HETEROCYCLES, Vol. 104, No. 5, pp. 855 - 877. © 2022 The Japan Institute of Heterocyclic Chemistry
Received, 12th August, 2021, Accepted, 7th February, 2022, Published online 16th February, 2022
DOI: 10.3987/COM-21-14535

SYNTHESIS, BIOLOGICAL EVALUATION, AND IN SILICO STUDIES OF NEW HETEROCYCLES INCORPORATING 4,5,6,7-TETRABROMOPHTHALIMIDE MOIETY AS POTENTIAL ANTIBACTERIAL AND ANTICANCER AGENTS

Magda H. Abdellattif,[1]* Hossam Nada,[2] and Ahmed Elkamhawy[2,3]

[1] Department of Chemistry, College of Science, Taif University, P.O. Box 11099, Taif, 21944, Saudi Arabia. [2] College of Pharmacy, Dongguk University-Seoul, Goyang 10326, Republic of Korea. [3] Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.
*Corresponding author E-mail: m.hasan@tu.edu.sa (M.H.A.)

Abstract – Cancer and infectious illnesses are currently the most significant public health issues in the globe. Phthalimide derivatives, including thalidomide (multi-target drug), have anti-inflammatory, analgesic, anticancer, antibacterial, and anticonvulsant biological activities. A new series of heterocyclic compounds incorporating 4,5,6,7-tetrabromophthalimide moiety were synthesized and biologically evaluated for potential antimicrobial and anticancer activities. While compounds **4a**–**c** and **11a**–**c** were the most active antimicrobial activities upon evaluation over *Aspergillus favus*, *E. coli*, *Staphylococcus*, and *Fusarium moniliform*, derivatives **4a** and **4cb** showed the most potent values over cervical and ovarian cancer (6.933–11.46 μg/mL). Further in silico studies, including a molecular docking investigation over HSP90 protein, were carried out to investigate the potential binding mode and toxicity profile(s) of the newly synthesized compounds.

INTRODUCTION

Cancer and infectious illnesses are currently the most significant public health issues in the globe. The emergence of multi-drug resistant microbial infections due to widespread antibiotic usage has fueled the hunt for new, safer, more powerful, and resistance-free antimicrobial drugs.[1-4] Furthermore, the development of new, selective, and more powerful antitumor medicines remains a critical problem for biologists and medicinal chemists. In this context, phthalimide derivatives have been shown to have anti-inflammatory, analgesic, anticancer, antibacterial, and anticonvulsant biological activities.[5,6] The

phthalimide ring system is an important pharmacophoric fragment, according to research investigations on the structure-activity relationship (SAR) of thalidomide metabolites and analogs.[5,7] Thalidomide is one famous example of a phthalimide-based multi-target drug that affects numerous cellular processes, including peptidase inhibition, COX inhibition, glucosidase inhibition, and androgen receptor antagonism.[8] Furthermore, heterocyclic hits are extremely useful in synthetic medications, insecticides, and biological effects, some of which were reported previously by our group.[9-14] Accordingly, in this study, a new series of 4,5,6,7-tetrabromophthalimide incorporating heterocycles were synthesized and biologically evaluated for potential antimicrobial and anticancer activities. Furthermore, further in silico studies, including a molecular docking investigation over celeblon (CRBN) was carried out to investigate the potential mechanism of action and the toxicity profile of the newly synthesized compounds.

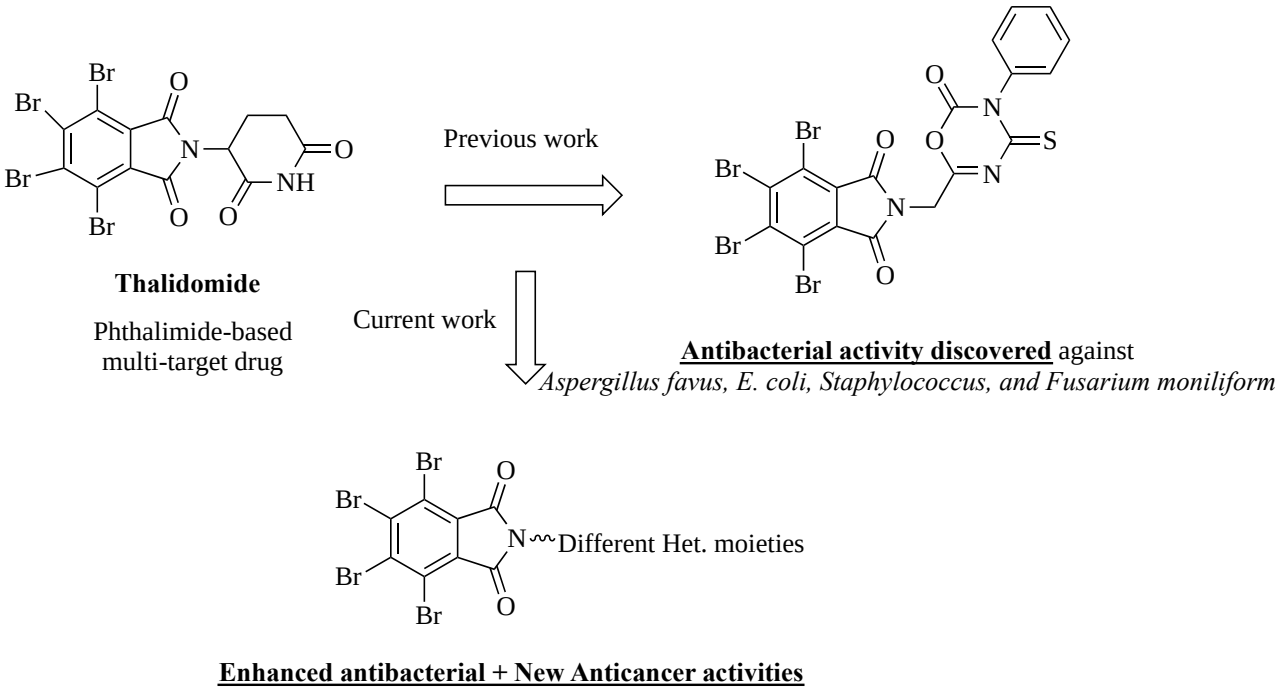
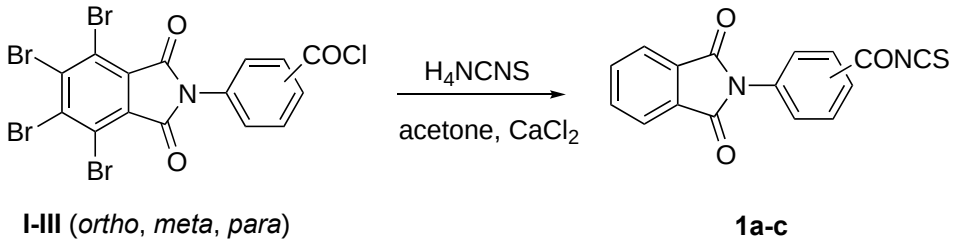


Figure 1. Graphical design for the approach of the current study based on thalidomide

RESULTS AND DISCUSSION

Chemistry

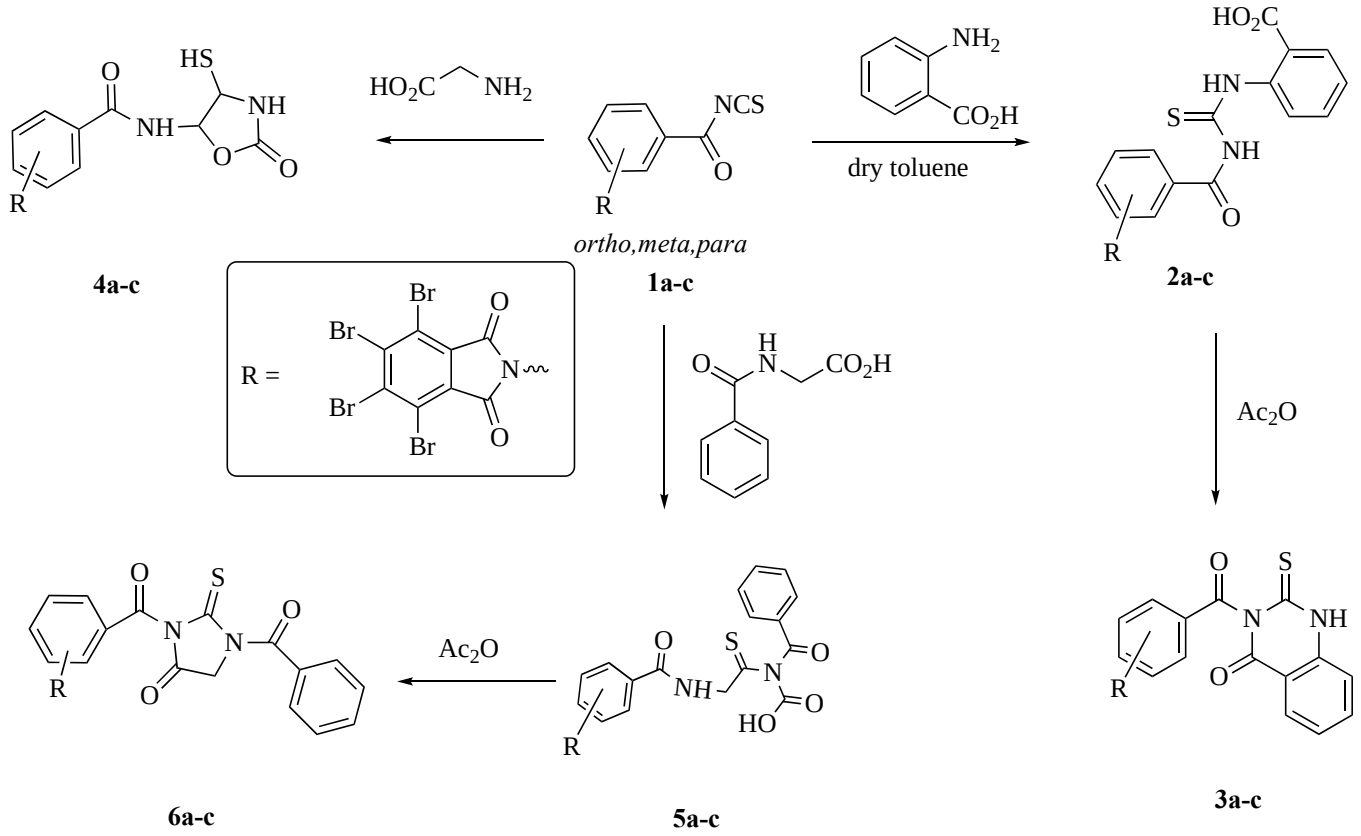
As illustrated in Scheme 1, compounds **1a**–**c** were prepared via reacting the appropriate derivative of (4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzoyl chloride and ammonium thiocyanate in dry acetone and the presence of anhydrous $CaCl_2$. The structure of compounds **1a**–**c** was proved by IR spectrum only because they can not be isolated from the reaction mixture, and they showed bands at 780-1770 cm^{-1}, 1730-1720 cm^{-1} v C=O imide, 1620-1610 cm^{-1} v C=N and 1290-1280 cm^{-1} v C=S.



