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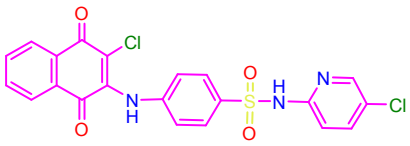
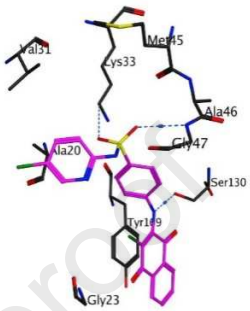
Graphical Abstract



compound **14**

IC_{50}= 9.90±0.61 μM (β5)



Design, Synthesis and Biological Evaluation of Novel Naphthoquinone-4-aminobenzensulfonamide/carboxamide Derivatives as Proteasome Inhibitors

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Abstract

A series of novel 4-aminobenzensulfonamide/carboxamide derivatives bearing naphthoquinone pharmacophore were designed, sythesized and evaluated for their proteasome inhibitory and antiproliferative activities against human breast cancer cell line (MCF-7). The structures of the synthesized compounds were confirmed by spectral and elemental analyses. The proteasome inhibitory activity studies were carried out using cell-based assay. The antiproteasomal activity results revealed that most of the compounds exhibited inhibitory activity with different percentages against the caspase-like (C-L, β1 subunit), trypsin-like (T-L, β2 subunit) and chymotrypsin-like (ChT-L, β5 subunit) activities of proteasome. Among the tested compounds, compound **14** bearing 5-chloro-2-pyridyl ring on the nitrogen atom of sulfonamide group is the most active compound in the series and displayed higher inhibition with IC_{50} values of 9.90±0.61, 44.83±4.23 and 22.27±0.15 µM against ChT-L, C-L and T-L activities of proteasome compared to the lead compound PI-083 (IC_{50}=12.47±0.21, 53.12±2.56 and 26.37±0.5 µM), respectively. The antiproliferative activity was also determined by MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay *in vitro*. According to the antiproliferative activity results, all of the compounds exhibited cell growth inhibitory activity in a range of IC_{50}= 1.72±0.14-20.8±0.5 µM and compounds **13** and **28** were found to be the most active compounds with IC_{50} values of 1.79±0.21 and 1.72±0.14 µM, respectively. Furthermore, molecular modeling studies were carried out for the compounds **13**, **14** and **28** to investigate the ligand-enzyme binding interactions.

Keywords: proteasome inhibitor; antiproliferative activity; naphthoquinone; sulfonamide/carboxamide; molecular docking; synthesis.

1. Introduction

The ubiquitin-proteasome system (UPS) is the main proteolytic pathway that plays a critical role in protein homeostasis in eukaryotic cells.[1-3] This system is involved in several fundamental cellular processes such as cell cycle regulation, DNA repair, apoptosis, immune and inflammatory responses.[4] 26S proteasome, the vital component of the UPS, is responsible for the ATP-dependent degredation of poly-ubiquitinated proteins and is composed of two 19S regulatory particles and one multicatalytic proteinase complex which is known as the 20S proteasome core particle.[5-7] The 20S proteasome possesses multiple catalytic activities, including caspase-like (C-L, β1 subunit), trypsin-like (T-L, β2 subunit) and chymotripsin-like (ChT-L, β5 subunit) activities. These β catalytic subunits have slightly different substrate specificity but they all perform a common mechanism of proteolysis by nucleophilic attack through a hydrophilic-γ-hydroxyl group of the N-terminal threonine.[8,9] Increased levels of proteasome and subsequent protein breakdown have been implicated in many disorders such as cancer, inflammation, neurodegenerative and immune diseases.[10,11] Proliferation and apoptosis pathways are tightly regulated in a cell by the UPS and alterations of this system may result in cellular transformation or other important pathologies especially cancer.[12] The high rate of protein production in cancer cells and the need for proteasome function in these cells for keeping up the cancer cell survival besides proliferation are making them more sensitive to proteasome inhibition than normal cells.[7,8] Therefore, the development of proteasome inhibitors has emerged as an attractive target for the treatment of cancer and other disorders.[13-16]

