometer (Santa Clara, CA) with an electron spray ionisation source.

4.1.2. Synthesis of 2-(2,6-dioxopiperidin-3-yl)isoquinoline-1,3(2H,4H)-dione (3a)

A mixture of homophthalic anhydride 1a (1.0 g, 6.2 mmol), 3-aminopiperidine-2,6-dione hydrochloride 2 (1.0 g, 6.1 mmol), and sodium acetate anhydrous (0.85 g, 6.2 mmol) was added in acetic acid 50 mL. The resulting mixture was heated to reflux for 24 h. After cooling to room temperature, acetic acid was evaporated and the residue was purified by flash column chromatography (methanol: dichloromethane = 1:20) to obtain compound 3a as a slightly yellow solid (1.5 g, 90%). $^1$H NMR (400 MHz, DMSO-$d_6$): 10.94 (s, 1H), 8.12–7.98 (m, 1H), 7.70 (t, $J=7.4$ Hz, 1H), 7.53–7.47 (m, 1H), 7.42 (d, $J=7.6$ Hz, 1H), 5.66–5.58 (m, 1H), 4.37–4.13 (m, 2H), 2.92–2.83 (m, 1H), 2.57–2.43 (m, 2H), and 1.96–1.90 (m, 1H); LCMS [M+H]$^+$: 273.03.
4.1.3. 2-(2,6-Dioxopiperidin-3-yl)-7-methoxyisoquinoline-1,3(2H,4H)-dione (3b)

It was prepared as for 3a as a yellow solid, 85% yield. \(^1\)H NMR (400 MHz, DMSO-d6): 8.26 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.86 (d, J = 8.5 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 5.88 (s, 1H), and 3.72 (s, 6H).

4.1.4. 2-(2,6-Dioxopiperidin-3-yl)-7-methoxy-4,4-dimethylisoquinoline-1,3(2H,4H)-dione (3c)

It was prepared as for 3a as a yellow solid, 85% yield. \(^1\)H NMR (400 MHz, DMSO-d6): 8.26 (d, J = 8.0 Hz, 1H), 7.74 (t, J = 8.0 Hz, 1H), 6.07 (s, 1H), and 3.63 (s, 6H).

4.1.5. Synthesis of 2-((3,1-dimethoxy-1,3-dioxopropan-2-yl)-6-nitrobenzoic acid (6a)

To a solution of dimethyl malonate, 15 mL was added 2-chloro-6-nitro-benzoic acid (6a) 1.4 g (10.8 mmol), and 3-aminopiperidine-2,6-dione hydrochloride (1.0 g, 2.5 mmol), TBTU (1.6 g, 5.0 mmol), DIPEA (1.3 g, 10.8 mmol), and sodium methanolate (0.8 g, 15.0 mmol) under nitrogen. The mixture was stirred at room temperature for 30 min, then added cuprous bromide (0.12 g, 0.8 mmol). The resulting mixture was heated at 80°C for 24 h. After cooling to room temperature, water 50 mL was added to the mixture followed by hexanes 50 mL. The aqueous layer was separated and added to toluene 50 mL, and the biphasic mixture was filtered through celite to remove insolubles. Then the aqueous layer was separated and acidified with 6 N aqueous HCl to pH 2–3, and then extracted twice with ethyl acetate. The combined organic phase was washed with brine and dried over anhydrous Na\(_2\)SO\(_4\). After filtration and evaporation, the crude residue was extracted twice with dichloromethane. The combined organic phase was combined and dried to give the crude compound 7a, which was used for the next reaction without further purification.

The compound 7a was dissolved in DMSO 5 mL and then added into 10% NaOH aqueous solution 1 mL. The mixture reacted under room temperature for 6 h, and then water 20 mL was added and acidified with concentrated HCl to pH 2. The white precipitate was filtered and washed twice with methanol 10 mL to obtain compound 8a (0.5 g, 44%) as a white solid. \(^1\)H NMR (400 MHz, DMSO-d6): 8.06 (s, 1H), 8.46 (s, 1H), 7.59 (s, 1H), 7.19 (s, 1H), 5.71 (s, 1H), 3.82–3.72 (m, 3H), 2.96–2.78 (m, 1H), 2.56–2.55 (m, 1H), and 1.90 (s, 1H).

4.1.10. 2-((2,6-Dioxopiperidin-3-yl)carbamoyl)-4-nitrophenyl)acetic acid (8b)

It was prepared as for 8a as a yellow solid, 53% yield. \(^1\)H NMR (400 MHz, DMSO-d6): 8.19 (d, J = 14.0 Hz, 1H), 8.70 (d, J = 42.8 Hz, 1H), 8.19 (s, 2H), 5.92–5.73 (m, 1H), 3.85 (d, J = 19.5 Hz, 3H), 2.94–2.84 (m, 1H), 2.60–2.50 (m, 1H), and 2.02–1.92 (m, 1H).