I-III (*ortho*, *meta*, *para*) **1a-c**

Scheme 1. Synthesis of compounds **1a**–**c**

In Scheme 2, compounds **1a**–**c** were then subjected to reaction with anthranilic acid in boiling dry toluene to give N-(tetrabromophthalimidobenzoyl)thioureas (**2a**–**c**). IR spectra of **2a**–**c** exhibits bands at 1740-1730 cm^{-1} due to ν CO of cyclic imide, 1680-1660 cm^{-1} due to ν CO of anilide, 3450-3250 cm^{-1} due to ν NH and ν OH,1290-1270 due to ν C=S, and ^{1}H NMR spectrum of **2a** showed the following signals at δ ppm 12.60 (1H, CO_2H), 6.50-8.00 (10H, ArH, + 2NH). When compounds **2a**–**c** were boiled in acetic anhydride, they afforded 3-(tetrabromophthalimidobenzoyl)quinazolin-4-one-2-thione derivatives (**3a**–**c**). IR spectra of **3a**–**c** exhibits bands at 1740-1720 cm^{-1} and 1680-1660 cm^{-1} due to different imides, 1280-1270 cm^{-1} due to ν C=S, 3250-3200 cm^{-1} due to ν NH. The reaction of isothiocyanates (**2a**–**c**) with glycine in dry toluene and few drops of pyridine afforded the corresponding 2-N-(tetrabromophthalimidobenzamido)-1,3-oxazolidin-5-one-2-thiol (**4a**–**c**). IR spectra of **4a**–**c** showed bands at 2450-2400 cm^{-1} due to ν SH, 3350-3250 cm^{-1} due to ν NH, 1760-1740 cm^{-1} due to (ν CO of lactone), and 1690-1680 cm^{-1} due to (ν CO of anilide), ^{1}H NMR spectrum of **4a** showed the following signals at δ ppm, 3.70 (2H, CH_2-CO), 7.20-8.00 (6H, ArH+2NH). Similarly, isothiocyanates (**1a**–**c**) reacted with benzoylglycine in dry toluene and few drops of pyridine to afford the corresponding N-(2-tetrabromophthalimidobenzoyl)-N-benzoyl-N-carboxymethylthiourea derivative (**5a**–**c**), which showed, in IR spectra, bands at 1700-1680 cm^{-1} due to ν CO, 3450-3250 cm^{-1} due to (ν OH and ν NH) and 1290-1270 cm^{-1} due to ν C=S. MS spectra of compound **5a** showed molecular ion peak at *m/z* M^+ = 800. When compounds **5a**–**c** were refluxed with acetic anhydride, they afforded the corresponding 1-(tetrabromophthalimidobenzoyl)-3-benzoyl-2-thiono-1,3-imidazol-5-one (**6a**–**c**) with IR spectra bands at 1730-1720 cm^{-1} due to ν CO, 1690-1680 cm^{-1} due to (ν CO of anilide), and 1290-1280 cm^{-1} due to ν C=S, Meanwhile no band for OH was observed.

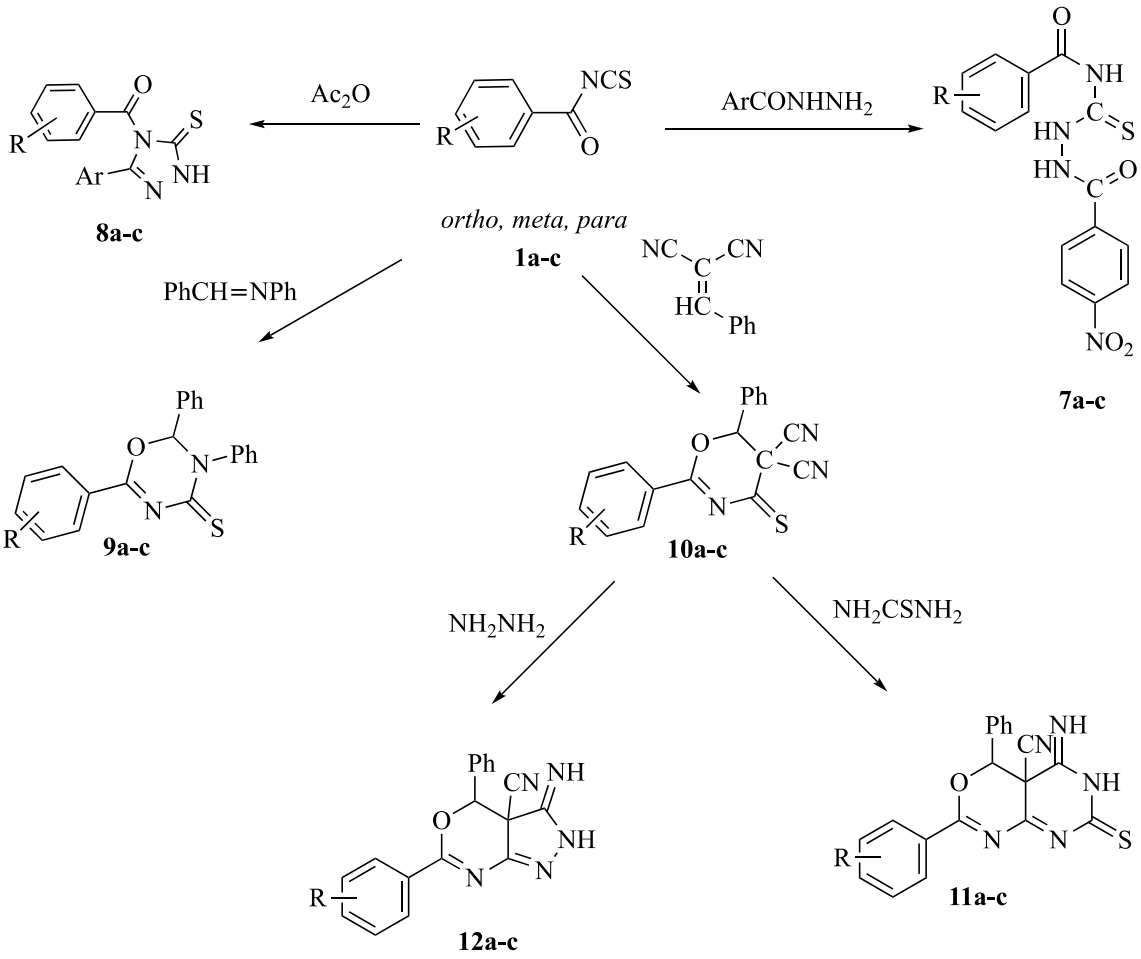


Scheme 2. Synthesis of compounds **2**–**6** (**a–c**)

As depicted in Scheme 3, the isothiocyanates **1a**–**c** reacted with *p*-nitrobenzohydrazide in dry toluene to afford the corresponding thiosemicarbazide derivatives (**7a**–**c**). IR spectra of compounds **7a**–**c** exhibited absorption bands at 1760-1720 cm^{-1} due to (ν CO of imide), 1690-1680 cm^{-1} due to (ν CO of anilide and hydrazide), 3350-3250 cm^{-1} due to ν NH and 1300-1290 due to ν C=S. When compounds (**7a–c**) were refluxed with acetic anhydride afforded 3-aryl-4-(tetrabromophthalimidobenzoyl)-Δ^2-1,2,4-triazoline-5-thione (**8a**–**c**). The structure of **8a–c** was confirmed from: Correct analytical data, IR spectrum of **8a**–**c** showed bands at 1630-1620 cm^{-1} due to ν C=N, 1750-1730 cm^{-1} due to (ν CO of imide), 1700-1680 cm^{-1} due to (ν CO of anilide), 3300-3250 cm^{-1} due to ν NH and 1300-1280 cm^{-1} due to ν C=S. The isothiocyanates (**1a**–**c**) reacted with benzylidene arylamine in dry toluene to afford 2-(tetrabromophthalimidophenyl)-5-aryl-6-phenyl-1,3,5-oxadiazine-4-thione (**9a**–**c**) via [4+2] cycloaddition. The structures of **9a**–**c** were confirmed by: Correct the analytical data, IR spectrum (**9a**–**c**) exhibited absorption bands at 1760-1740 cm^{-1} due to (ν CO of imide), 1620-1600 cm^{-1} due to ν C=N,1070-1060 cm^{-1} due to ν C-O-C, 1290-1280 cm^{-1} due to ν C=S, MS spectra (**9a**) showed molecular ion peak at *m/z* = 805.

When isothiocyanates (**1a**–**c**) were treated with α-cyanocinnamonitrile in dry toluene afforded the corresponding 2-(tetrabromophthalimidophenyl-5,5-disubstituted-1,3-oxazine-4-thiones (**10a**–**c**) the

reaction proceeds via [4+2] cycloaddition, Scheme 3. The structures (**10a**–**c**) were confirmed from: correct analytical data. IR spectrum for **10a**–**c** exhibits absorption bands at 2250-2200 cm^{-1} due to ν C≡N, 1630-1610 cm^{-1} due to ν C=N 1280-1270 cm^{-1} due to ν C=S 1060-1040 cm^{-1} due to ν C-O-C and 1750-1630 cm^{-1} due to ν CO. When compounds (**10a**–**c**) allowed to react with thiourea in boiling *n*-butanol they gave pyrimidine derivatives (**11a**–**c**), Scheme 3. The structures (**11a**–**c**) were confirmed by: correct analytical data, IR spectrum for **11a**–**c** exhibits absorption bands at 1680-1610 cm^{-1}due to ν C=N 2260-2120 cm^{-1} due to ν C≡N 1280-1270 cm^{-1} due to ν C=S, 3540-3300 cm^{-1} due to ν NH, ^{1}H NMR spectrum of **12c** showed the following signals (δ ppm), 6.8-8(11H, ArH+2NH) and (1H, Ph, CH), The MS spectrum of **11a** showed a molecular ion peak at *m/z* = 820. When compounds (**10a**–**c**) were allowed to react with hydrazine hydrate in boiling *n*-butanol, pyrazole derivatives were afforded (**12a**–**c**), The structures (**12a**–**c**) were confirmed data: correct the analytical data, IR spectrum for **12a**–**c** exhibited absorption bands at 1600-1670 cm^{-1} due to ν C=N, 2260-2120 cm^{-1} due to ν C≡N 3540-3300 cm^{-1} due to n NH, ^{1}H NMR spectra (**12a**–**c**) showed the following signals at δ ppm, 7-8 (11H, ArH+2NH) and (1H, PhCH).



Scheme 3. Synthesis of compounds **7**–**12** (**a**–**c**)

Biological evaluation

Antibacterial activity

The representative compounds were screened against *Aspergillus favus*, *E. coli*, *Staphylococcus*, and *Fusarium moniliform*, to evaluate the potential antimicrobial activity of the synthesized compounds over Gram-positive and Gram-negative bacteria. From the obtained biological data in Table 1, an evident elevated level of activity was demonstrated by derivatives **2a**, **4a**, **4b**, **4c**, **8a**, **10a**, **10b**, and **11a–c** in evaluation with *A. favus* and *E. coli*. For *Staphylococcus*, compounds **4a**, **4b**, **10a**, and **11a–c** exhibited significant antimicrobial activities. Moreover, **2a**, **4a**, **4c**, **10b**, and **11a–c** were active in assessment with *F. moniliforme*. Thus, compounds **11a–c** were concluded to have the most broadspectrum antimicrobial activity among all evaluated compounds.