Bortezomib, Ixazomib and Carfilzomib have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of multiple myeloma (MM) (Figure 1).[17] All of the approved inhibitors carry a peptide backbone containing an electrophilic warhead and they covalently bound to the N-terminal threonine residue in the catalytic subunits of the proteasome. Despite the remarkable success of these inhibitors in the clinic, intrinsic defects such as chemical instability, poor membrane permeability and acquired drug resistance are the major problems.[18] In addition, these inhibitors have also been unsuccessful in the treatment of solid cancers.[19] The peptide backbone and the electrophilic reactive groups of these agents are thought to be the major cause of side effects, acquired drug resistance and unsatisfactory pharmacokinetic profiles.[20] Therefore, much efforts have been dedicated for the development of non-covalent and non-peptidic proteasome inhibitors with different scaffolds which may limit some of these intrinsic pharmacokinetic drawbacks and may translate into a broader clinical profile in recent years.[21-23] As a result of these studies, several classes of proteasome inhibitors bearing distinct pharmacophoric groups have been developed.[24-26] In 2009, Kazi et al. discovered PI-083 compound, a novel non-peptide proteasome inhibitor, that possesses a unique naphthoquinone skeleton by screening compounds from the NCI (National Cancer Institute) chemical libraries and is proved to have a broader antitumor activity and is more selective against cancer cells compared to Bortezomib *in vitro* and *in vivo*.[27] Furthermore, studies regarding the non-peptide PI-083 and its analogues suggest the potential interaction of the γ-hydroxyl group of the catalytic threonine residue with the 2-chloronaphthoquinone unit. PI-083 and its naphthoquinone scaffold have been acknowledged as promising structural phenotypes for the development of new proteasome inhibitors.[28]

According to the existing literature evaluation on proteasome inhibitors, PI-083 was selected as the lead compound and a series of novel 4-aminobenzensulfonamide/carboxamide derivatives bearing naphthoquinone pharmacophoric group have been designed by molecular modification method. As illustrated in Figure 2, the design of the compounds was constructed based on the suggested hypothetical structure as a result of docking and the structure-activity relationship studies on PI-083 by Xu et al.[29] Accordingly, pharmacophoric unit chloronaphthoquinone was linked to hydrogen bond acceptor sulfonamide or carboxamide group by phenylamine linker. Then, different carbocyclic/heterocyclic rings bearing substituents with distinct electronic and steric properties were substituted to amide nitrogen. Herein, the synthesis, antiproliferative and proteasome inhibitory activities of the designed compounds were reported. Also molecular modeling studies were carried out for the active compounds **13**, **14** and **28** to investigate the ligand enzyme binding interactions.

2. Results and Discussion

2.1. Chemistry

In this study, novel thirty-three naphthoquinone-4-aminobenzensulfonamide/carboxamide derivatives have been synthesized to evaluate their antiproliferative and proteasome inhibitory activities against MCF-7 cell line. PI-083 lead compound was also synthesized in order to compare the proteasome inhibitory activity.

The synthesis of the target compounds was illustrated in Schemes 1 and 2. As shown in Scheme 1, the sulfonamide derivatives were synthesized in three steps according to the literature.[28,30,31] First, acetanilide and chlorosulfonic acid were reacted to yield 4-acetamidobenzensulfonyl chloride. After this compound was reacted with appropriate amines by the nucleophilic substitution (S_N2) reaction, 4-amino-*N*-substitutedbenzenesulfonamide intermediates were obtained by the deacetylation reaction. Finally, 4-amino-*N*-substitutedbenzenesulfonamides were treated with 2,3-dichloro-1,4-naphthoquinone to give the title compounds.

The carboxamide derivatives were prepared in two-step synthesis described in Scheme 2. Initially, 2,3-dichloro-1,4-naphthoquinone was coupled with 4-aminobenzoic acid to obtain 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)benzoic acid. After this carboxylic acid derivative was converted to acyl halide by using $SOCl_2$ reagent, it

was reacted with corresponding amines to effort the carboxamide products (compounds **18**, **20-25**, **27**, **32** and **33**).[32,33] For the synthesis of compounds **19**, **26**, **28-31**, carboxylic intermediate 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)benzoic acid was converted to the ester functional group in the presence of NMM and IBCF, before it was treated with appropriate amines.[34]

The structures of the target compounds were confirmed by spectral (IR, ^{1}H NMR, ^{13}C NMR and Mass) and elemental analyses. According ^{1}H and ^{13}C NMR spectra, the proton and carbon resonance signals of aromatic and aliphatic groups were observed at the expected regions. The mass spectra of the title compounds were verified by ESI or APCI spectra where the m/z values of molecular ion peaks were in complete agreement with the calculated molecular weight for each compound. The purity levels of compounds were determined by elemental analysis (C, H, N and S) and the results were within ± 0.4% of the calculated values. The compounds **2, 3, 7, 11, 12, 15-17** and **21-25** had water of crystallization (see Experimental section).

