DESIGN, MOLECULAR MODELING AND SYNTHESIS OF NEW IMMUNOMODULATORY AGENTS FOR BIOLOGICAL STUDIES

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ABSTRACT

Cancer is the second leading cause of death worldwide. This work is an effort to find new effective and safe anticancer agents. In accordance with thalidomide features as immunomodulator anticancer drug, we designed and synthesized ten new thalidomide analogs. The synthesized compounds were biologically evaluated for their anti-tumor activity against three human cancer cell lines namely; hepatocellular carcinoma (HepG2), prostate cancer (PC3) and breast cancer (MCF-7). Thalidomide was used as a reference drug. The obtained data showed that compound **10a** is far better than thalidomide against the three cancer cell lines. It exhibited IC_{50} = 3.89, 4.01 and 2.91 µg/mL against the cell lines, respectively. While thalidomide exhibited IC_{50} values of 11.26, 14.58, and 16.87 µg/mL against the three cell lines, respectively. Moreover, compounds **7a, 6a** and **8** were found to be better than thalidomide against MCF-7 cell line. As they showed IC_{50} values of 10.32, 12.15 and 15.32 µg/mL, respectively. Furthermore, compounds **6a, 7a**, and **8** showed strong activity against the three cell lines. Results of docking studies showed that our compounds can accommodate the pocket of CRBN with binding energies too close to that of thalidomide.

Key words: Anticancer, immunomodulator, thalidomide

1- Introduction

Cancer is a life threatening disease and is reported as the second leading cause of death worldwide(Siegel, Miller, and Jemal 2020)(Jemal et al. 2008). At the same time nonselective chemotherapeutic agents are known to cause severe side effects (Mac Donald 2012). These two points strongly encourage us to develop new effective and safe anticancer agents. One of the promising safe and effective approach to cancer treatment is the development of immunomodulatory agents (Fernández-Lázaro et al. 2018).

Thalidomide (I) (**Fig. 1**) is an immunomodulator anticancer drug of a particular significance (Jin et al. 2013). It is a synthetic glutamic acid derivative originally marketed as a sedative and antiemetic in 1954. However, in 1961 it was quickly withdrawn from distribution when its teratogenic properties were discovered (Ito et al. 2010). Several years later the serendipitous finding that thalidomide could allay the symptoms of erythema nodosum leprosum (ENL) led to its re-emergence as a treatment for various pro-inflammatory and autoimmune conditions (Okafor 2003). In 1994, speculation that thalidomide teratogenicity is linked to the repression of angiogenesis (Ito, Ando, and Handa 2011) resulted in a new wave of clinical investigations that expanded the use of thalidomide for the treatment of various malignancies, including multiple myeloma (MM) (Singhal *et al.* 1999), melanoma, renal-cell carcinoma and prostate cancer (Eleutherakis-Papaiakovou, Bamias, and Dimopoulos 2004). Thalidomide was given FDA approval for the treatment of acute ENL in 1998, after further investigations found an immunological basis for this effect. It also received FDA approval in 2006 for the treatment of newly diagnosed MM (Diggle 2001).

Modification of thalidomide afforded anticancer drugs of significant activity e.g. lenalidomide (**II**) (Lopez-Girona *et al.* 2012), pomalidomide (**III**) (Lopez-Girona *et al.* 2012) and CC-122 (**IV**) (**Fig.1**) (Hagner *et al.* 2015).

lenalidomide (**II**) was found to be more potent than thalidomide as an inhibitor of TNF-α (Akobeng and Stokkers 2009). In 2006, it was approved by FDA for treatment of MM (Attal *et al.* 2012).

pomalidomide (**III**) was 10-fold more potent than lenalidomide (**2**) as a TNF-α inhibitor and interleukin-2 (IL-2) stimulator (Galustian and Dalgleish 2011). It also showed better anti-angiogenic results than thalidomide (**1**) and lenalidomide (**2**). In 2013, FDA approved pomalidomide for the treatment of MM (Offidani *et al.* 2014).
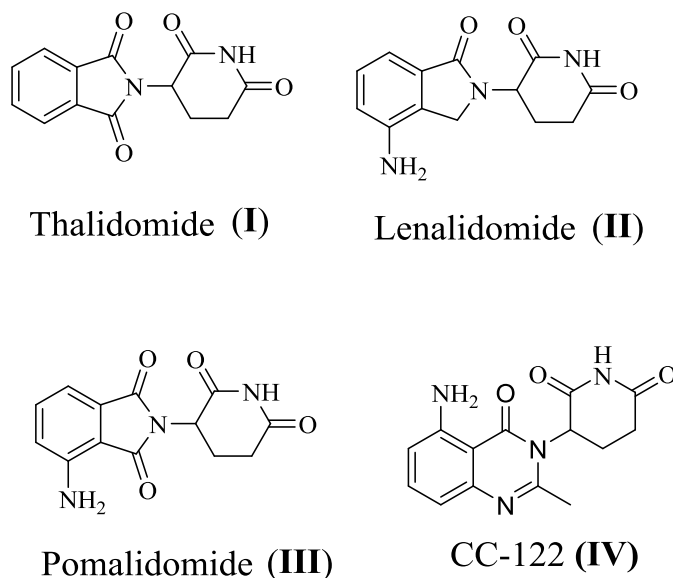
Figure 1: Structure of thalidomide and some of its analogs

CC-122 draw attention as one of thalidomide analogs. It showed potent anti-proliferative, immunomodulatory and anti-angiogenic activities with a potentially broader range of activity than lenalidomide (P. *et al.* 2014). It was found to be effective against diffuse large B-cell lymphoma (DLBCL), multiple myeloma (MM) and solid tumors (Hagner *et al.* 2015) , (T. *et al.* 2010). In line with the approach of thalidomide modification, we designed new analogs for anticancer testing.