Table 1. Antibacterial screening results; activity (A) and minimum inhibitory concentration (MIC)

Compound Number	*Aspergillus favus*		*E. coli*		*Staphylococcus aureus*		*Fusarium moniliform*	
	A	**MIC**	**A**	**MIC**	**A**	**MIC**	**A**	**MIC**
2a	+	250	-	-	-	-	+	250
4a	++	125	+	125	+	125	+	250
4b	++	250	+	125	+	125	-	-
4c	+++	250	+	250	-	-	++	250
8a	-	-	+	250	-	250	-	-
10a	++	125	-	-	+	250	-	-
10b	++	125	+	125	-	-	+	125
11a	++	250	+	250	++	250	+	250
11b	++	250	+	250	++	250	+	250
11c	++	125	+	125	+	125	+	125

The zone width of inhibition indicates the potency antimicrobial activity; (-) no activity, (+) mild activity with diameter zone of (0.5-0.7 Cm), (++) moderate activity with diameter zone of (1.1-1.2 Cm); (+++) marked with diameter zone of (1.6-1.8 Cm)

Anticancer activity

The anticancer activity was evaluated against two cell lines, OVCAR-3 for ovarian cancer and **Hela** for cervical cancer. The results obtained in Table 2 indicated that compounds IC_{50} of OVCAR-3 cell lines for compounds **11a** and **11c** are the most potent compounds. The graphical statistical analysis is represented in Figure 2.

Table 2. IC_{50} values in µg/mL for the tested compounds against two cancer cell lines (OVCAR-3 and Hela)

Cpd.	OVCAR-3		HeLa	
	IC_{50}	± SD	IC_{50}	± SD
2a	12.43	0.518	15.63	0.023
4a	16.56	0.125	14.26	0.329
4b	9.114	0.533	16.62	0.021
4c	10.16	0.859	7.339	0.024
8a	17.960	0.138	14.19	0.93
10a	15.36	0.148	14.90	0.017
10b	11.47	0.335	9.485	0.078
11a	8.349	0.301	7.803	0.006
11b	11.46	0.06	6.933	0.054
11c	8.421	0.368	9.004	0.056

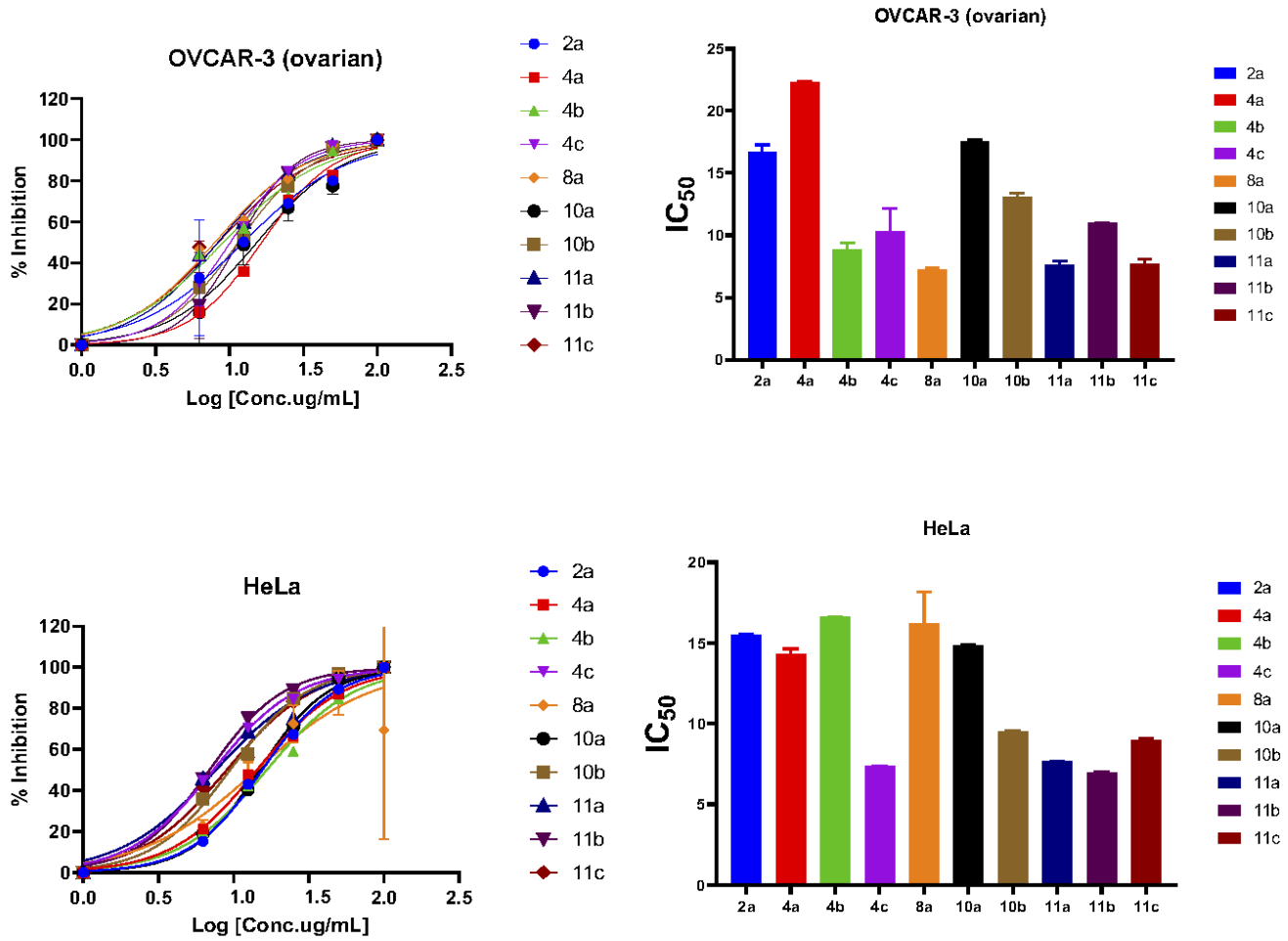


Figure 2. The graphical statistical analysis of the anticancer activities of the selected compounds

MOLECULAR DOCKING

Immunomodulatory drugs (IMiDs) are useful in the treatment of multiple myeloma and myelodysplastic syndrome. Binding of IMiDs to cereblon (CRBN), the substrate receptor of the CRL4CRBN E3 ubiquitin ligase, causes cancer cell death by degrading key neo-substrates. The target molecule of thalidomide and its derivatives has recently been identified as celeblon (CRBN), and the biological actions and mechanisms of thalidomide-related compounds are increasingly becoming clearer. The thalidomide derivatives have been shown to bind to CRBN and inhibit the CRBN-HSP90 interaction, reducing HSP90's chaperone function and helping to combat cancer. Accordingly, a molecular docking study was conducted to predict the inhibitory activity of the synthesized compounds on cereblon. The validity of the docking protocol used herein was calculated by reproducing the co-crystal pose of the S-thalidomide ligand. The co-crystal ligand binding pose was reproduced accurately within 0.30 Å heavy atom RMSD (Table 3) indicating that our docking model was sufficiently accurate.

Table 3. Molecular docking validation

Co-crystal ligand	Crystal structure PDB	Glide RMSD to input
S-Thalidomide (EF2)	4CI1	0.30 Å

The docking scores of the synthesized compounds predicted that compounds **10a–b** and **11a–c** to possess potent inhibitory activity on the cereblon protein. On the other hand, compounds **2a**, **4a**–**c** and **8a** were predicted to possess moderate to low activity as demonstrated by their moderated docking scores in Table 3. Compounds **10a**, **10b** and **11a** exhibited the highest docking scores of -8.12, -8.92 and -7.86 Kcal/mol, respectively. Compound **10b** possessed the highest predicted docking score of -8.92, which may be due to the high number of strong interactions it formed with the active site of the cereblon protein. Compound **10b** established six hydrogen bonds (Figures 3a and b) with the amino acid residues; HIS355, HIS380, HIS359, ASN353, SER377 and TRP402. Compound **11a** established a hydrogen bond with HIS355 and a π–sulfur bond with TRP402, Figure 3d. However, the high docking score of compound **11a** may stem from the fact that it exhibited a good fit inside the active site residue, indicating a strong binding affinity. The binding of compounds **10b** and **11a** is illustrated in Figure 3.

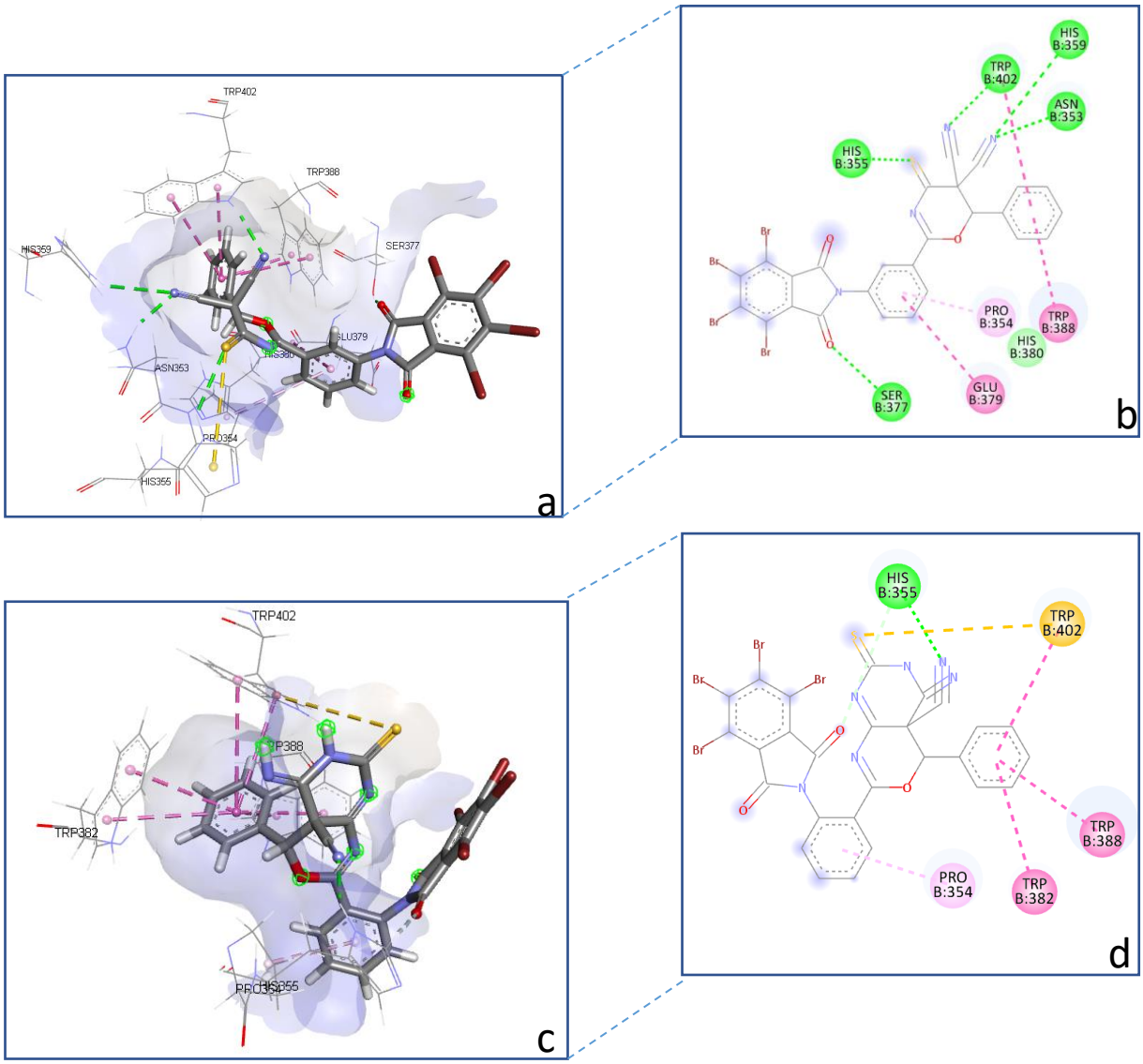


Figure 3. Predicted interactions of compounds **10b** and **11a** into the active site residues of cerebelon (PDB: 4CI1). **10b(a)** and **11a(c)** 3D structural views of the ligands inside the active site structural. **10b(b)** and **11a(d)** represent ligand interaction diagram; green lines represent hydrogen bonds, orange lines represent π–sulfur interactions and violet lines represent π–cation interactions.