Regarding the literature survey, only compound **14** was reported previously. However, except for predicted antimalarial activity using constructed QSAR models *in silico*, its synthesis and detailed spectral data have not been described.[35] The synthesis and spectral data of the title compounds were reported for the first time in this study. The details of synthesis protocol and structural characterization are reported in the Experimental section.

2.2. Biological activity

2.2.1. Cellular proliferation inhibition assay

Considering the fundamental role of proteasome in cell proliferation, we investigated the antiproliferative activity of all the compounds on MCF-7 human breast cancer cell line by MTT method compared to the reference drug doxorubicin. The activity results are listed in Table 1. The results revealed that all compounds exhibited cell growth inhibitory activity at different ratios with the IC_{50} values varying from 1.72±0.14 to 20.8±0.5 µM. The most active compound in sulfonamide series (1-17) is compound **13** bearing 5-methyl-2-pyridyl ring on the nitrogen atom of sulfonamide group with an IC_{50} value of 1.79±0.21 µM. In terms of carboxamide derivatives, the most active compound is compound **28** that bearing 4-methyl-2-pyridyl ring on the nitrogen atom of carboxamide group with an IC_{50} value of 1.72 ±0.14 µM. These two most active compounds in both series showed slightly better antiproliferative activity compared to the lead compound PI-083 with an IC_{50} value of 1.96±0.14 µM. The rest of carboxamide derivatives did not produce any better activity than the lead compound PI-083, whereas most of the compounds in sulfonamide series exhibited antiproliferative activities comparable or superior to the lead compound PI-083. Replacing the sulfonamide group with a carboxamide group decreased the antiproliferative activity. In general, sulfonamides had better antiproliferative activity profile than carboxamides except for compounds **19**, **23**, **24**, **27-29**, **31** and **32**. In terms of compound **17** where nitrogen atom of sulfonamide group is converted into member of an aliphatic cyclic ring provided a favorable effect on the activity. In addition, introduction of methyl substituent into the 2-pyridyl ring on the nitrogen atom of amide group afforded the most active compounds in both series. These results indicated that the structure of the group attached on the amide group has considerable effect on the antiproliferative activity.

2.2.2. Cell-based proteasome inhibition assay

The proteasome inhibitory activities of the synthesized compounds were evaluated for the three activities (ChT-L, C-L and T-L) of proteasome by cell-based assay using specific fluorogenic substrates (Suc-LLVY-AMC for the ChT-L, Z-LLE-AMC for the C-L and Boc-LRR-AMC for the T-L) on MCF-7 human breast cancer cell line. MG132 was used as a positive control. Inhibition potency of lead compound PI-083 against ChT-L, C-L and T-L activities of proteasome was also determined for the comparison of biological activity. In initial screening, the percentage of ChT-L, C-L and T-L proteasome inhibitory activities of the compounds were measured and on the basis of these results a dose-response IC_{50} values were obtained for compounds that displayed higher or comparable inhibition percentage than the lead compound PI-083 at 10 µM. The antiproteasomal activity results of the compounds are reported in Tables 2 and 3.

Generally, most of the compounds possessed inhibitory activity with varying ratios against the ChT-L, C-L and T-L activities of proteasome. Among the tested compounds, only one sulfonamide (compound **13**) and one carboxamide derivative (compound **23**) did not display any inhibitory activity against C-L activity of proteasome, while seven carboxamide derivatives (compounds **19**, **25**, **27-31**) have been found inactive against T-L activity of proteasome. Therefore, it can be concluded that sulfonamide derivatives possessed slightly better inhibitory activity than carboxamide derivatives against all three proteasomal activities.

Under the set of the compounds studied, compound **14** bearing 5-chloro-2-pyridyl ring on the nitrogen atom of sulfonamide group is the most active compound with 42.31%, 28.89% and 35.02% inhibition values against all three ChT-L, C-L and T-L activities of proteasome, respectively. Additionally, this compound exhibited higher inhibitory potency than lead compound PI-083 bearing 2-pyridyl ring on the nitrogen atom of sulfonamide group having 36.76%, 18.81% and 29.59% inhibition values against ChT-L, C-L and T-L activities of proteasome, respectively. The introduction of chlorine atom into the 5-position of 2-pyridyl ring resulted in an increase on the inhibition of all three proteasomal acitivities and produced the most active compound in the series, whereas the replacement of chlorine at the 5-position on 2-pyridyl ring with methyl led to a decrease or loss in activity (compound **13**). The positional change

of this methyl group from the 5-position to 4 or 3 positions on 2-pyridyl ring did not cause a significant improvement on activity. On the other hand, the conversion of sulfonamide group of the most active compound (compound **14**) to carboxamide counterpart (compound **30**) led to a significant decrease in ChT-L and C-L inhibitory activities and loss of T-L inhibitory activity. These findings suggested that the characterization of substituent on the 2-pyridyl ring and amide is important for the inhibitory activity potency.