4.1.11. 2-((2,6-Dioxopiperidin-3-yl)carbamoyl)-5-methylphenyl)acetic acid (8c)

It was prepared as for 8a as a yellow solid, 44% yield. \(^1\)H NMR (400 MHz, DMSO-d6): 11.00 (s, 1H), 8.48–8.43 (m, 2H), 7.89 (s, 1H), 5.64–5.53 (m, 1H), 3.68 (s, 2H), 2.91–2.82 (m, 1H), 2.58–2.39 (m, 2H), and 1.99–1.91 (m, 1H).

4.1.12. 2-((2,6-Dioxopiperidin-3-yl)carbamoyl)-6-nitrophenyl)acetic acid (8d)

It was prepared as for 8a as a yellow solid, 40% yield. \(^1\)H NMR (400 MHz, DMSO-d6): 10.90 (s, 1H), 8.48–8.43 (m, 2H), 7.89 (s, 1H), 5.64–5.53 (m, 1H), 3.68 (s, 2H), 2.91–2.82 (m, 1H), 2.58–2.39 (m, 2H), and 1.99–1.91 (m, 1H).

4.1.13. Synthesis of 2-((2,6-dioxopiperidin-3-yl)-8-nitrosoisoquinoline-1,3(2H,4H)-dione (9a)

Compound 8a (1.0 g, 3.0 mmol) was added into acetic acid 30 mL and stirred at reflux for 12 h. After cooling to room temperature, acetic acid was evaporated and the residue was purified by flash column chromatography (methanol:dichloromethane = 1:20) to obtain compound 9a (0.8 g, 85%) as a slightly yellow solid. \(^1\)H NMR (400 MHz, DMSO-d6): 10.95 (d, J = 7.8 Hz, 1H), 7.89 (t, J = 7.7 Hz, 1H), 7.87 (t, J = 9.9 Hz, 1H), 7.66 (m, 1H), 5.58 (s, 1H), 4.50–4.32 (m, 2H), 3.37 (s, 1H), 2.88–2.79 (m, 1H), 2.57–2.49 (m, 1H), 2.41–2.31 (m, 1H), and 1.95–1.88 (m, 1H); LCMS [M + H]^+ : 318.01.
It was prepared as for 9a as a yellow solid, 74% yield. $^1$H NMR (400 MHz, DMSO-d$_6$): 10.98 (s, 1H), 8.54–8.41 (m, 2H), 7.80–7.76 (m, 1H), 5.65–5.61 (m, 1H), 4.67–4.46 (m, 2H), 2.93–2.84 (m, 1H), 2.58–2.49 (m, 2H), and 1.99–1.92 (m, 1H).

4.1.17. Synthesis of 8-amino-2-(2,6-dioxopiperidin-3-yl)isoquinoline-1,3(2H,4H)-dione (10a) 

Compound 9a (100 mg, 0.32 mmol) and stannous chloride dihydrate (360 mg, 1.6 mmol) were added into methanol 5 mL and stirred at reflux for 6 h. The solution was evaporated and added into water 20 mL, and alkali was added to pH = 7. The aqueous solution was extracted by dichloromethane (3 × 20 mL), then the combined organic phase was washed with brine and dried over anhydrous Na$_2$SO$_4$. After filtration and evaporation, the crude residue was purified by flash column chromatography (methanol:dichloromethane = 1:20) to obtain 10a (27 mg, 33%) as a yellow solid. $^1$H NMR (400 MHz, DMSO-d$_6$): 10.89 (s, 1H), 7.28–7.22 (m, 1H), 7.10 (s, 1H), 6.69 (dd, $J$ = 16.0, 8.4 Hz, 1H), 6.41 (dd, $J$ = 7.2, 0.9 Hz, 1H), 5.61–5.52 (m, 1H), 4.12–3.92 (m, 2H), 2.89–2.82 (m, 1H), 2.56–2.37 (m, 2H), and 1.97–1.87 (m, 1H); LCMS [M + H]$^+$: 288.08.

4.1.18. 7-Amino-2-(2,6-dioxopiperidin-3-yl)isoquinoline-1,3(2H,4H)-dione (10b) 

It was prepared as for 10a as a yellow solid, 30% yield. $^1$H NMR (400 MHz, DMSO-d$_6$): 10.91 (d, $J$ = 8.0 Hz, 1H), 7.32–7.18 (m, 1H), 7.06 (d, $J$ = 11.2 Hz, 1H), 6.91 (d, $J$ = 8.0 Hz, 1H), 5.63–5.55 (m, 1H), 4.07–3.89 (m, 2H), 2.91–2.82 (m, 1H), 2.57–2.38 (m, 2H), and 1.94–1.87 (m, 1H); LCMS [M + H]$^+$: 288.08.