2- Rationale and design

Modification of thalidomide was carried out at three positions as illustrated in **Fig. 2**. The first position is phthalimido moiety which was replaced by benzodiazine (quinoxaline) which is a bioisostere to quinazoline of CC-122. This is because thalidomide is not a flexible compound. As glutarimido moiety is almost perpendicular to phthalimido moiety and the rotation around the sigma bond between them is highly restricted due to steric effect. To increase the rotation and flexibility of our compounds the five membered heteroaromatic ring was replaced with six membered ring. The second site of modification was the glutarimido moiety. It was replaced with sulfonylpiperazine, piperazinocarboxamide and piperazinocarboxylate. These moieties are able to form Hydrogen. bonds as glutarimide moiety with better flexibility. The third modification was the addition of a terminal hydrophobic group to study the effect of this extension on activity. Several compounds with similar extensions were reported to have promising activities (see **Fig.3**).
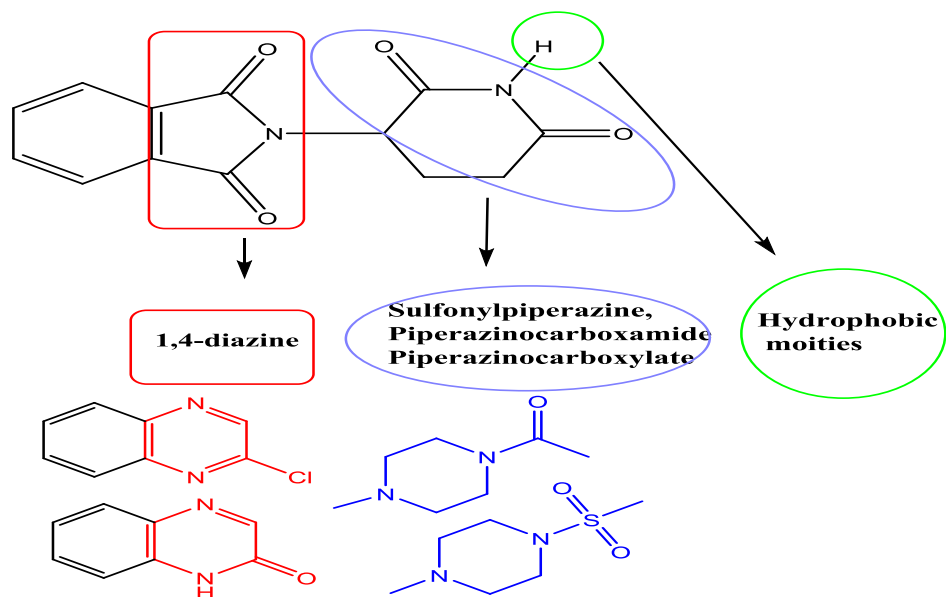
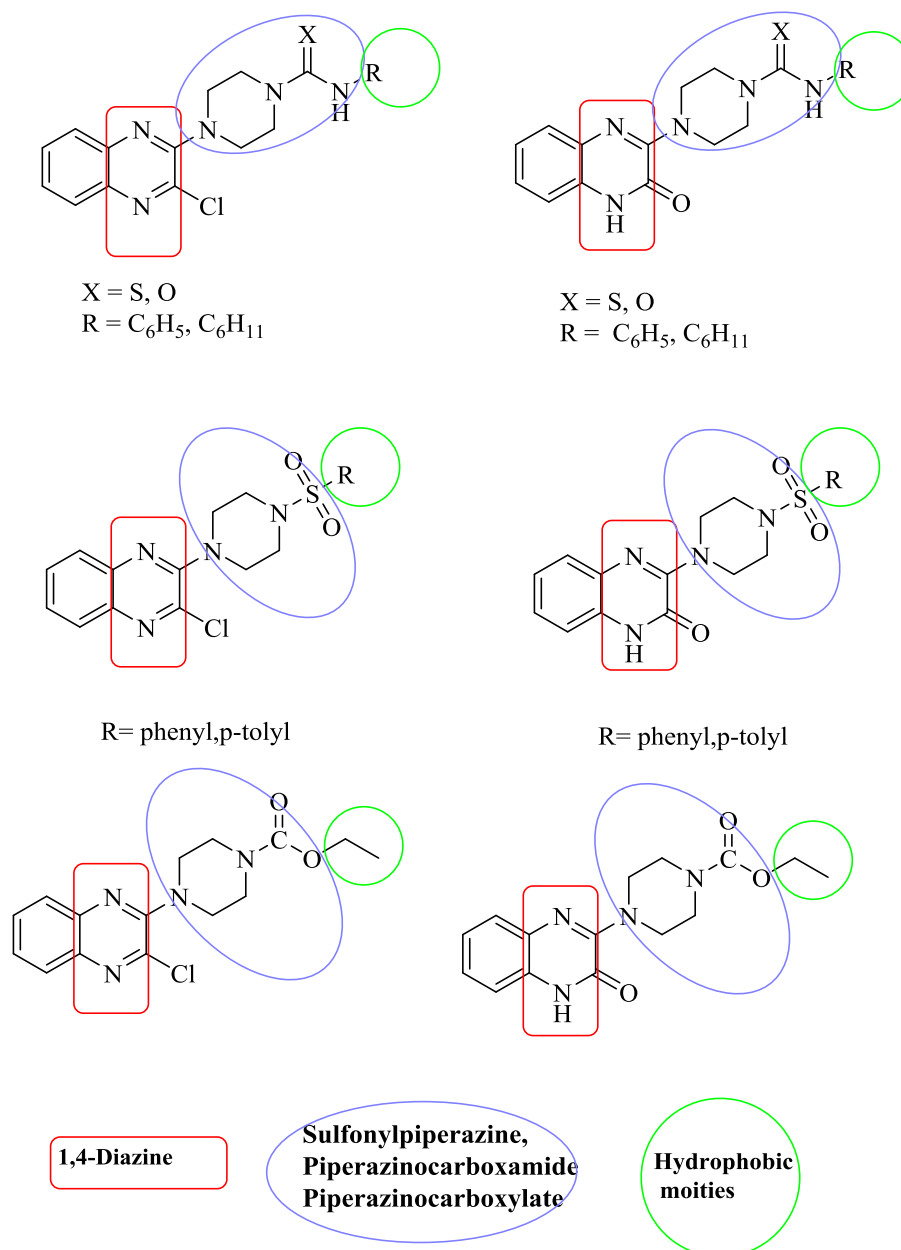Figure 2: The proposed modification of thalidomide as a lead compound in our molecules design.