N-phenylcarboxamide derivative, compound **21**, bearing 4-nitrophenyl ring on the amide nitrogen is the second most active compound in the series with 35.34%, 22.77% and 30.52% inhibition values against ChT-L, C-L and T-L activities of proteasome, respectively and exhibited nearly similar potency with the lead compound PI-083. The introduction of an electron-withdrawing nitro group on 4-position of *N*-phenyl ring dramatically enhanced the inhibition potency compared to non-substituted *N*-phenylcarboxamide derivative, compound **18**. Overall, in the *N*-phenylcarboxamide derivatives, the presence of a substituent on the *N*-phenyl ring appeared to be benefical for the potency when non-substituted derivative **18** is compared to other compounds with different substituents on the *N*-phenyl ring, except for compounds **19** and **23** which exhibit low or no inhibition against C-L and T-L activities.

On the other hand, 3-chloro and 4-nitro substitutions on the *N*-phenyl ring in the *N*-phenylsulfonamide derivatives yielded the most active compounds and rest of the substituents studied, namely 2-ethyl, 2-isopropyl, 3,5-dimethoxy, 2-nitro, 3-nitro and 4-bromo, produced compounds with nearly equal activity (see Table 2). Interestingly, same activity trend was observed in the *N*-phenylcarboxamide derivatives. 3-Chloro and 4-nitro substitution patterns on the *N*-phenyl ring seems to give superior results than other substitutions in each series.

Concerning heterocyclic substitution on amide nitrogen in both series, the inhibitory effect of non-substituted or substituted heterocyclic ring depended on its substitution nature and pattern. In general, the sulfonamide derivatives (compounds **11**-**16**) exhibited varying inhibition percentage against the three proteasome subunits, whereas heterocyclic ring substituted carboxamide derivatives did not follow similar trend except for compounds **26**, **32** and **33**. These carboxamide derivatives displayed higher ChT-L inhibitory activity than C-L inhibitory activitiy and most of them did not show any inhibitory activity against T-L proteasomal activity. Therefore, it can be speculated that the carboxamide derivatives are selective against ChT-L activity of proteasome.

According to the literature survey, a compound that possesses high antiproliferative activity is expected to have good antiproteasomal activity. However, 5-methyl-2-pyridyl substituted sulfonamide derivative (compound **13**) and 4-methyl-2-pyridyl substituted carboxamide derivative (compound **28**) exhibited high antiproliferative activity, whilst they possessed weak proteasomal inhibitory activity. The discrepancy between antiproliferative activity and cell-based proteasome inhibitory activity can be attributed to the fact that compounds exhibit cytotoxicity through a molecular mechanism other than proteasome inhibition.

In continuation of our work, IC_{50} values were determined for the most active compounds (compounds **14** and **21)** which played higher or comparable inhibition percentage than the lead compound PI-083 against all three activities of proteasome. The results suggested that compound **14** showed higher inhibitory activity with IC_{50} values of 9.90±0.61, 44.83±4.23 and 22.27±0.15 µM against ChT-L, C-L and T-L activities of proteasome, respectively than the lead compound PI-083 with IC_{50} values of 12.47±0.21, 53.12±2.56 and 26.37±0.5 µM.

Since compound **14** showed the lowest IC_{50} value on the activity of ChT-L among tested proteasome catalytic subunits and PI-083 has been previously determined as a chymotrypsin-like reversible proteasome inhibitor[27], we next determined the expression level of 20S proteasome subunit β5 which is related to ChT-L activity using MCF-7 cells treated with compound **14**, PI-083 or solvent control (DMSO) at IC_{50} concentrations for 12 hours. Our immunoblotting results showed that neither compound **14** nor PI-083 caused significant change on the expression levels of β5 subunit suggesting the inhibition of ChT-L activity by compound **14** does not depend on the expression level of target protein (Figure 3A). On the other hand, the evaluation of K48-linked polyubiquitinated protein levels clearly revealed that treatment of cells with compound **14** at IC_{50} concentration caused significantly more accumulation of polyubiquitinated proteins compared to treatment of cells with PI-083 at IC_{50} concentration (Figure 3B). To sum, our data suggested that compound **14** potently inhibits ChT-L catalytic activity without affecting 20S proteasome subunit β5 protein expression.

Under the set of the compounds studied, the most active compound in the series had *N*-heterocyclic ring substituted sulfonamide structure, whereas the second most active compound possessed *N*-phenyl ring substituted carboxamide structure. Acccording to the contribution of substitution on amide nitrogen in both series, 5-chloro substitution of 2-pyridyl ring and 4-nitro substitution of phenyl ring seemed to yield superior compounds.