Table 4. The docking scores of the synthesized compounds

Cpd.	Docking score (Kcal/mol)
2a	-7.47
4a	-6.48
4b	-5.91
4c	-5.45
8a	-6.32
10a	-8.12
10b	-8.92
11a	-7.86
11b	-7.82
11c	-7.0847

ADMT study

A potent antagonistic interaction of inhibitors with a receptor protein or enzyme cannot guarantee the ability of an inhibitor as a drug; therefore, ADME assessment is essential in drug development. ADME is founded on Lipinski's rule of five and aid in the endorsement of inhibitors for biological systems. One of the main reasons for the failure of many medicines during their clinical experiments is having poor ADME characteristics and unfavorable toxicology. Using the SwissADME server, we subjected the synthesized compounds to an in silico ADME study. From Figure 4, we can conclude that all compounds have no inhibitory effect on the cytochrome P450 enzymes, predicting their safety and interaction profile.

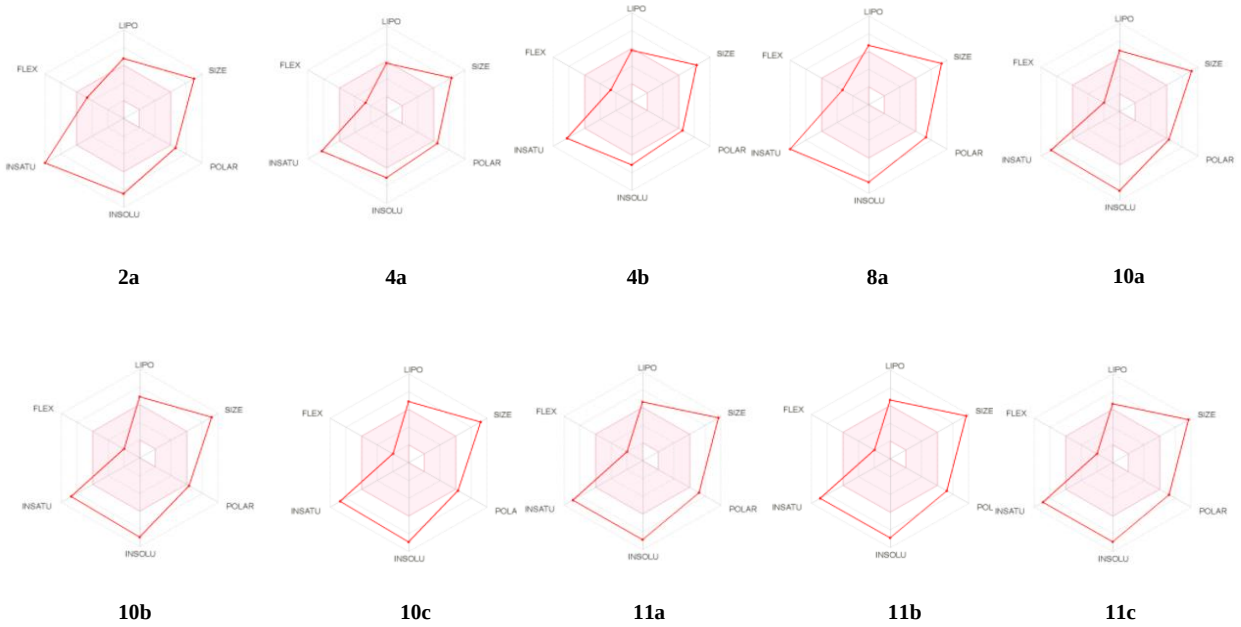


Figure 4. Toxicity profiles of the evaluated compounds using SwissADME server

CONCLUSION

In summary, as an extension to our recent report,[5] a novel series of heterocyclic compounds containing the 4,5,6,7-tetrabromophthalimide moiety, was synthesized and physiologically investigated for potential antibacterial and anticancer properties. While compounds **11a**–**c** were the most active antibacterial activity against *A. favus*, *E. coli*, *Staphylococcus*, and *F. moniliform*, derivatives **11a** and **11c** had a whole range (6.933–11.46 μg/mL) against cervical and ovarian cancer cell lines. To examine the possible binding mechanism and toxicity profiles of the newly synthesized chemical, additional in silico experiments were conducted, including molecular docking research over cereblon protein where compounds **10**–**b** and **11a**–**c** possessed excellent binding affinity to the cereblon active site. Additionally, no negative impact on cytochrome P450 enzymes was predicted for all the evaluated compounds.

EXPERIMENTAL

Chemistry

The general protocol of the chemical experiments is presented in detail in the supplementary material.

Synthesis of (4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzoyl isothiocyanate derivatives (1a–c)

A solution of the appropriate tertabromo-*N*-phthalimidebenzoyl chloride (0.01 mol) and ammonium thiocyanate (0.02 mmol) in dry acetone was refluxed for 3 h, in the presence of calcium chloride. After cooling, the crude product was used in the next step without further purification.

Synthesis of compounds 2a–c

A solution of isothiocyanates (**1a**–**c**, 0.01 mmol) in dry acetone and anthranilic and (0.01 mmol) in 30 mL of dry toluene was refluxed for 1–1.5 h. After cooling, the separated products were crystallized from the proper solvents to give compounds **2a**–**c**.

2a: 2-(3-(2-(4,5,6,7-Tetrabromo-1,3-dioxoisoindolin-2-yl)benzoyl)thioureido)benzoic acid

Brown solid, mp 175-177 ˚C, solvent: EtOH, yield 55%, IR (KBr) υ cm^{-1} 1740-1730 CO of cyclic imide, 1680-1660 CO of anilide, 3450-3250 NH and OH,1290-1270 C=S. ^{1}H NMR δ: 8.09 (s, 1H), 8.00 (ddd, *J* = 14.5, 7.6, 1.6 Hz, 2H), 7.93 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.87 (dd, *J* = 6.4, 1.4 Hz, 1H), 7.63 (ddd, *J* = 7.2, 6.6, 1.5 Hz, 1H), 7.53 (td, *J* = 7.9, 1.5 Hz, 1H), 7.28 (td, *J* = 7.2, 1.3 Hz, 1H), 7.22 (td, *J* = 7.9, 1.4 Hz, 1H). ^{13}C NMR δ: 178.23, 170.05, 167.75, 165.29, 164.85, 141.30, 140.98, 135.41, 133.95, 133.74, 133.03, 132.83, 131.63, 131.20, 126.10, 125.81, 125.27, 123.24, 122.00, 120.29, 120.06, 118.98, 118.55. Anal. Calcd for C:36.3; H:1.5; N:5.5. Found C:35.8; H:1.3; N:5.3%.

2b: 2-(3-(3-(4,5,6,7-Tetrabromo-1,3-dioxoisoindolin-2-yl)benzoyl)thioureido)benzoic acid

Brown solid, mp 110-112 ˚C, solvent: benzene, yield 75%, IR (KBr) υ cm^{-1} 1740-1730 CO of cyclic imide, 1680-1660 CO of anilide, 3450-3250 NH and OH,1290-1270 C=S. ^{1}H NMR δ: 8.21 (t, *J* = 2.1 Hz, 1H), 7.82 (ddd, *J* = 7.4, 2.1, 1.3 Hz, 1H), 7.68 (dd, *J* = 7.5, 6.5 Hz, 1H), 7.62 (ddd, *J* = 6.6, 2.1, 1.3 Hz, 1H), 7.03 (q, *J* = 6.3 Hz, 1H), 6.72 (d, *J* = 2.1 Hz, 1H), 6.07 (d, *J* = 2.1 Hz, 1H), 1.93 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR δ: 176.53, 166.78, 166.34, 164.98, 141.45, 138.43, 134.47, 134.02, 133.00, 132.00, 129.84, 128.00, 126.09, 125.78, 122.77, 122.15, 95.64, 15.74. Anal. Calcd for C:36.3; H:1.5; N:5.5. Found C:35.9; H:1.4; N:5.2%.

2c: 2-(3-(4-(4,5,6,7-Tetrabromo-1,3-dioxoisoindolin-2-yl)benzoyl)thioureido)benzoic acid

Brown solid, mp 139-140 ˚C, solvent: EtOH, yield 60%, IR (KBr) υ cm^{-1} 1740-1730 CO of cyclic imide, 1680-1660 CO of anilide, 3450-3250 NH and OH,1290-1270 C=S. ^{1}H NMR δ: 8.21 (t, *J* = 2.1 Hz, 2H),

7.99 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.93 (dd, *J* = 8.0, 1.3 Hz, 2H), 7.82 (ddd, *J* = 7.5, 2.1, 1.3 Hz, 2H), 7.68 (dd, *J* = 7.5, 6.5 Hz, 2H), 7.62 (ddd, *J* = 6.6, 2.1, 1.3 Hz, 2H), 7.53 (td, *J* = 7.9, 1.5 Hz, 2H), 7.22 (td, *J* = 7.9, 1.4 Hz, 2H). ^{13}C NMR δ: 178.46, 170.05, 165.98, 164.98, 140.98, 138.19, 134.02, 133.00, 132.00, 131.63, 131.20, 129.84, 128.00, 126.09, 122.77, 122.15, 122.00, 120.06, 118.55. Anal. Calcd for C:36.3; H:1.5; N:5.5. Found C:35.7; H:1.4; N:5.1%.

Synthesis of compounds 3a–c

Compound **2a–c** (0.01 mmol) was refluxed in 10 mL of acetic anhydride for 30 min. Then, the reaction mixture was cooled and poured into ice-cold water. The solid product obtained was filtered and crystallized from suitable solvents to give the corresponding derivative **3a**–**c**.