Figure 3: The synthesized compounds showed different structure modification

3- Results and discussion

3.1 Chemistry

Quinoxaline-2,3-dione **3** was obtained by refluxing of *o*-phenylenediamine and oxalic acid in 4N HCl. Quinoxaline-2,3-dione was then heated in $POCl_3$ to afford 2,3-dichloroquinoxaline (**4**). Reaction of compound **4** with piperazine was carried out in ethanol under reflux temperature as illustrated in **Scheme 1.**
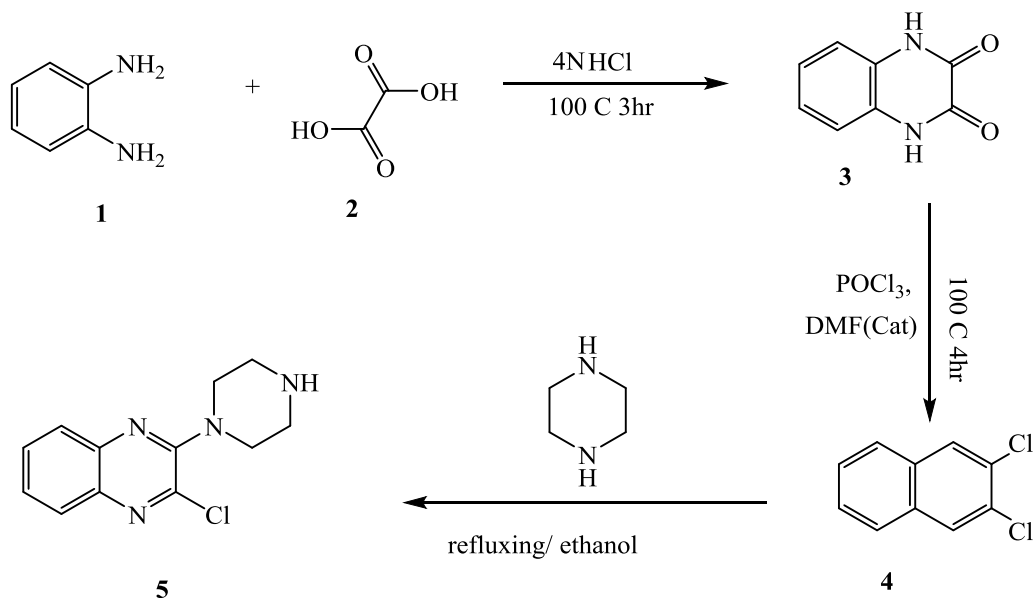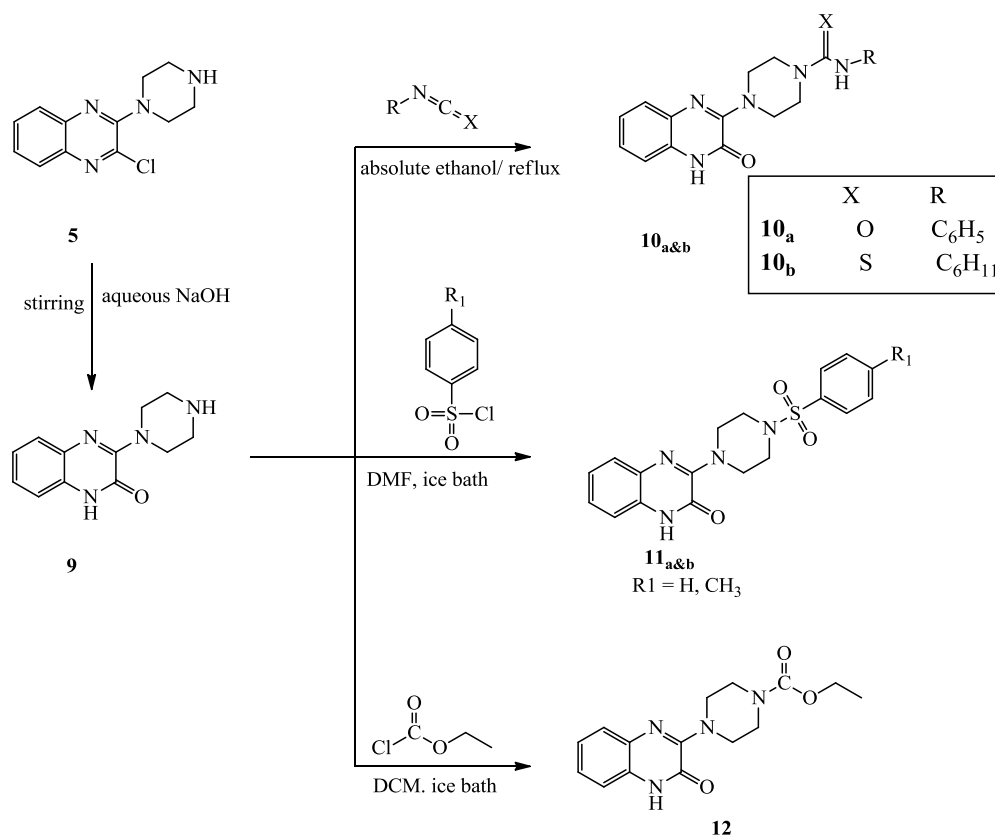
Scheme 1: Chemical synthesis of the target compound 5

2-Chloro-3-piperazinoquinoxaline (**5**) was used as starting material for the preparation of final compounds **6$_{a\&b}$, 7$_{a\&b}$**, and **8** as shown in **scheme2**. Refluxing of compound 5 with phenyl or cyclohexyl isocynate afforded the final compounds **6$_{a\&b}$,** respectively. While addition of benzenesulfonyl chloride or 4-methylbenzenesulfonyl chloride dropwise to compound **5** in DMF gave the final compounds **7$_{a\&b}$.** At the same manner addition of ethyl chloroformate dropwise to compound **5** in DCM gave compound **8.**

	X	R
6$_a$	O	C_6H_5
6$_b$	S	C_6H_{11}
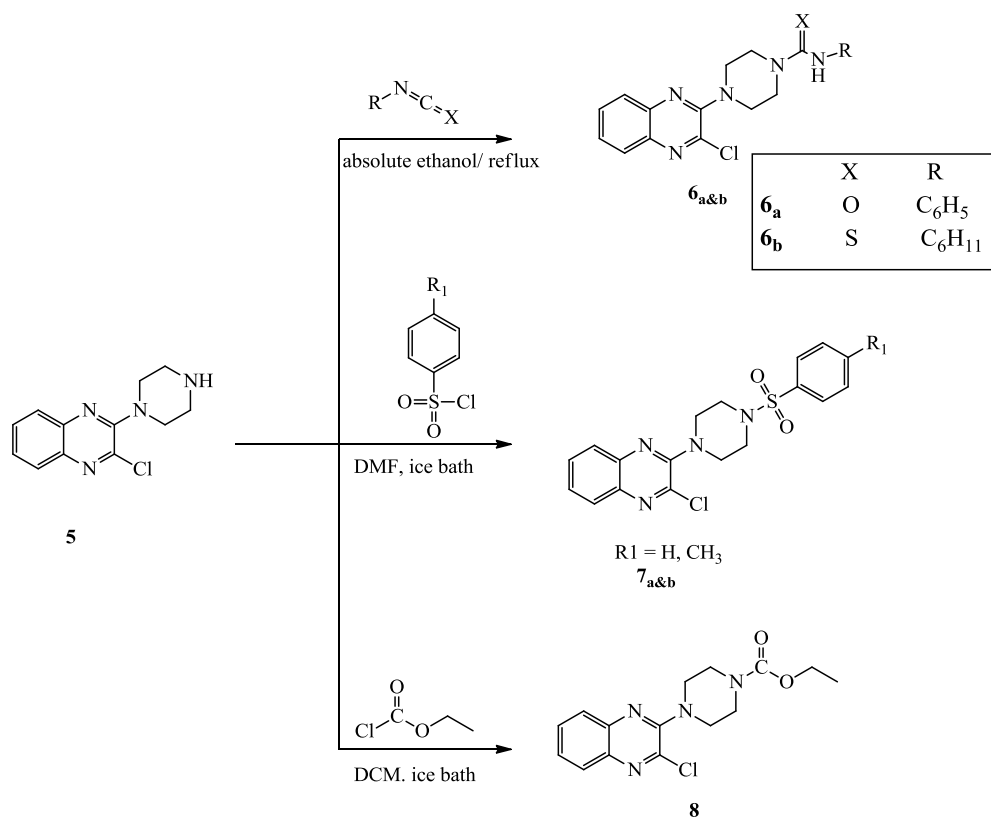
Scheme 2: Chemical synthesis of the target compounds 6a&b, 7a&b and 8

