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 IC₅₀ = 3.89, 4.01 and 2.91 µg/mL against the cell lines, respectively. While thalidomide exhibited IC₅₀ 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 IC₅₀ 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 However, in 1961 it was quickly withdrawn from distribution when its teratogenic properties were discovered (Ito et al. 2010).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).
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, piperazinocarboxamid 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 POCI\(_3\) 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\textsubscript{a&b}, 7\textsubscript{a&b} and 8 as shown in scheme 2. Refluxing of compound 5 with phenyl or cyclohexyl isocyanate afforded the final compounds 6\textsubscript{a&b} respectively. While addition of benzenesulfonyl chloride or 4-methylbenzenesulfonyl chloride dropwise to compound 5 in DMF gave the final compounds 7\textsubscript{a&b}. At the same manner addition of ethyl chloroformate dropwise to compound 5 in DCM gave compound 8.
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 10a&b respectively. At the same time the final compounds 11a&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 \( \text{IC}_{50} = 3.89, 4.01 \text{ and } 2.91 \mu\text{g/mL} \) against the cell lines, respectively. While thalidomide exhibited \( \text{IC}_{50} \) values of 11.26, 14.58, and 16.87 \( \mu\text{g/mL} \) 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 \( \text{IC}_{50} \) values of 10.32, 12.15 and 15.32 \( \mu\text{g/mL} \), 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\textsubscript{50} of the final compounds and thalidomide against HepG2, PC3 and MCF-7 cell lines.

| Serial | Comp. no. | HePG2 (µg/mL) | PC3 (µg/mL) | MCF-7 (µg/mL) |
|--------|-----------|---------------|-------------|---------------|
| 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       | 15.93±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).
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 6a&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 6a&b, respectively.

4.1.1.1 4-(3-Chloroquinoxalin-2-yl)-N-phenylpiperazine-1-carboxamide (6a) (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), 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 (6b) (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 (dd, 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$_{22}$ClN$_5$S (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 (2.01 mmol), namely benzenesulfonl 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, 130.03, 128.37, 127.85, 127.21, 48.41, 45.99; Anal. Calcd. for C$_{18}$H$_{17}$ClN$_4$O$_2$S (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, 2CH$_2$ piperazine), 3.10 (t, J = 4.8 Hz, 4H, 2CH$_2$ piperazine), 2.40 (s, 3H, CH$_3$); Anal. Calcd. for C$_{19}$H$_{19}$ClN$_4$O$_2$S (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, 2.01 mmol) 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
C₁₅H₁₇ClN₄O₂ (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 10a&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 10a&b, respectively.

4.1.4.1 4-(3-Oxo-3,4-dihydroquinoxalin-2-yl)-N-phenylpiperazine-1-carboxamide (10a) (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); ¹H NMR (DMSO-d₆, 400 MHz) δ (ppm): 12.16 (s, 1H , NH quinoxaline, D₂O exchangeable), 8.58 (s, 1H, CONH, D₂O 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, 2CH₂ piperazine), 3.67 – 3.52 (m, 4H, 2CH₂ piperazine); Anal. Calcd. for C₁₉H₁₉N₃O₂ (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-carboxthioamide (10b) (yield 81.49 %); m.p. 235-237 °C; IR (KBr, cm⁻¹): 3379,3344 (2NH), 3055 (C-H aromatic), 2981-2777 (C-H aliphatic), 1662 (C=O amide); ¹H NMR (DMSO-d₆, 400 MHz) δ (ppm): 12.13 (s, 1H, NH quinoxaline, D₂O exchangeable), 7.41 (d, J = 7.4 Hz, 1H, CSNH, D₂O 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, 5CH₃cyclohexyl); ¹³C NMR (DMSO-d₆, 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 C₁₉H₂₅N₃O₅ (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 11a&b:

To a solution of 3-(piperazin-1-yl)quinoxalin-2(1H)-one (9) (2.01 mmol) and Et₃N (0.37 g, 0.50 ml, 3. 62 mmol) in DMF (15 mL), an appropriate 4-(un)substituted -benzenesulfonyl chloride (2.01 mmol), 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 (11a&b) respectively.