4.1.19. 6-Amino-2-(2,6-dioxopiperidin-3-yl)isoquinoline-1,3(2H,4H)-dione (10c) 

It was prepared as for 10a as a yellow solid, 33% yield. $^1$H NMR (400 MHz, DMSO-d$_6$): 10.85 (s, 1H), 7.89–7.62 (m, 1H), 6.80–6.23 (m, 2H), 5.59–5.50 (m, 1H), 4.18–3.96 (m, 2H), 2.87–2.81 (m, 1H), 2.54–2.43 (m, 2H), and 1.87 (brs, 1H); LCMS [M + H]$^+$: 288.08.

4.1.20. 5-Amino-2-(2,6-dioxopiperidin-3-yl)isoquinoline-1,3(2H,4H)-dione (10d) 

It was prepared as for 10a as a yellow solid, 28% yield. $^1$H NMR (400 MHz, DMSO-d$_6$): 10.91 (s, 1H), 7.38–7.16 (m, 2H), 6.96–6.94 (m, 1H), 5.63–5.56 (m, 1H), 3.88–3.76 (m, 2H), 2.91–2.81 (m, 1H), 2.57–2.39 (m, 2H), and 1.94–1.88 (m, 1H); LCMS [M + H]$^+$: 288.08.

4.1.21. 8-Amino-2-(2,6-dioxopiperidin-3-yl)-4,4-dimethylisoquinoline-1,3(2H,4H)-dione (12a) 

It was prepared as for 10a as a yellow solid, 34% yield. $^1$H NMR (400 MHz, DMSO-d$_6$): 10.89 (s, 1H), 7.34–7.29 (m, 1H), 6.77–6.70 (m, 2H), 5.75–5.59 (m, 1H), 2.97–2.88 (m, 1H), 2.74–2.67 (m, 1H), 2.47–2.32 (m, 1H), 1.95–1.90 (m, 1H), and 1.52 (dd, $J$ = 31.1, 7.2 Hz, 6H); LCMS [M + H]$^+$: 316.06.
and PI staining). Stained cells were analysed on a Flow Cytometer (BD Biosciences, Franklin Lakes, NJ), and the data were analysed using the Cell Quest software.

4.2.2. TNF-α ELISA assay

PBMC (4 × 10^5 cells) were incubated in the 96-well culture plate and stimulated by 2 μg/mL LPS. The level of TNF-α was checked by standard ELISA (R&D Systems, Minneapolis, MN). Analysis was performed by following the manufacturer’s procedure for each ELISA kit. The test samples were assessed in triplicate and absorbance was taken on a Microplate Reader (ELx-800, BioTek Instruments, Winooski, VT) at 450 nm. The IC50 value for each compound was calculated by comparison with standard curves with purified recombinant TNF-α using GraphPad Prism 5.0 software (San Diego, CA).

4.2.3. Immunoblot analysis

For immunoblot analysis, NCI-H929 cells were treated with the studied compounds at the indicated concentrations for various times, collected, and lysed in RIPA buffer in the presence of protease inhibitors on ice for 30 min. The protein from each sample was quantitated using a BCA protein assay kit, and was separated by SDS-PAGE and then transferred onto the PVDF membrane. The membranes were probed with specific primary antibodies at 4°C overnight, followed by incubation with IRDye®680 goat anti-mouse secondary antibodies. The signals were acquired using the Odyssey Infrared Imaging System (LI-COR, Lincoln, NE).

4.2.4. TR-FRET assay

His-tagged CRBN-DDB1 complex (Abcam, Cambridge, UK, catalogue no. ab235611) 60 nM was mixed with Eu-anti-His tag antibody 3 nM (Thermo Fisher, Waltham, MA, catalogue no. PV5596) in a final buffer containing 20 mM HEPES pH 7.0, 150 mM NaCl, and 0.005% Tween-20. The solution was then mixed with Cy5-labeled thalidomide 10 nM and various concentrations of compounds, and then was incubated at room temperature for 1 h. FRET signals were measured on an EnVision plate reader by exciting at 340 nm and recording emission at both 615 nm (no FRET control) and 665 nm (FRET signals) with a 60 μs delay. FRET efficiency was calculated by the ratio of 665 nm/615 nm. Quantitative loss of FRET efficiency as a function of compound concentrations was fitted by GraphPad Prism 5.0 (San Diego, CA) and calculated the IC50.

4.3. Molecular docking studies

The docking studies were done using AutoDock 4.2.6. During the docking simulations, the pdb file of CRBN (pdb: 4CI2) was downloaded from protein data bank (http://www.rcsb.org/pdb) and the ligand and its single bonds were moved freely within the potential binding pocket. Discovery studio and PyMOL softwares were used to visualise the binding interaction. The result of the docking study of the compound 10a is represented in Figure 7.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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