Scheme 3 shows that stirring of compound **5** in aqueous NaOH solution afforded 3-piperazinoquinoxaline-2-one (**9**). Refluxing of compound **9** with phenyl isocyanate and cyclohexyl isothiocynate afforded the final compounds **10$_{a\&b}$** respectively. At the same time the final compounds **11$_{a\&b}$** were obtained by addition of benzenesulfonyl chloride or 4-methylbenzenesulfonyl chloride to compound **9.** While addition of ethyl chloroformate dropwise to compound **9** in DCM gave compound **12.**

Scheme 3: Chemical synthesis of the target compounds **10a&b**, **11a&b** and **12**

3.2 Biology

In vitro Antiproliferative assay

All the final compounds were tested for their antitumor activity against three human cancer cell lines namely; hepatocellular carcinoma (HepG2), prostate cancer (PC3) and breast cancer (MCF-7). Thalidomide was tested as a reference drug. The data presented in **Table 1** show that compound **10b** is the most active candidate with very potent antitumor activity against the three cell lines. We can see that it is far better than thalidomide in this test. As it showed IC_{50} = 3.89, 4.01 and 2.91 µg/mL against the cell lines, respectively. While thalidomide exhibited IC_{50} values of 11.26, 14.58, and 16.87 µg/mL against the three cell lines, respectively. It was found that compounds **7a, 6a,** and **8** were better than thalidomide against MCF-7 cell line, as they showed IC_{50} values of 10.32, 12.15 and 15.32 µg/mL, respectively. Also it can be seen that compound **7a** demonstrated nearly the same activity of thalidomide against HepG-2. Moreover, compounds **6a, 7a, 8** showed strong activity against the three cell lines. While the other compounds showed moderate activities. No compound was found to be weak or

inactive. Furthermore, the breast cancer cell line was found to be the most sensitive to the effect of our compounds.

Table 1: IC_{50} of the final compounds and thalidomide against ; HepG2, PC3 and MCF-7 cell lines.

Serial	Comp. no.	IC_{50} (µg/mL)		
		HePG2	PC3	MCF-7
1	**6a**	17.01±1.3	19.51±1.4	12.15±1.2
2	**6b**	22.72±1.7	31.07±1.23	25.07±1.53
3	**7a**	11.81±0.82	19.28±1.3	10.32±0.93
4	**7b**	35.07±1.63	41.07±1.73	30.07±1.33
5	**8**	16.37±1.7	19.23±1.6	15.32±1.4
6	**10a**	23.10±1.62	27.50±1.09	22.35±1.07
7	**10b**	3.89±0.25	4.01±0.35	2.91±0.05
8	**11a**	16.93±1.49	28.07±1.3	20.50±1.32
9	**11b**	25.67±1.8	35.08±1.23	22.49±1.5
10	**12**	21.56±1.56	28.12±1.28	24.66±1.51
11	**Thalidomide**	11.26±0.54	14.58±0.57	16.87±0.7

## 3.3 Molecular modeling

Docking studies

To get insights on the binding mode of our compounds and their binding energy to Cereblon (CRBN), we carried out docking studies using molecular operating environment software (MOE). CRBN is the main target of thalidomide and its analogs(Mori *et al.* 2018) (Lopez-Girona *et al.* 2012). It is composed of three domains; 1- the amino terminal domain (NTD), 2- the α-helical bundle domain (HBD), and 3- the carboxy-terminal domain (CTD) (Fuchs et al. 2014). CRBN is a part of damage-specific DNA binding protein 1 (DDB1)/cullin4 E3 ubiquitin ligase complex and plays pivotal role in the ubiquitination of substrate proteins, thus promoting their degradation. The ubiquitin ligase activity of this complex is altered by thalidomide CRBN binding (Matyskiela *et al.* 2016). The crystal structure of CRBN is now available at protein data bank (PDB) with ID 4TZC. Redocking of thalidomide into the protein showed root mean square deviation (RMSD) 1.34 Å and docking score of -14.22 kcal/mol (see **Fig. 4**). The binding mode of thalidomide to CRBN is shown in **Fig.5**. We can see that glutarimide moiety of thalidomide was able to accommodate the pocket and form two hydrogen bonds with His380 and Trp382 (see **Fig. 6**). Further cation π interaction was observed between the nitrogen of phthalimide moiety and phenyl ring of Trp388.
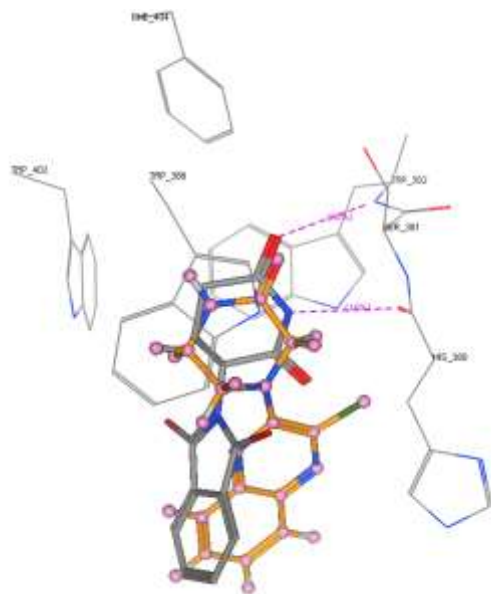
Figure 4: The overlay of the re-docked thalidomide molecule (grey colored molecule) and chloropiprazine derivative (the ligand) crystallized with CRBN (orange colored molecule).
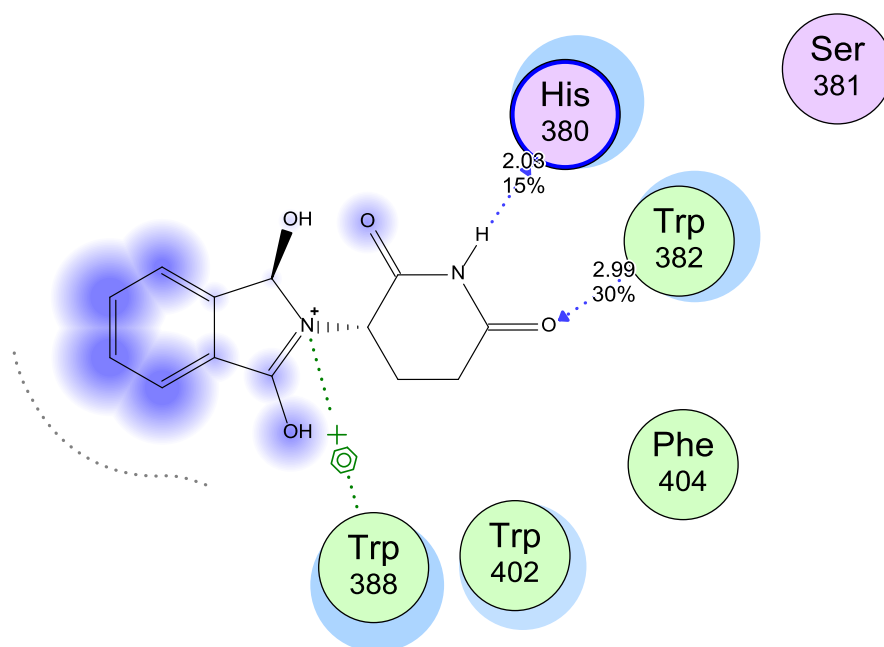
Figure 5: 2D diagram represents the accommodation and interaction of glutarimide moiety of thalidomide molecule inside the tri-Trp pocket of CRBN protein.
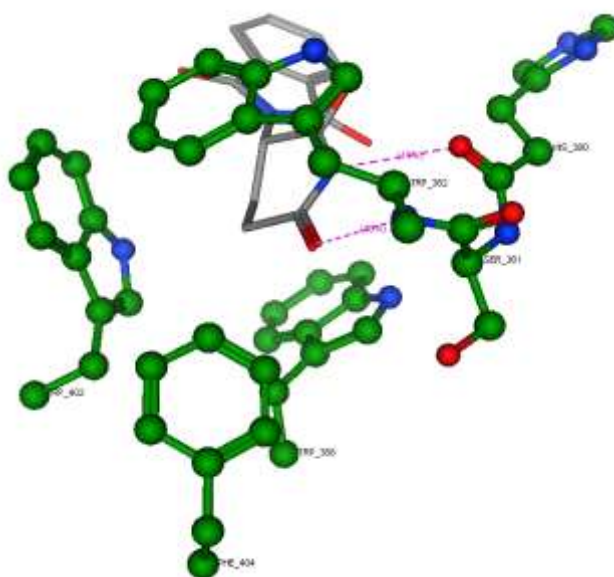
Figure 6: 3D diagram represents the accommodation and interaction of glutarimide moiety of thalidomide molecule inside the tri-Trp pocket of CRBN protein.