3a: 4,5,6,7-Tetrabromo-2-(2-(4-oxo-2-thioxo-1,2,3,4-tetrahydroquinazoline-3-carbonyl)phenyl)isoindoline-1,3-dione

Yellow solid, mp 200-202 °C, solvent: EtOH, yield 65%, IR (KBr) υ cm^{-1}: 1740-1720 and 1680-1660, different imides, 1280-1270 v C=S, 3250-3200 v NH, ^{1}H NMR δ: 8.05 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.97 (dd, *J* = 7.4, 1.5 Hz, 1H), 7.84 (dd, *J* = 6.5, 1.4 Hz, 1H), 7.65 (td, *J* = 7.9, 1.5 Hz, 1H), 7.57 (ddd, *J* = 8.1, 6.0, 1.4 Hz, 2H), 7.36 – 7.26 (m, 2H). ^{13}C NMR δ: 175.90, 168.94, 164.81, 162.59, 140.35, 134.90, 133.44, 133.24, 132.83, 132.67, 129.84, 127.34, 126.89, 125.46, 123.83, 123.10, 122.71, 117.77, 117.28. Anal. Calcd for $C_{23}H_9N_3O_4Br_4$; C: 37.3; H: 1.2; N: 5.7. Found C: 36.6; H: 1.00; N: 5.5%.

3b: 4,5,6,7-Tetrabromo-2-(3-(4-oxo-2-thioxo-1,2,3,4-tetrahydroquinazoline-3-carbonyl)phenyl)isoindoline-1,3-dione

Brown solid, mp 145-147 °C, solvent: EtOH, yield 80%, IR (KBr) υ cm^{-1}: 1740-1720 and 1680-1660, different imides, 1280-1270 v C=S, 3250-3200 v NH. ^{1}H NMR δ: 8.05 (dd, *J* = 8.4, 1.5 Hz, 1H), 8.02 – 7.97 (m, 2H), 7.65 (td, *J* = 7.9, 1.5 Hz, 1H), 7.57 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.51 – 7.46 (m, 2H), 7.29 (ddd, *J* = 8.4, 7.8, 1.5 Hz, 1H). ^{13}C NMR δ: 176.13, 167.59, 164.72, 162.73, 140.35, 135.38, 134.90, 133.14, 133.05, 132.94, 129.84, 127.34, 125.21, 123.10, 122.12, 117.77, 117.52. Anal. Calcd for C: 37.3; H: 1.2; N: 5.7. Found C: 36.9; H: 1.1; N: 5.6%.

3c: 4,5,6,7-Tetrabromo-2-(4-(4-oxo-2-thioxo-1,2,3,4-tetrahydroquinazoline-3-carbonyl)phenyl)isoindoline-1,3-dione

Brown solid, mp 160-162 °C, solvent: EtOH, yield 70%, IR (KBr) υ cm^{-1}: 1740-1720 and 1680-1660, different imides, 1280-1270 v C=S, 3250-3200 v NH. ^{1}H NMR δ: 8.05 (dd, *J* = 8.4, 1.5 Hz, 1H), 8.02 – 7.97 (m, 2H), 7.65 (td, *J* = 7.9, 1.5 Hz, 1H), 7.57 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.51 – 7.46 (m, 2H), 7.29 (ddd,

J = 8.4, 7.8, 1.5 Hz, 1H). 13C NMR δ: 176.13, 167.59, 164.72, 162.73, 140.35, 135.38, 134.90, 133.14, 133.05, 132.94, 129.84, 127.34, 125.21, 123.10, 122.12, 117.77, 117.52. Anal. Calcd for C: 37.3; H: 1.2; N: 5.7. Found C: 36.7; H: 1.00; N: 5.4%.

Synthesis of compounds 4a–c

A solution of isothiocyanates (**1a**–**c**, 0.01 mmol) in dry acetone was added to glycine (0.01 mmol) in 30 mL of dry toluene and few drops of dry pyridine. The reaction mixture was refluxed for 1–1.5 h, then cooled to room temperature. The solid product formed was filtered off and crystallized from suitable solvents to give (**4a**–**c**).

4a: N-(4-Mercapto-2-oxooxazolidin-5-yl)-2-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzamide

Brown solid, mp 106-108 ˚C, solvent: benzene, yield 55%, IR (KBr) υ cm^{-1}: 2450-2400, SH, 3350-3250 NH, 1760-1740 v CO of lactone, and 1690-1680 v CO of anilide. ^{1}H NMR δ: 7.92 – 7.84 (m, 2H), 7.63 (ddd, *J* = 7.2, 6.5, 1.7 Hz, 1H), 7.56 (td, *J* = 7.2, 1.4 Hz, 1H), 7.41 (d, *J* = 5.5 Hz, 1H), 6.99 (d, *J* = 5.3 Hz, 1H), 5.80 (dd, *J* = 5.5, 2.0 Hz, 1H), 4.48 (ddd, *J* = 5.5, 4.5, 2.0 Hz, 1H), 2.99 (s, 1H). ^{13}C NMR δ: 169.90, 164.86, 157.98, 134.05, 133.99, 133.46, 132.99, 129.84, 128.48, 125.25, 123.30, 122.67, 80.55, 59.25. Anal. Calcd for C: 30.9; H: 1.3; N: 6 Found C: 30.1; H: 1.3; N: 5.7%.

4b: N-(4-Mercapto-2-oxooxazolidin-5-yl)-3-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzamide

Brown solid, mp 158-160 ˚C, solvent: benzene, yield 75%, IR (KBr) υ cm^{-1}: 2450-2400, SH, 3350-3250 NH, 1760-1740 v CO of lactone, and 1690-1680 v CO of anilide. ^{1}H NMR δ: 8.12 (t, *J* = 2.1 Hz, 1H), 7.79 (ddd, *J* = 7.4, 2.1, 1.2 Hz, 1H), 7.68 (dd, *J* = 7.6, 6.5 Hz, 1H), 7.62 (ddd, *J* = 6.6, 2.1, 1.2 Hz, 1H), 7.51 (d, *J* = 5.5 Hz, 1H), 6.99 (d, *J* = 5.3 Hz, 1H), 5.77 (dd, *J* = 5.6, 1.9 Hz, 1H), 4.50 (ddd, *J* = 5.5, 4.4, 2.0 Hz, 1H), 2.99 (s, 1H). ^{13}C NMR δ: 166.46, 164.98, 157.98, 137.32, 134.13, 133.00, 132.03, 129.84, 127.95, 125.53, 122.39, 122.15, 80.72, 59.18. Anal. Calcd for C: 30.9; H: 1.3; N: 6. Found C: 30.9; H: 1.1; N: 5.8%.

4c: N-(4-Mercapto-2-oxooxazolidin-5-yl)-4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzamide

Brown solid, mp 140-142 ˚C, solvent: benzene, yield 60%, IR (KBr) υ cm^{-1}: 2450-2400, SH, 3350-3250 NH, 1760-1740 v CO of lactone, and 1690-1680 v CO of anilide. ^{1}H NMR δ: 8.06 – 8.00 (m, 2H), 7.59 (d, *J* = 5.5 Hz, 1H), 7.51 – 7.45 (m, 2H), 6.99 (d, *J* = 5.3 Hz, 1H), 5.77 (dd, *J* = 5.5, 2.0 Hz, 1H), 4.50 (ddd, *J* = 5.5, 4.4, 2.0 Hz, 1H), 2.99 (s, 1H). ^{13}C NMR δ: 166.41, 164.72, 157.98, 134.96, 133.14, 131.89, 131.49, 129.84, 125.17, 122.12, 80.62, 59.18. Anal. Calcd for C: 30.9; H: 1.3; N: 6. Found C: 30.4; H: 1.2; N: 5.9%.

Synthesis of compounds 5a–c

A solution of isothiocyanates (**1a**–**c**, 0.01 mol) in dry acetone was added to benzoylglycine (0.01 mol) in 30 mL of dry toluene and few drops of dry pyridine. The reaction mixture was refluxed for 1–1.5 h, then cooled to room temperature. The solid product formed was filtered off and crystallized from the suitable solvent to give compounds **5a**–**c**.

5a: Benzoyl(2-(2-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzamido)ethanethioyl)carbamic acid

Grey solid, mp 196-198 °C, solvent: benzene, yield 75%, IR (KBr) υ cm^{-1}, 1700-1680 CO, 3450-3250 OH and NH and 1290-1270 C=S. ^{1}H NMR δ: 9.19 (t, *J* = 4.7 Hz, 1H), 7.92 – 7.84 (m, 2H), 7.84 – 7.78 (m, 2H), 7.63 (ddd, *J* = 7.2, 6.6, 1.7 Hz, 1H), 7.59 – 7.46 (m, 4H), 4.64 (d, *J* = 4.7 Hz, 2H). ^{13}C NMR δ: 195.87, 169.43, 169.05, 164.83, 137.00, 134.66, 134.18, 133.46, 132.97 (d, *J* = 4.5 Hz), 132.34, 129.84, 129.16, 128.44, 127.24, 124.74, 123.27, 122.67, 51.98. Anal. Calcd for C: 37.4; H: 1.6; N: 5.2. Found C: 37.1; H: 1.4; N: 5.1%.

5b: Benzoyl(2-(4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzamido)ethanethioyl)carbamic acid

Brown solid, mp 206-208 °C, solvent: benzene, yield 65%, IR (KBr) υ cm^{-1}, 1700-1680 CO, 3450-3250 OH and NH and 1290-1270 C=S. ^{1}H NMR δ: 8.90 (t, *J* = 4.6 Hz, 1H), 8.13 (t, *J* = 2.2 Hz, 1H), 7.80 (tdd, *J* = 7.0, 2.4, 1.4 Hz, 3H), 7.67 (dd, *J* = 7.5, 6.5 Hz, 1H), 7.62 (ddd, *J* = 6.6, 2.1, 1.3 Hz, 1H), 7.58 – 7.50 (m, 1H), 7.53 – 7.46 (m, 2H), 4.62 (d, *J* = 4.7 Hz, 2H). ^{13}C NMR δ: 195.87, 169.43, 169.05, 164.83, 137.00, 134.66, 134.18, 133.46, 132.97 (d, *J* = 4.5 Hz), 132.34, 129.84, 129.16, 128.44, 127.24, 124.74, 123.27, 122.67, 51.98. Anal. Calcd for C: 37.4; H: 1.6; N: 5.2. Found C: 37.0; H: 1.3; N: 5.3%.

5c: Benzoyl(2-(3-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzamido)ethanethioyl)carbamic acid

Brown solid, mp 105-107 °C, solvent: benzene, yield 75%, IR (KBr) υ cm^{-1}, 1700-1680 CO, 3450-3250 OH and NH and 1290-1270 C=S, ^{1}H NMR δ: 8.90 (t, *J* = 4.6 Hz, 1H), 8.13 (t, *J* = 2.2 Hz, 1H), 7.80 (tdd, *J* = 7.0, 2.4, 1.4 Hz, 3H), 7.67 (dd, *J* = 7.5, 6.5 Hz, 1H), 7.62 (ddd, *J* = 6.6, 2.1, 1.3 Hz, 1H), 7.58 – 7.50 (m, 1H), 7.53 – 7.46 (m, 2H), 4.62 (d, *J* = 4.7 Hz, 2H). ^{13}C NMR δ: 195.87, 169.43, 169.05, 164.83, 137.00, 134.66, 134.18, 133.46, 132.97 (d, *J* = 4.5 Hz), 132.34, 129.84, 129.16, 128.44, 127.24, 124.74, 123.27, 122.67, 51.98. Anal. Calcd for C: 37.4; H: 1.6; N: 5.2. Found C: 37.1; H: 1.5; N: 5.0%.