4.1.5.1 3-(4-Phenylsulfonyl)piperazin-1-yl)quinoxalin-2(1H)-one (11a) (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 (SO₂ sulfonamide); ¹H NMR (DMSO-d₆, 400 MHz) δ (ppm): 12.16 (s, 1H, NH, D₂O 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, 2CH₂ piperazine), 3.04 (t, J = 5.0 Hz, 4H, 2CH₂ piperazine); ¹³C NMR (DMSO-d₆, 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_{4}O_{3}S (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(1H)-one (11b) (Yield 90.19 %); m.p. 245-247 °C; IR (KBr, cm⁻¹): 3417 (NH), 3020 (C-H aromatic), 2981-2777 (C-H aliphatic), 1693 (C=O amide), 1489, 1332 (SO₂ sulfonamide); ¹H NMR (DMSO-d₆, 400 MHz) δ (ppm): 12.15 (s, 1H, NH, D₂O 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, 2CH₂ piperazine), 3.00 (t, J = 4.9 Hz, 4H, 2CH₂ piperazine), 2.39 (s, 3H, CH₃); ¹³C NMR (DMSO-d₆, 400 MHz) δ (ppm): 152.33, 151.21, 144.32, 132.33, 131.52, 130.36, 129.81, 128.12, 125.67, 125.58, 123.7, 114.89, 46.19, 46.16, 21.45; Anal. Calcd. for C_{19}H_{20}N_{4}O_{3}S (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 (2.01 mmol) and Et₃N (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), 1767 (C=O amide); ¹H NMR (DMSO-d₆, 400 MHz) δ (ppm): 12.17 (s, 1H, NH, D₂O 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, OCH₂-CH₃), 3.92 – 3.82 (m, 4H, 2CH₂ piperazine), 3.50 (t, J = 5.1 Hz, 4H, 2CH₂ piperazine), 1.21 (t, J = 7.1 Hz, 3H, CH₃); Anal. Calcd. for C₁₅H₂₀ClN₄O₂ (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% CO₂ incubator. The cells were seeded in a 96-well plate at a density of 1.0x10⁴ cells/well. at 37 C for 48 h under 5% CO₂. 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 وتنمجه جزيئية وتشير عوامل جديدة منظمة لجهاز المناعة لدرستها بيولوجيا

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الملخص العربي

السرطان هو السبب الرئيسي الثاني للوفاة في جميع أنحاء العالم. هذا العمل هو محاولة لإيجاد عوامل جديدة فعالة وأمانة مضادة للسرطان. وفقاً لخصائص التاليدوميد كدواء مضاد للسرطان مناعي، فضلاً بتصميم وتصنيع عشرة نظائر جديدة للتاليدوميد. تم تقييم المركبات المحصورة بيولوجيا لتشكلها المضادة للورم ضد ثلاثة خطوط خلايا سرطانية بشرية وهي: سرطان الخلايا الكبدية (HepG2) وسرطان البروستاتا (PC3) والثدي (MCF-7). تم استخدام التاليدوميد كدواء مرجع. أثبتت نتائج الاختبارات البيولوجية أن المركب رقم 10 أقوى من عقار التاليدوميد ضد خلايا سرطان الكبد والثدي و البروستاتا. كذلك أثبتت النتائج أن ثلاثة من هذه المركبات الجديدة أقوى من عقار التاليدوميد ضد خلايا سرطان الثدي. و كانت النتائج المذكورة جزيئية متماثلة مع الكرنب البشري. أظهرت نتائج دراسات الالتحام أن مركباتنا يمكن أن تبدو جيب.

الكلمات المفتاحية: مضاد للسرطان، منظم لجهاز المناعة، عقار التاليدوميد