Our compounds docking results showed that all compounds exhibited binding energies too close to that of thalidomide (**Table 2**).

Table 2: Binding energies of our compounds and thalidomide to CRBN protein

serial	Compound No.	Binding energy (Kcal./mol.)
1	**6a**	-13.2
2	**6b**	-10.6
3	**7a**	-12.7
4	**7b**	-13.2
5	**8**	-13.1
6	**10a**	-13.1
7	**10b**	-10.3
8	**11a**	-12.4
9	**11b**	-14.1
10	**12**	-12.3
11	**Thalidomide**	-14.6

With respect to binding modes of our compounds to CRBN, it was found that our compounds were able to accommodate the pocket to form interactions with the essential amino acids but in a manner differs than that of thalidomide. **Fig. 7** shows that compound **10a** formed one H.B. as well as π π interaction with the essential residue His380. The H.B. was via the NH of quinoxalinone nucleus. We can see also further H.B. between the amidic carbonyl of **10a** and Trp402. Cation π interaction between the terminal phenyl ring and His399 can also be noticed (see **Fig. 8**).
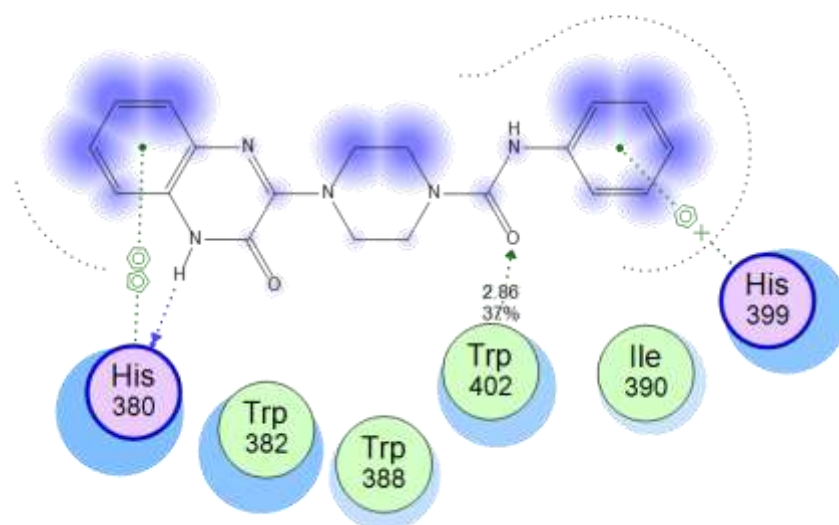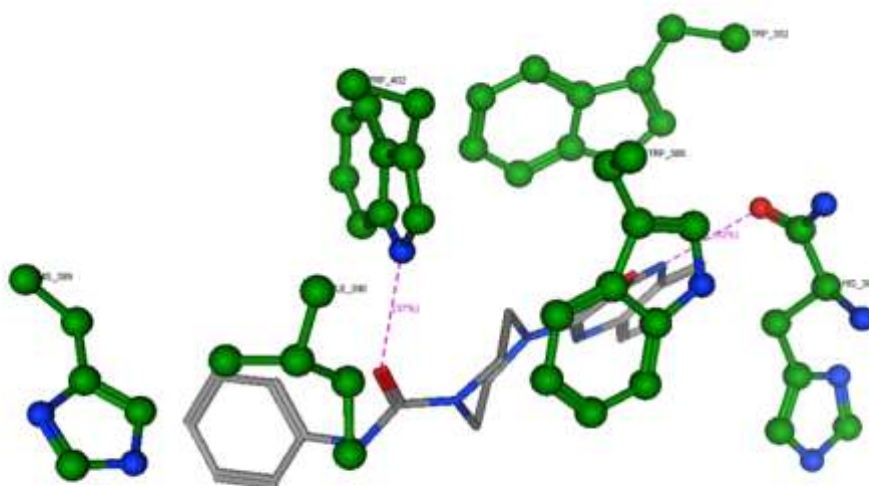
Figure 7: 2D diagram for binding mode of compound 10a

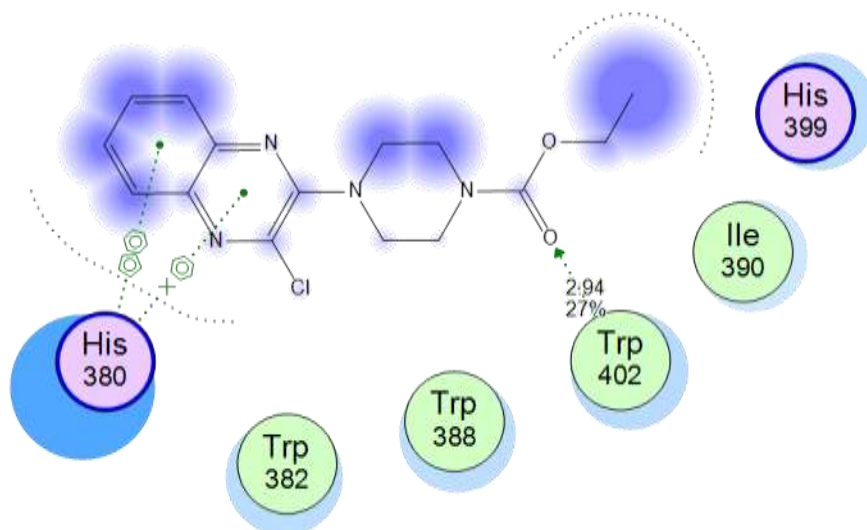**Figure 8: 3D diagram for binding mode of compound 10a**