Synthesis of compounds 6a–c

Compounds **5a**–**c** (0.01 mol) were refluxed in 10 mL of acetic anhydride for 30 min. The reaction mixture was then cooled and poured on ice-cold water. The solid product obtained was filtered and crystallized from proper solvents to give compounds **6a**–**c**.

6a: 2-(2-(3-Benzoyl-5-oxo-2-thioxoimidazolidine-1-carbonyl)phenyl)-4,5,6,7-tetrabromoisoindoline-1,3-dione

Brown solid, mp 265-267 °C, solvent: EtOH, yield 62%, IR (KBr) υ cm^{-1}: 1730-1720 CO, 1690-1680 CO of anilide, 1290-1280 C=S. ^{1}H NMR δ: 7.98 (dd, *J* = 7.4, 1.6 Hz, 1H), 7.89 – 7.82 (m, 3H), 7.60 – 7.49 (m, 3H), 7.52 – 7.47 (m, 1H), 7.32 (td, *J* = 7.3, 1.4 Hz, 1H), 5.00 (s, 2H). ^{13}C NMR δ: 180.95, 169.73, 169.13, 166.75, 164.81, 134.55, 133.44, 132.83, 132.42, 132.07, 129.84, 128.74, 128.01, 127.16, 125.47, 123.83, 122.71, 50.24. Anal. Calcd for C: 38.2; H: 1.4; N: 5.4. Found C: 37.3; H: 1.1; N: 5.1%.

6b: 2-(3-(3-Benzoyl-5-oxo-2-thioxoimidazolidine-1-carbonyl)phenyl)-4,5,6,7-tetrabromoisoindoline-1,3-dione

Brown solid, mp 205-207 °C, solvent: EtOH, yield 75%, IR (KBr) υ cm^{-1}: 1730-1720 CO, 1690-1680 CO of anilide, 1290-1280 C=S. ^{1}H NMR δ: 8.17 (dq, *J* = 1.6, 0.9 Hz, 1H), 7.88 – 7.83 (m, 2H), 7.75 – 7.68 (m, 2H), 7.62 – 7.54 (m, 1H), 7.54 – 7.47 (m, 2H), 4.99 (s, 2H). ^{13}C NMR δ: 180.83, 169.74, 169.14, 167.16, 164.86, 138.87, 134.55, 133.24, 133.00, 132.98, 132.07, 129.84, 128.74, 128.53, 128.01, 126.55, 122.73, 122.15, 50.16. Anal. Calcd for C: 38.2; H: 1.4; N: 5.4. Found C: 37.7; H: 1.3; N: 5.3%.

6c: 2-(2-(3-Benzoyl-5-oxo-2-thioxoimidazolidine-1-carbonyl)phenyl)-4,5,6,7-tetrabromoisoindoline-1,3-dione

Brown solid, mp 160-163 °C, solvent: benzene, yield 70%, IR (KBr) υ cm^{-1}: 1730-1720 CO, 1690-1680 CO of anilide, 1290-1280 C=S. ^{1}H NMR δ: 7.98 (dd, *J* = 7.4, 1.6 Hz, 1H), 7.89 – 7.82 (m, 3H), 7.60 – 7.49 (m, 3H), 7.52 – 7.47 (m, 1H), 7.32 (td, *J* = 7.3, 1.4 Hz, 1H), 5.00 (s, 2H). ^{13}C NMR δ: 180.95, 169.73, 169.13, 166.75, 164.81, 134.55, 133.44, 132.83, 132.42, 132.07, 129.84, 128.74, 128.01, 127.16, 125.47, 123.83, 122.71, 50.24. Anal. Calcd for C: 38.2; H: 1.4; N: 5.4. Found C: 37.6; H: 1.21; N: 5.2%.

Synthesis of compounds 7a–c

The solution of isothiocyanates (**1a**–**c**, 0.01 mol) in dry acetone was added to 4-nitrobenzohydrazide (0.01 mol) in 30 mL of dry toluene. The reaction mixture was refluxed for 1–1.5 h. The solid product was filtered and crystallized from suitable solvents to give **7a**–**c**.

7a: N-(2-(4-Nitrobenzoyl)hydrazine-1-carbonothioyl)-2-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzamide

Brown solid, mp 150-152 °C, solvent: EtOH, yield 58%, IR (KBr) υ cm^{-1}: 1760-1720 CO of imide, 1690-1680 ν CO of anilide and hydrazide, 3350-3250 NH and 1300-1290 C=S. ^{1}H NMR δ: 8.02 (dd, *J* = 7.2, 1.6 Hz, 1H), 7.87 (dd, *J* = 6.5, 1.3 Hz, 1H), 7.82 – 7.76 (m, 2H), 7.63 (ddd, *J* = 7.2, 6.6, 1.5 Hz, 1H), 7.28 (td,

J = 7.2, 1.3 Hz, 1H), 7.19 – 7.13 (m, 2H). ^{13}C NMR δ: 179.31, 167.63, 165.20, 164.82, 146.36, 135.42, 133.97, 133.46, 133.03, 129.84, 129.48, 127.47, 126.06, 125.97, 125.32, 123.24, 122.67. Anal. Calcd for C: 34.3; H: 1.4; N: 8.7. Found C: 33.9; H: 1. 1; N: 8.3%.

7b: N-(2-(4-Nitrobenzoyl)hydrazine-1-carbonothioyl)-3-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzamide

Brown solid, mp 160-162 °C, solvent: toluene, yield 80%, IR (KBr) υ cm^{-1}: 1760-1720 CO of imide, 1690-1680 ν CO of anilide and hydrazide, 3350-3250 NH and 1300-1290 C=S. ^{1}H NMR δ: 8.02 (dd, *J* = 7.2, 1.6 Hz, 1H), 7.87 (dd, *J* = 6.5, 1.3 Hz, 1H), 7.82 – 7.76 (m, 2H), 7.63 (ddd, *J* = 7.2, 6.6, 1.5 Hz, 1H), 7.28 (td, *J* = 7.2, 1.3 Hz, 1H), 7.19 – 7.13 (m, 2H). ^{13}C NMR δ: 179.31, 167.63, 165.20, 164.82, 146.36, 135.42, 133.97, 133.46, 133.03, 129.84, 129.48, 127.47, 126.06, 125.97, 125.32, 123.24, 122.67. Anal. Calcd for C: 34.3; H: 1.4; N: 8.7. Found C: 33.9; H: 1. 2; N: 8.4%.

7c: N-(2-(4-Nitrobenzoyl)hydrazine-1-carbonothioyl)-4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzamide

Brown solid, mp 90-92 °C, solvent: benzene, yield 60%, IR (KBr) υ cm^{-1}: 1760-1720 CO of imide, 1690-1680 ν CO of anilide and hydrazide, 3350-3250 NH and 1300-1290 C=S. ^{1}H NMR δ: 8.02 (dd, *J* = 7.2, 1.6 Hz, 1H), 7.87 (dd, *J* = 6.5, 1.3 Hz, 1H), 7.82 – 7.76 (m, 2H), 7.63 (ddd, *J* = 7.2, 6.6, 1.5 Hz, 1H), 7.28 (td, *J* = 7.2, 1.3 Hz, 1H), 7.19 – 7.13 (m, 2H). ^{13}C NMR δ: 179.31, 167.63, 165.20, 164.82, 146.36, 135.42, 133.97, 133.46, 133.03, 129.84, 129.48, 127.47, 126.06, 125.97, 125.32, 123.24, 122.67. Anal. Calcd for C: 34.3; H: 1.4; N: 8.7. Found C: 34; H: 1. 1; N: 8.6%.

Synthesis of compounds 8a–c

A solution of (**7a**–**c**, 0.01 mol) was refluxed for 30 min in 10 mL of acetic anhydride. The reaction mixture was cooled and poured on ice-cold water. The solid product which had separated was filtered and crystallized from suitable solvents to give **8a**–**c**.

8a: 4,5,6,7-Tetrabromo-2-(2-(3-(4-nitrophenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-4-carbonyl)phenyl)isoindoline-1,3-dione

Brown solid, mp 269-271 °C, solvent: EtOH, yield 58%, IR (KBr) υ cm^{-1}: 1630-1620 C=N, 1750-1730 ν CO of imide, 1700-1680 ν CO of anilide, 3300-3250 cm^{-1} NH and 1300-1280 C=S. ^{1}H NMR δ: 8.32 – 8.26 (m, 2H), 8.03 – 7.95 (m, 3H), 7.84 (dd, *J* = 6.5, 1.4 Hz, 1H), 7.57 (ddd, *J* = 7.2, 6.6, 1.5 Hz, 1H), 7.32 (td, *J* = 7.3, 1.4 Hz, 1H).^{13}C NMR δ: 173.97, 166.61, 164.80, 150.31, 148.60, 133.44, 133.35, 132.82, 132.65,

132.31, 129.84, 126.78, 126.61, 125.45, 124.76, 123.83, 122.71. Anal. Calcd for C: 35.1; H: 1.2; N: 8.9. Found C: 33.9; H: 1. 1; N: 8.3%.

8b: 4,5,6,7-Tetrabromo-2-(3-(3-(4-nitrophenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-4-carbonyl)phenyl)isoindoline-1,3-dione

Brown solid, mp 278-280 ˚C, solvent: EtOH, yield 80%, IR (KBr) υ cm^{-1}: 1630-1620 C=N, 1750-1730 ν CO of imide, 1700-1680 ν CO of anilide, 3300-3250 cm^{-1} NH and 1300-1280 C=S. ^{1}H NMR δ: 8.17 – 8.12 (m, 1H), 7.96 (dd, *J* = 16.7, 1.4 Hz, 1H), 7.83 (ddq, *J* = 16.6, 8.2, 0.9 Hz, 1H), 7.75 – 7.68 (m, 2H), 7.62 – 7.55 (m, 1H), 7.27 (dp, *J* = 8.2, 1.4 Hz, 1H), 2.05 (t, *J* = 1.2 Hz, 3H).^{13}C NMR δ: 173.72, 168.57, 164.86, 156.45, 141.31, 135.96, 133.24, 133.00, 132.98, 131.00, 129.84, 128.50, 128.11, 126.54, 122.83, 122.15, 121.88, 14.58. Anal. Calcd for C: 35.1; H: 1.2; N: 8.9. Found C: 34.5; H: 1; N: 8.5%.

8c: 4,5,6,7-Tetrabromo-2-(4-(3-(4-nitrophenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-4-carbonyl)phenyl)isoindoline-1,3-dione

Brown solid, mp 199-202 ˚C, solvent: EtOH, yield 60%, IR (KBr) υ cm^{-1}: 1630-1620 C=N, 1750-1730 ν CO of imide, 1700-1680 ν CO of anilide, 3300-3250 cm^{-1} NH and 1300-1280 C=S. ^{1}H NMR δ: 8.32 – 8.26 (m, 1H), 8.03 – 7.96 (m, 2H), 7.51 – 7.46 (m, 1H).^{13}C NMR δ: 173.81, 167.15, 164.72, 150.66, 148.60, 135.38, 133.14, 133.03, 132.43, 132.24, 129.84, 126.63, 125.21, 124.76, 122.12. Anal. Calcd for C: 35.1; H: 1.2; N: 8.9. Found C: 34.8; H: 1.1; N: 8.7%.