Compound **8** was found to form one π π interaction and one cation π interaction with the essential amino acid His380 as illustrated in **Fig. 9**. It can be seen that one H.B. is formed between its carbonyl and Trp402 (see **Fig. 10**).
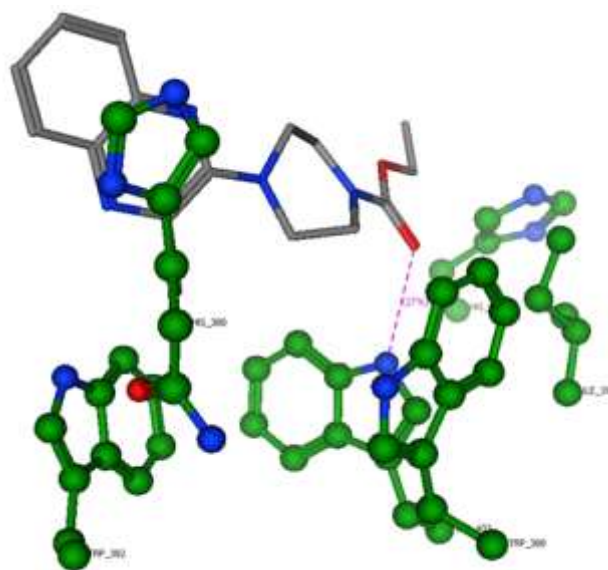
Figure 9: 2D diagram for binding mode of compound 8

Figure 10: 3D diagram for binding mode of compound 8

3.4 Conclusion

In our effort to develop new effective anticancer agents, we designed, synthesized and biologically evaluated ten new thalidomide analogs. Biological data showed that compound **10b** is a promising thalidomide analog. So that this work suggests that compound **10b** should be considered for further evaluation. It can also serve as a lead compound for future development of new effective anticancer agents.

4- Materials and methods

4.1 Chemistry

All melting points were carried out by open capillary method on a Gallen lamp Melting point apparatus and were uncorrected. Infrared spectra were recorded on Pye Unicam SP 1000 IR spectrophotometer (KBr discs) and were expressed in wave number (cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER 100 MHZ-NMR Spectro-photometer. TMS was used as internal standard in deuterated DMSO and chemical shifts were measured in δ ppm. Progresses of the reaction was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp.

4.1.1 General method for synthesis of compounds 6$_{a\&b}$: A mixture of the 2-Chloro-3-piperazin-1-yl-quinoxaline (1 mmol) and an appropriate isocyanate and/or isothiocyanate, namely, phenyl isocyanate and cyclohexyl isothiocyanate (1.2 mmol) was refluxed in absolute ethanol (50 ml) for 3 h. The reaction mixture was cooled. The formed solid was filtered and recrystallized from ethanol to afford the corresponding compound 6$_{a\&b}$, respectively.

4.1.1.1 4-(3-Chloroquinoxalin-2-yl)-*N*-phenylpiperazine-1-carboxamide (6$_a$) (yield 87.62 %); m.p. 218-220 °C; IR (KBr, cm^{-1}): 3302 (NH), 3059 (C-H aromatic), 2908, 2881, 2846 (C-H aliphatic), 1635 (CO amide); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 8.64 (s, 1H, CONH) (D$_2$O exchangeable), 7.90 (d, J = 8.2 Hz, 1H, Ar-H quinoxaline), 7.85 (d, J = 8.3 Hz, 1H, Ar-H quinoxaline), 7.75 (dd, J = 7.7, 7.7 Hz, 1H, Ar-H quinoxaline), 7.64 (dd, J = 7.7, 7.7 Hz, 1H, Ar-H quinoxaline), 7.49 (d, J = 8.0 Hz, 2H, Ar-H), 7.25 (dd, J = 7.7, 7.7 Hz, 2H, Ar-H), 6.95 (t, J = 7.3 Hz, 1H, Ar-H), 3.68 (t, J = 4.5 Hz, 4H 2CH$_2$ piperazine), 3.54 (t, J = 4.5 Hz, 4H, 2CH$_2$ piperazine); Anal. Calcd. for C$_{19}$H$_{18}$ClN$_5$O (367.84): C, 62.04; H, 4.93; N, 19.04. Found: C, 61.89; H, 5.12; N, 19.23%.

4.1.1.2 4-(3-Chloroquinoxalin-2-yl)-*N*-cyclohexylpiperazine-1-carbothioamide (6$_b$) (yield 84.09 %); m.p. 145-147 °C; IR (KBr, cm^{-1}): 3286 (NH), 3039 (C-H aromatic), 2981-2823 (C-H aliphatic); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 7.90 (dd, J = 8.2, 1.4 Hz, 1H, Ar-H), 7.84 (dd, J = 8.3, 1.4 Hz, 1H, Ar-H), 7.76 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H, Ar-H), 7.64 (ddd, J = 8.3, 6.9, 1.4 Hz, 1H, Ar-H), 7.39 (d, J = 7.8 Hz, 1H, CSNH) (D$_2$O exchangeable), 4.23 (m, , 1H, CH of cyclohexyl), 4.07 − 3.90 (m, 4H, 2CH$_2$ piperazine), 3.70 − 3.49 (m, 4H, 2CH$_2$ piperazine), 1.90-0.96 (m, 10H, 5CH$_2$ of cyclohexyl); ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ (ppm): 181.19, 152.36, 141.32, 139.84, 138.01, 131.07, 128.02, 127.83, 127.16, 55, 48.56, 47.12, 32.53, 25.75, 25.58 Anal.

Calcd. for $C_{19}H_{24}ClN_5S$ (389.95): C, 58.52; H, 6.20; N, 17.96. Found: C, 58.73; H, 6.30; N, 18.12%.

4.1.2 General method for synthesis of compounds 7$_{a\&b}$:

To a solution of 2-chloro-3-piperazin-1-yl-quinoxaline (0.5 g, 2.01 mmol) and Et$_3$N (0.37 g, 0.50 ml, 3. 62 mmol) in DMF (15 mL), the appropriate 4-(un)substituted-benzenesulfonyl chloride (2.01mmol), namely benzenesulfonyl chloride and 4-methyl was added in a drop wise manner while stirring in ice-salt bath for 1 h. then the reaction mixture was slowly poured into ice-water with stirring. The obtained solid was filtered, washed with water and crystallized from ethanol to give the corresponding final compounds **7$_{a\&b}$** respectively.

4.1.2.1 2-Chloro-3-(4-(phenylsulfonyl)piperazin-1-yl)quinoxaline (7$_a$) (yield 85.38 %); m.p. 178-180 °C; IR (KBr, cm^{-1}): 3039 (C-H aromatic), 2920-2854 (C-H aliphatic), 1425, 1346 (SO$_2$ sulfonamide); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 7.88 (d, J = 8.2 Hz, 1H, Ar-H), 7.81 (d, J = 7.7 Hz, 3H, Ar-H,), 7.75 (d, J = 7.0 Hz, 2H, Ar-H), 7.70 (dd, J = 7.4, 5.9 Hz, 2H, Ar-H), 7.64 (t, J = 7.6 Hz, 1H, Ar-H), 3.57 (t, J = 4.8 Hz, 4H, 2CH$_2$ piperazine), 3.22 – 2.99 (m, 4H, 2CH$_2$ piperazine); ^{13}C NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 152.23, 141.56, 139.69, 138.16, 135.25, 133.94, 131.12, 130.03, 128.37, 128.07, 127.85, 127.21, 48.41, 45.99; Anal. Calcd. for $C_{18}H_{17}ClN_4O_2S$ (388.87): C, 55.60; H, 4.41; N, 14.41. Found: C, 55.82; H, 4.62; N, 14.65%.

4.1.2.2 2-Chloro-3-(4-tosylpiperazin-1-yl)quinoxaline (7$_b$)   (yield 92.01 %); m.p. 185-187 °C; IR (KBr, cm^{-1}): 3062 (C-H aromatic), 2995-2850 (C-H aliphatic), 1427, 1342 (SO$_2$ sulfonamide); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 7.86 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.80 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.73 (t, *J* = 7.7 Hz, 1H, Ar-H), 7.68 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.62 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.48 (d, *J* = 7.9 Hz, 2H, Ar-H, Ar-H), 3.56 (t, *J* = 4.8 Hz, 4H, 2CH2 piperazine), 3.10 (t, *J* = 4.8 Hz, 4H, 2CH2 piperazine), 2.40 (s, 3H, CH$_3$); Anal. Calcd. for $C_{19}H_{19}ClN_4O_2S$ (402.90): C, 56.64; H, 4.75; N, 13.91. Found: C, 56.78; H, 4.81; N, 14.15%.

4.1.3 General method for synthesis of compound 8

To a solution of 2-chloro-3-piperazin-1-yl-quinoxaline (0.5 g, 2.01mmol) and Et$_3$N (0.41 g, 0.56 ml, 4.02 mmol) in DCM (15 mL), ethyl chloroformate (0.24 g, 0.21 ml, 2.21 mmol) was added drop wise over a period of 0.5 h. while stirring in ice salt bath. The reaction mixture was stirred for further 0.5 h. The solvent was removed under vacuum and the obtained residue was washed with water, dried and crystallized from methanol.

4.1.3.1 Ethyl-4-(3-chloroquinoxalin-2-yl)piperazine-1-carboxylate (8) (Yield 90.01 %); m.p. 132-134 °C; IR (KBr, cm^{-1}): 3016 (C-H aromatic), 2985-2847 (C-H aliphatic), 1693 (C=O amide); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 7.77 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.71 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.64 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.55 (t, *J* = 7.6 Hz, 1H, Ar-H), 4.04 (q, *J* = 7.1 Hz, 2H, OCH$_2$-CH$_3$), 3.52 (m,4H, 2CH$_2$ piperazine), 3.44 – 3.33 (m, 4H, 2CH$_2$ piperazine) , 1.17 (t, *J* = 7.1 Hz, 3H, CH$_3$); Anal. Calcd. for

$C_{15}H_{17}ClN_4O_2$ (320.78): C, 56.17; H, 5.34; N, 17.47. Found: C, 56.39; H, 5.51; N, 17.64%.

4.1.4 General method for synthesis of compounds 10$_{a\&b}$: A mixture of 3-(piperazin-1-yl)quinoxalin-2(1H)-one (**9**) (1 mmol) and an appropriate isocyanate and/or isothiocyanate, namely, phenyl isocyanate and cyclohexyl isothiocyanate (1.2 mmol) was refluxed in absolute ethanol (50 ml) for 3 h. The reaction mixture was cooled. The formed solid was filtered and recrystallized from ethanol to afford the corresponding compound **10$_{a\&b}$**, respectively.

4.1.4.1 4-(3-Oxo-3,4-dihydroquinoxalin-2-yl)-*N*-phenylpiperazine-1-carbox-amide (10$_a$) (yield 84.42 %); m.p. 237-239 °C; IR (KBr, cm^{-1}): 3390 (2NH), 3062 (C-H aromatic), 2978-2715 (C-H aliphatic), 1670, 1647 (C=O amide); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 12.16 (s, 1H , NH quinoxaline, D$_2$O exchangeable), 8.58 (s, 1H, CONH, D$_2$O exchangeable), 7.53 − 7.45 (m, 2H, Ar-H), 7.43 (ddd, *J* = 7.5, 1.2 Hz, 1H, Ar-H), 7.30 − 7.22 (m, 2H, Ar-H), 7.20 (dd, *J* = 3.7, 1.1 Hz, 2H, Ar-H), 7.16 (ddd, *J* = 7.4, 5.1, 3.8 Hz, 1H, Ar-H), 6.99 − 6.91 (m, 1H, Ar-H), 4.02 − 3.84 (m, 4H, 2CH$_2$ piperazine), 3.67 − 3.52 (m, 4H, 2CH$_2$ piperazine); Anal. Calcd. for $C_{19}H_{19}N_5O_2$ (349.39): C, 65.32; H, 5.48; N, 20.04. Found: C, 65.18; H, 5.69; N, 19.87%.

4.1.4.2   *N*-Cyclohexyl-4-(3-oxo-3,4-dihydroquinoxalin-2-yl)piperazine-1-carbo-thioamide (10$_b$) (yield 81.49 %); m.p. 235-237 °C; IR (KBr, cm^{-1}): 3379,3344 (2NH), 3020 (C-H aromatic), 2981-2777 (C-H aliphatic), 1662 (C=O amide); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 12.13 (s, 1H, NH quinoxaline, D$_2$O exchangeable), 7.41 (d, *J* = 7.4 Hz, 1H, CSNH, D$_2$O exchangeable), 7.29 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.23 − 7.08 (m, 3H, Ar-H), 4.21 (m, 1H, CH cyclohexyl), 3.93 (m, *J* = 5.6 Hz, 8H, 4CH2 piperazine), 1.90-1.04 (m, 10H, 5CH$_2$cyclohexyl); ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ (ppm): 180.79, 152.5, 151.34, 132.69, 129.68, 125.58, 125.2, 123.68, 114.87, 54.95, 47.14, 45.98, 32.55, 25.73, 25.58; Anal. Calcd. for $C_{19}H_{25}N_5OS$ (371.50): C, 61.43; H, 6.78; N, 18.85. Found: C, 61.60; H, 6.71; N, 19.02%.

4.1.5 General method for synthesis of compounds 11$_{a\&b}$:

To a solution of 3-(piperazin-1-yl)quinoxalin-2(1*H*)-one (**9**) (2.01 mmol) and Et$_3$N (0.37 g, 0.50 ml, 3. 62 mmol) in DMF (15 mL), an appropriate 4-(un)substituted - benzenesulfonyl chloride (2.01mmol), namely benzenesulfonyl chloride and 4-methylbenzenesulfonyl chloride was added in a drop wise manner while stirring in ice-salt bath for 1 h. then the reaction mixture was slowly poured into ice-water with stirring. The obtained solid was filtered, washed with water and crystallized from ethanol to give the corresponding final compounds (**11$_{a\&b}$**) respectively.