Synthesis of compounds 9a–c

The solution of benzylidene arylamine (0.01 mol) in 30 mL dry toluene was added to a solution of the equivalent amount (0.01 mol) of isothiocyanates (**1a–c**) in dry acetone, and the mixture was heated to cool to room temperature and the solid product formed was crystallized from suitable solvents to give **9a–c**.

9a: 4,5,6,7-Tetrabromo-2-(2-(2,3-diphenyl-4-thioxo-3,4-dihydro-2H-1,3,5-oxadiazin-6-yl)phenyl)isoindoline-1,3-dione

Brown solid, mp 137-140 ˚C, solvent: benzene yield 65%, IR (KBr) υ cm^{-1}: 1760-1740 ν CO of imide, 1620-1600 C=N,1070-1060 C-O-C,1290-1280 C=S. ^{1}H NMR δ: 8.02 (dd, *J* = 6.5, 1.5 Hz, 1H), 7.76 (t, *J* = 0.8 Hz, 1H), 7.68 (dd, *J* = 6.5, 1.4 Hz, 1H), 7.62 – 7.55 (m, 2H), 7.50 (td, *J* = 6.8, 1.5 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.43 – 7.36 (m, 1H), 7.38 – 7.27 (m, 5H), 7.08 (tt, *J* = 7.6, 1.4 Hz, 1H).^{13}C NMR δ: 181.84, 170.25, 164.43, 138.98, 136.53, 136.47, 133.46, 132.78, 132.49, 129.84, 129.30, 129.14, 128.66, 128.16, 126.15, 125.92, 125.76, 125.40, 125.02, 122.67, 87.94. Anal. Calcd for C: 43.3; H: 1.9; N: 5.2. Found C: 43; H: 1.7; N: 5.2%.

9b: 4,5,6,7-Tetrabromo-2-(3-(2,3-diphenyl-4-thioxo-3,4-dihydro-2H-1,3,5-oxadiazin-6-yl)phenyl)isoindoline-1,3-dione

Brown solid, mp 160-162 ˚C, solvent: toluene, yield 60%, IR (KBr) υ cm^{-1}: 1760-1740 ν CO of imide, 1620-1600 C=N,1070-1060 C-O-C,1290-1280 C=S. ^{1}H NMR δ: 8.02 (dd, *J* = 6.5, 1.5 Hz, 1H), 7.76 (t, *J* = 0.8 Hz, 1H), 7.68 (dd, *J* = 6.5, 1.4 Hz, 1H), 7.62 – 7.55 (m, 2H), 7.50 (td, *J* = 6.8, 1.5 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.43 – 7.36 (m, 1H), 7.38 – 7.27 (m, 5H), 7.08 (tt, *J* = 7.6, 1.4 Hz, 1H).^{13}C NMR δ: 181.84, 170.25, 164.43, 138.98, 136.53, 136.47, 133.46, 132.78, 132.49, 129.84, 129.30, 129.14, 128.66, 128.16, 126.15, 125.92, 125.76, 125.40, 125.02, 122.67, 87.94. Anal. Calcd for C: 43.3; H: 1.9; N: 5.2 Found C: 42.9; H: 1.6; N: 4.9%.

9c: 4,5,6,7-Tetrabromo-2-(4-(2,3-diphenyl-4-thioxo-3,4-dihydro-2H-1,3,5-oxadiazin-6-yl)phenyl)isoindoline-1,3-dione

Yellow solid, mp 85-87 ˚C, solvent: benzene, yield 55%, IR (KBr) υ cm^{-1}: 1760-1740 ν CO of imide, 1620-1600 C=N,1070-1060 C-O-C,1290-1280 C=S. ^{1}H NMR δ: 8.02 (dd, *J* = 6.5, 1.5 Hz, 1H), 7.76 (t, *J* = 0.8 Hz, 1H), 7.68 (dd, *J* = 6.5, 1.4 Hz, 1H), 7.62 – 7.55 (m, 2H), 7.50 (td, *J* = 6.8, 1.5 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.43 – 7.36 (m, 1H), 7.38 – 7.27 (m, 5H), 7.08 (tt, *J* = 7.6, 1.4 Hz, 1H).^{13}C NMR δ: 181.84, 170.25, 164.43, 138.98, 136.53, 136.47, 133.46, 132.78, 132.49, 129.84, 129.30, 129.14, 128.66, 128.16, 126.15, 125.92, 125.76, 125.40, 125.02, 122.67, 87.94. Anal. Calcd for C: 43.3; H: 1.9; N: 5.2. Found C: 42.8; H: 1.6; N: 4.9%.

Synthesis of compounds 10a–c

Isothiocyanate (**1a**–**c**, 0.01 mol) in dry acetone was added to α-cyanocinnamonitrile (0.01 mol) in 30 mL dry toluene. The reaction mixture was heated under reflux for 1–1.5 h. The solid product formed was filtered off and crystallized from suitable solvents to give **10a**–**c**.

10a: 6-Phenyl-2-(2-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)phenyl)-4-thioxo-4H-1,3-oxazine-5,5(6H)-dicarbonitrile

Brown solid, mp 94-96 ˚C, solvent: benzene, yield 62%, IR (KBr) υ cm^{-1}: 2250-2200, C=N, 1630-1610 C=N 1280-1270 C=S 1060-1040, C-O-C and 1750-1630 CO. ^{1}H NMR δ: 8.32 – 8.26 (m, 2H), 8.03 – 7.95 (m, 3H), 7.84 (dd, *J* = 6.5, 1.4 Hz, 1H), 7.57 (ddd, *J* = 7.2, 6.6, 1.5 Hz, 1H), 7.32 (td, *J* = 7.3, 1.4 Hz, 1H).^{13}C NMR δ: 212.46, 169.18, 164.54, 136.68, 133.46, 133.30, 132.76, 132.66, 129.84, 129.37, 128.38, 128.24, 127.50, 125.89, 124.92, 122.67, 108.88, 86.93, 47.56. Anal. Calcd for C: 40.1; H: 1.3; N: 7.2. Found C: C: 39.7; H: 1.2; N: 7.0%.

10b: 6-Phenyl-2-(3-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)phenyl)-4-thioxo-4H-1,3-oxazine-5,5(6H)-dicarbonitrile

Yellow solid, mp 105-107 ˚C, solvent: benzene, yield 75%, IR (KBr) υ cm^{-1}: 2250-2200, C=N, 1630-1610 C=N 1280-1270 C=S 1060-1040, C-O-C and 1750-1630 CO. ^{1}H NMR δ: 8.04 (dd, *J* = 6.5, 1.5 Hz, 1H), 7.69 (dd, *J* = 6.5, 1.4 Hz, 1H), 7.54 – 7.45 (m, 3H), 7.42 – 7.35 (m, 1H), 7.35 – 7.27 (m, 3H), 7.18 (d, *J* = 0.7 Hz, 1H).^{13}C NMR δ: 212.46, 169.18, 164.54, 136.68, 133.46, 133.30, 132.76, 132.66, 129.84, 129.37, 128.38, 128.24, 127.50, 125.89, 124.92, 122.67, 108.88, 86.93, 47.56. Anal. Calcd for C: 40.1; H: 1.3; N: 7.2. Found C: 39.8; H: 1.2; N: 7%.

10c: 6-Phenyl-2-(4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)phenyl)-4-thioxo-4H-1,3-oxazine-5,5(6H)-dicarbonitrile

Brown solid, mp 180-182 ˚C, solvent: toluene, yield 60%, IR (KBr) υ cm^{-1}: 2250-2200, C=N, 1630-1610 C=N 1280-1270 C=S 1060-1040, C-O-C and 1750-1630 CO. ^{1}H NMR δ: 8.04 (dd, *J* = 6.5, 1.5 Hz, 1H), 7.69 (dd, *J* = 6.5, 1.4 Hz, 1H), 7.54 – 7.45 (m, 3H), 7.42 – 7.35 (m, 1H), 7.35 – 7.27 (m, 3H), 7.18 (d, *J* = 0.7 Hz, 1H).^{13}C NMR δ: 212.46, 169.18, 164.54, 136.68, 133.46, 133.30, 132.76, 132.66, 129.84, 129.37, 128.38, 128.24, 127.50, 125.89, 124.92, 122.67, 108.88, 86.93, 47.56. Anal. Calcd for C: 40.1; H: 1.3; N: 7.2. Found C: 39.5; H: 1.1; N:6.9%.

Synthesis of pyrimidine derivatives 11a–c

A mixture of (**10a–c**) (0.01 mol) with thiourea (0.01 mol) in 30 mL *n*-butanol was refluxed for 5 h. After cooling, the separated products were crystallized from the proper solvents to give pyrimidine derivatives (**11a–c**).

11a: 5-Imino-4-phenyl-2-(2-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)phenyl)-7-thioxo-6,7-dihydro-4H-pyrimido[4,5-d][1,3]oxazine-4a(5H)-carbonitrile

Orange solid, mp 130-132 ˚C, solvent: toluene, yield 65%, IR (KBr) υ cm^{-1}: 1680-1610 C=N 2260-2120 C=N, 1280-1270 C=S. ^{1}H NMR δ: 8.04 (dd, *J* = 6.5, 1.5 Hz, 1H), 7.69 (dd, *J* = 6.5, 1.4 Hz, 1H), 7.54 – 7.45 (m, 3H), 7.42 – 7.35 (m, 1H), 7.35 – 7.27 (m, 3H), 7.18 (d, *J* = 0.7 Hz, 1H)· ^{13}C NMR δ: 180.40, 174.35, 164.43, 162.71, 150.64, 136.28, 134.76, 133.46, 132.76, 131.63, 129.84, 128.40, 128.37, 128.24, 126.84, 125.89, 125.01, 123.33, 122.67, 81.82, 48.24. Anal. Calcd for C: 39.5; H: 1.5; N: 10.2. Found C: 38.9; H: 1.4; N: 9.9%.

11b: 5-Imino-4-phenyl-2-(3-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)phenyl)-7-thioxo-6,7-dihydro-4H-pyrimido[4,5-d][1,3]oxazine-4a(5H)-carbonitrile

Brown solid, mp 278-280 ˚C, solvent: benzene, yield 50%, IR (KBr) υ cm^{-1}: 1680-1610 C=N 2260-2120 C=N, 1280-1270 C=S. ^{1}H NMR δ: 9.60 (s, 1H), 8.29 (t, *J* = 2.2 Hz, 1H), 7.92 (ddd, *J* = 6.9, 2.1, 1.2 Hz, 1H), 7.79 (t, *J* = 6.7 Hz, 1H), 7.60 (ddd, *J* = 6.6, 2.1, 1.2 Hz, 1H), 7.45 – 7.38 (m, 1H), 7.35 – 7.27 (m, 3H), 7.06 (d, *J* = 0.8 Hz, 1H). ^{13}C NMR δ: 180.38, 174.87, 164.98, 156.06, 150.64, 134.82, 134.75, 134.14, 133.00, 132.12, 129.84, 128.37, 128.24, 126.91, 126.84, 126.54, 123.29, 122.20, 122.15, 81.72, 39.79. Anal. Calcd for C: 39.5; H: 1.5; N: 10.2. Found C: 38.5; H: 1.3; N: 9.8%.

11c: 5-Imino-4-phenyl-2-(4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)phenyl)-7-thioxo-6,7-dihydro-4H-pyrimido[4,5-d][1,3]oxazine-4a(5H)-carbonitrile

Brown solid, mp 199-202 ˚C, solvent: EtOH, yield 60%, IR (KBr) υ cm^{-1}: 1680-1610 C=N, 2260-2120 C=N, 1280-1270 C=S. ^{1}H NMR δ: 9.60 (s, 1H), 8.29 (t, *J* = 2.2 Hz, 1H), 7.92 (ddd, *J* = 6.9, 2.1, 1.2 Hz, 1H), 7.79 (t, *J* = 6.7 Hz, 1H), 7.60 (ddd, *J* = 6.6, 2.1, 1.2 Hz, 1H), 7.45 – 7.38 (m, 1H), 7.35 – 7.27 (m, 3H), 7.06 (d, *J* = 0.8 Hz, 1H). ^{13}C NMR δ: 180.38, 174.87, 164.98, 156.06, 150.64, 134.82, 134.75, 134.14, 133.00, 132.12, 129.84, 128.37, 128.24, 126.91, 126.84, 126.54, 123.29, 122.20, 122.15, 81.72, 39.79. Anal. Calcd for C: 39.5; H: 1.5; N: 10.2. Found C: 38.9; H: 1.4; N: 10%.

Synthesis of pyrazole derivatives 12a–c

A mixture of **10a–c** (0.01 mol) with hydrazine hydrate (0.01 mol) in 30 mL of *n*-butanol was refluxed for 5 h. After cooling, the separated products were crystallized from the proper solvents to give pyrazole derivatives **12a–c**.

12a: 3-Imino-4-phenyl-6-(2-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)phenyl)-2,3-dihydropyrazolo[3,4-d][1,3]oxazine-3a(4H)-carbonitrile

Yellow solid, mp 195-197 ˚C, solvent: benzene, yield 75%, IR (KBr) υ cm^{-1}: 1730-1720 CO, 1690-1680 CO of anilide, 1290-1280 C=S. ^{1}H NMR δ: 9.28 (s, 1H), 8.02 (dd, *J* = 6.4, 1.6 Hz, 1H), 7.69 (dd, *J* = 6.4, 1.3 Hz, 1H), 7.50 (td, *J* = 6.9, 1.5 Hz, 1H), 7.44 – 7.35 (m, 2H), 7.35 – 7.27 (m, 3H), 7.05 (d, *J* = 0.6 Hz, 1H). ^{13}C NMR δ: 164.43, 163.48, 162.59, 152.82, 136.38, 135.09, 133.46, 132.76, 131.63, 129.84, 128.37, 128.24, 127.98, 126.79, 125.89, 125.01, 122.67, 114.90, 80.78, 49.59. Anal. Calcd for C: 40.2; H: 1.6; N: 10.9. Found C: 39.7; H: 1.5; N: 10.5%.

12b: 3-Imino-4-phenyl-6-(3-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)phenyl)-2,3-dihydropyrazolo[3,4-d][1,3]oxazine-3a(4H)-carbonitrile

Brown solid, mp 150-152 ˚C, solvent: toluene, yield 70%, IR (KBr) υ cm^{-1}: 1600-1670 C=N, 2260-2120 C=N 3540-3300 NH. ^{1}H NMR δ: 9.28 (s, 1H), 8.19 (t, *J* = 2.1 Hz, 1H), 7.86 (ddd, *J* = 6.9, 2.1, 1.2 Hz, 1H),

7.78 (t, *J* = 6.7 Hz, 1H), 7.60 (ddd, *J* = 6.6, 2.1, 1.2 Hz, 1H), 7.45 – 7.38 (m, 1H), 7.35 – 7.27 (m, 3H), 7.02 (d, *J* = 0.6 Hz, 1H). ^{13}C NMR δ: 164.98, 163.17, 156.91, 152.82, 135.14, 134.82, 133.92, 133.00, 132.16, 129.84, 128.37, 128.24, 126.91, 126.79, 126.68, 122.17, 122.15, 114.91, 80.59, 49.34. Anal. Calcd for C: 40.2; H: 1.6; N: 10.9. Found C: 39.8; H: 1.4; N: 10.4%.

12c: 3-Imino-4-phenyl-6-(4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)phenyl)-2,3-dihydropyrazolo[3,4-d][1,3]oxazine-3a(4H)-carbonitrile

Brown solid, mp 163-165 ˚C, solvent: EtOH, yield 65%, IR (KBr) υ cm^{-1}: 1600-1670 C=N, 2260-2120 C=N 3540-3300 NH. ^{1}H NMR δ: 9.28 (s, 1H), 8.33 – 8.27 (m, 2H), 7.68 – 7.62 (m, 2H), 7.44 – 7.38 (m, 1H), 7.35 – 7.27 (m, 3H), 7.02 (d, *J* = 0.6 Hz, 1H).^{13}C NMR δ: 164.72, 163.21, 155.87, 152.82, 135.78, 135.14, 133.14, 132.04, 129.84, 128.76, 128.37, 128.24, 126.79, 124.68, 122.12, 114.91, 80.56, 49.34. Anal. Calcd for C: 40.2; H: 1.6; N: 10.9. Found C: 39.6; H: 1.4; N: 10.4%.

Biological studies

Biological evaluations were performed according to reported standard literature protocols,[5] and as detailed in the supporting material.

Molecular docking study

Crystal structure of Ddb1-Crbn E3 Ubiquitin Ligase in Complex with thalidomide (PDB ID: 4CI1, Resolution: 2.98 Å) was obtained from the protein data bank (www.pdb.org) . The protein preparation wizard of Schrödinger 2021 version 3 was used to prepare the downloaded crystal structure at the default setting and 7.4 pH value. ChemDraw Professional 16.0 was employed to sketch the synthesized compounds. Ligand preparation and geomotery optimization was carried out using the Ligprep module of Schrödinger. Validation of the docking was carried out by re-docking X-ray ligands and calculating the RMSD value. Docking was carried out for all minimized conformations of the ligands using Glide’s extra precision module to produce ten poses for each ligand, then choosing the pose with the most engative score. The docking figures were produced using the Discovery Studio Client 2021 package.

In Silico Pharmacokinetic Study

The Swiss ADME server was used to predict various pharmacokinetic properties of the synthesized molecules, such as Log P (partition coefficient), Lipinski’s rule of 5 violations, TPSA (topological polar surface area) and water solubility. These factors can then be utilized to determine a compound's oral availability and the likelihood of causing CNS side effects.

ACKNOWLEDGEMENTS

The authors would like to thank Taif University Researchers Supporting Project number (TURSP-2020/91), Taif University, Saudi Arabia.

REFERENCES

1. Q. J. Lin, F. Yang, C. Jin, and D. L Fu, *World J. Gastroenterol.*, 2015, **21**, 7988.
2. P. C. Appelbaum and P. A Hunter, *Int. J. Antimicrob. Agents*, 2000, **16**, 5.
3. J. M. Frère, *Mol. Microbiol.*, 1995, **16**, 385.
4. G. Devasahayam, W. M. Scheld, and P. S. Hoffman, *Expert Opin. Investig. Drugs*, 2010, **19**, 215.
5. M. H. Abdellattif, O. A. Ali, M. M. H. Arief, and M. A. Hussien, *Curr. Org. Synth.*, 2020, **17**, 230.
6. I. M. M. Othman, M. A. M. Gad-Elkareem, M. El-Naggar, E. S. Nossier, and A. E. E. Amr, *J. Enzyme Inhib. Med. Chem.*, 2019, **34**, 1259.
7. P. F. Lamie, J. N. Phillopes, A. O. El-Gendy, L. Rarova, and J. Gruz, *Molecules*, 2015, **20**, 16620.
8. Y. Hashimoto, *Arch. Pharm.*, 2008, **341**, 536.
9. S. T. Xue, H. F. Guo, M. J. Liu, J. Jin, D. H. Ju, Z. Y. Liu, and Z. R. Li, *Eur. J. Med. Chem.*, 2015, **96**, 151.
10. S. Alsaedi, B. A. Babgi, M. H. Abdellatif, A.-H. Emwas, M. Jaremko, M. G. Humphrey, and M. A. Hussien, *J. Inorg. Organomet. Polym. Mater.*, 2021.
11. M. Abdellattif, M. Hussien, and E. Alzahrani, *Int. J. Pharm. Sci*, 2018, **9**, 1000.
12. A. Elkamhawy, A. N. I. Viswanath, A. N. Pae, H. Y. Kim, J. C. Heo, W. K. Park, C. O. Lee, H. Yang, K. H. Kim, D. H. Nam, H. J. Seol, H. Cho, and E. J. Roh, *Eur. J. Med. Chem.*, 2015, **103**, 210.
13. M. M. Al-Sanea, A. Elkamhawy, S. Paik, K. Lee, A. M. El Kerdawy, B. Syed Nasir Abbas, E. J. Roh, W. M. Eldehna, H. A. H. Elshemy, R. B. Bakr, I. Ali Farahat, A. I. Alzarea, S. I. Alzarea, K. S. Alharbi, and M. A. Abdelgawad, *Bioorg. Med. Chem.*, 2020, **28**, 115525.
14. M. M. Al -Sanea, A. Elkamhawy, A. Zakaria, B. S. Park, Y. Kwon, S. H. Lee, S. W. Lee, and I. T. Kim, *Molecules*, 2015, **20**, 1031.
15. L. Li, N. N. Chen, Q. D. You, and X. L. Xu, *Expert Opin. Ther. Pat.*, 2021, **31**, 67.
16. G. Garg, A. Khandelwal, and B. S. Blagg, *Adv. Cancer Res.*, 2016, **129**, 51.
17. Z. Zhou, X. Li, Y. Qian, C. Liu, X. Huang, and M. Fu, *Biochem. J.*, 2020, **477**, 3923.
18. J. Beliakoff and L. Whitesell, *Anticancer Drugs*, 2004, **15**, 651.
19. D. B. Solit, H. I. Scher, and N. Rosen, *Semin. Oncol.*, 2003, **30**, 709.
20. S. Sharp and P. Workman, *Adv. Cancer Res.*, 2006, **95**, 323.
21. S. M. Roe, C. Prodromou, R. O'Brien, J. E. Ladbury, P. W. Piper, and L. H. Pearl, *J. Med. Chem.*, 1999, **42**, 260.

22. C. Garcie, S. Tronnet, A. Garénaux, A. J. McCarthy, A. O. Brachmann, M. Pénary, S. Houle, J.-P. Nougayrède, J. Piel, P. W. Taylor, C. M. Dozois, P. Genevaux, E. Oswald, and P. Martin, *J. Infect. Dis.*, 2016, **214**, 916.