4.1.5.1 3-(4-(Phenylsulfonyl)piperazin-1-yl)quinoxalin-2(1*H*)-one (11$_a$) (Yield 82.37 %); m.p. 238-240 °C; IR (KBr, cm^{-1}): 3448 (NH), 3055 (C-H aromatic), 2985-2762 (C-H aliphatic), 1662 (C=O amide), 1492, 1346 (SO$_2$ sulfonamide); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 12.16 (s, 1H, NH, D$_2$O exchangeable), 7.87 − 7.77 (m, 2H, Ar-H), 7.48 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.37 (dd, *J* = 7.9, 1.4 Hz, 1H, Ar-H), 7.23 − 7.08 (m, 4H, Ar-H), 3.98 (t, *J* = 4.8 Hz, 4H, 2CH$_2$ piperazine), 3.04 (t, *J* = 5.0 Hz, 4H, 2CH2 piperazine); ^{13}C NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 166.44, 163.93, 152.34, 151.19,

132.33, 131.55, 131.52, 131.22,131.12, 129.81, 125.68, 125.57, 123.69, 117.26, 117.03, 114.88, 46.16, 45.69; Anal. Calcd. for $C_{18}H_{18}N_4O_3S$ (370.43): C, 58.36; H, 4.90; N, 15.13. Found: C, 58.58; H, 5.11; N, 15.40%.

4.1.5.2 3-(4-Tosylpiperazin-1-yl)quinoxalin-2(1*H*)-one (11$_b$) (Yield 90.19 %); m.p. 245-247 °C; IR (KBr, cm^{-1}): 3417 (NH), 3020 (C-H aromatic), 2981-2777 (C-H aliphatic), 1666 (C=O amide), 1489, 1332 (SO$_2$ sulfonamide); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 12.15 (s, 1H, NH, D$_2$O exchangeable), 7.65 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.41 (m, 3H, Ar-H), 7.22 − 7.06 (m, 3H, Ar-H), 3.96 (t, *J* = 4.9 Hz, 4H, 2CH$_2$ piperazine), 3.00 (t, *J* = 4.9 Hz, 4H, 2CH$_2$ piperazine), 2.39 (s, 3H, CH$_3$); ^{13}C NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 152.33, 151.21, 144.32, 132.33, 132.08, 130.36, 129.81, 128.12, 125.67, 125.58, 123.7, 114.89, 46.19, 45.7, 21.45; Anal. Calcd. for $C_{19}H_{20}N_4O_3S$ (384.45): C, 59.36; H, 5.24; N, 14.57. Found: C, 59.54; H, 5.33; N, 14.79%.

4.1.6 General method for synthesis of compound 12

To a solution of 3-(piperazin-1-yl)quinoxalin-2(1H)-one (2.01mmol) and Et$_3$N (0.41 g, 0.56 ml, 4.02 mmol) in DCM (15 mL), ethyl chloroformate (0.24 g, 0.21 ml, 2.21 mmol) was added drop wise over a period of 0.5 h. while stirring in ice salt bath. The reaction mixture was stirred for further 0.5 h. The solvent was removed under vacuum and the obtained residue was washed with water, dried and crystallized from methanol.

4.1.6.1 Ethyl 4-(3-oxo-3,4-dihydroquinoxalin-2-yl)piperazine-1-carboxylate (12) (yield 95.00 %); m.p. 203-205 °C; IR (KBr, cm^{-1}): 3425(NH), 3093 (C-H aromatic), 2981-2777 (C-H aliphatic), 1708 (C=O ester), 1674 (C=O amide); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ (ppm): 12.17 (s, 1H, NH, D$_2$O exchangeable), 7.41 (dt, J = 7.5, 1.2 Hz, 1H, Ar-H), 7.17 (m, 3H, Ar-H), 4.08 (q, *J* = 7.1 Hz, 2H, OCH$_2$-CH$_3$), 3.92 − 3.82 (m, 4H, 2CH$_2$ piperazine), 3.50 (t, *J* = 5.1 Hz, 4H, 2CH2 piperazine), 1.21 (t, *J* = 7.1 Hz, 3H, CH$_3$); Anal. Calcd. for $C_{15}H_{17}ClN_4O_2$ (320.78): C, 59.59; H, 6.00; N, 18.53. Found: C, 59.80; H, 6.18; N, 18.87%.

4.2 Biological testing

Cell lines

The cell lines were obtained from ATCC *via* Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Chemical reagents

The reagents used are RPMI-1640 medium, MTT and DMSO (sigma co., St. Louis, USA) and Fetal Bovine serum (GIBCO).

Antiproliferative assay

The different cell lines mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is

based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37 C in a 5% CO_2 incubator. The cells were seeded in a 96-well plate at a density of 1.0×10^4 cells/well. at 37 C for 48 h under 5% CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100.

4.3 Molecular modeling

To study the binding interaction and measure the binding energy of the newly synthesized compounds within CRBN protein, docking studies for these compounds were performed. Crystal structures of CRBN protein is available on protein data bank (PDB) website. Docking studies were done on CRBN (PDB ID: 4TZC) by MOE software. Protein was downloaded from PDB website to be opened on MOE. Water molecules deletion, energy minimization and addition of hydrogen atoms were done. The re-docking of the co-crystallized thalidomide molecule into CRBN protein validated the docking process.

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تصميم ونمذجه جزيئية وتشيد عوامل جديدة منظمة لجهاز المناعة لدرستها بيولوجيا

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الملخص العربى

السرطان هو السبب الرئيسي الثاني للوفاة في جميع أنحاء العالم. هذا العمل هو محاولة لإيجاد عوامل جديدة فعالة وآمنة مضادة للسرطان. وفقًا لخصائص الثاليدومايد كدواء مضاد للسرطان مناعي ، قمنا بتصميم وتصنيع عشرة نظائر جديدة للثاليدومايد. تم تقييم المركبات المحضرة بيولوجيا لنشاطها المضاد للورم ضد ثلاثة خطوط خلايا سرطانية بشرية وهي: سرطان الخلايا الكبدية (HepG2) وسرطان البروستاتا (PC3) وسرطان الثدي (MCF-7). تم استخدام الثاليدومايد كدواء مرجعي. أثبتت نتائج الاختبارات البيولوجية أن المركب رقم ١٠أ أقوى من عقار الثاليدوميد ضد خلايا سرطان الكبد و الثدى و البروستاتا. كذلك أثبتت النتائج أن ثلاثة من هذه المركبات الجديدة أقوى من عقار الثاليدوميد ضد خلايا سرطان الثدى. و كانت نتائج النمذجة الجزيئية متماشية مع النتائج البيولوجية. أظهرت نتائج دراسات الالتحام أن مركباتنا يمكن أن تستوعب جيب CRBN.

الكلمات المفتحية : مضاد للسرطان, منظم لجهاز المناعة و عقار الثاليدوميد

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