2.3. Docking Studies

Docking is a very effective method to propose the binding interactions between the ligand and the receptor. In this study, we have chosen the most active compound (compound **14**) against β1, β2 and β5 subunits of human 20S proteasome for molecular modeling studies in order to propose the non-covalent binding interactions between compound **14** and the active sites. Also docking studies were carried out for compounds **13** and **28** which possessed the highest antiproliferative but low antiproteasomal activity to suggest the reason of discrepancy between the two activities in terms of binding interactions.

The proposed binding pose of compound **14** in complex with β1 subunit has three important H-bonds (Figure 4A). Two of these H-bonds are located between the carbonyl groups of naphthoquinone ring and Ala49 and Gly23 amino acid residues. These H-bonds demonstrate that the core structure of the ligand is stabilized by the two key amino acids of the active site. Ala49 draws attentions with the H-bonds that forms between the important inhibitors like PI-083 and Bortezomib.[28,36] The third H-bond of compound **14** is observed between the NH of the sulfonamide group of the side chain and Asp51 amino acid.

On the other hand, the proposed binding pose of compound **14** in complex with β2 subunit has four H-bonds: two H-bonds between the carbonyl groups of naphthoquinone ring and Ala20 and Gly47 amino acid residues, one H- bond between the NH group adjacent to the naphthoquinone ring and Thr21 amino acid and one H-bond between the NH of the sulfonamide group of the side chain and Asp166 amino acid (Figure 4B). Three of these H-bonds are formed in the active site and two H-bonds formed are remarkable as they were formed with Thr21 and Gly47 key amino acids. These two amino acids take place in forming binding interactions with some important proteasome inhibitors in molecular modeling studies.[27]

Binding interactions of compound **14** and the β5 subunit of proteasome are different from the other proposed binding poses. Naphthoquinone ring makes a strong π-π interaction with Tyr169 which is heading out of the pocket, NH group located next to the ring forms a H-bond with Ser130 and also SO group of the sulfonamide on the side chain stabilizes with Gly47, one of the key amino acids (Figure 4C).

Docking studies of compound **13** and compound **28** in complex with β1, β2 and β5 subunits of human 20S proteasome reveal different binding interactions and different orientations in the active sites than compound **14** (Figure 5). In β1 subunit's active site, compounds **13** and **28** show similar orientation but settled in a different geometry than compound **14**. Compound **13** has one hydrogen bond formed between SO group and Ala50 amino acid residue. Compound **28** also has one hydrogen bond formed between NH of carboxamide and Thr22 (Figures 5A, 6 and 7).

Similar case can be observed in β2 subunit's active site for compounds **13** and **28**. They both settled in a different geometry than compound **14** but relatively similar orientation in each other (Figure 5B). Compound **13** has two hydrogen bonds which are located between SO group and Thr1 and pyridine-N and Thr21. Compound **28** formed only one hydrogen bond between the carbonyl group of naphthoquinone ring and Gly128 amino acid residue (Figures 8 and 9).

In case of the proposed binding modes of compound **13** and **28** in the active site of β5 subunit, they both have different orientations in the active gorge than compound **14** and also in themselves (Figure 5C). Compound **13** has three hydrogen bonds which of two are formed between Thr1 and NH and SO of sulfonamide. The third bond was formed between the carbonyl group of naphthoquinone ring andAla49. Compound **28** formed two hydrogen bonds with Thr 21 and the carbonyl group of naphthoquinone ring and NH group next to the naphthoquinone ring (Figures 10 and 11). See supplementary data for figures (5-11) of the proposed binding poses of the compounds **13** and **28**.

In the light of these proposed binding poses and the binding interactions, it can be observed that compound **14** forms important bonds with naphthoquinone pharmacophoric group and the side chains while compound **13** and compound **28** settles in different orientations and forms their binding interactions mostly with the side chains or linker parts. It can be speculated that the proteasome inhibitory activity difference may be explained by the geometry of the compounds in the active sites and the difference of binding interactions.

2.3.1. Molecular Dynamics Simulations

Molecular dynamics (MD) simulations are an effective technique usually used to investigate the binding interactions between the ligand and the active site, dynamically. The purpose of this technique is to simulate the body enviroment and test if the ligand is stable in the active site and the binding interactions of the proposed binding pose are conserved or not in a time period.

With this aim, free molecular dynamics simulations was performed in order to test the stability of β1, β2 and β5 subunits of human 20S proteasome, compound **14** in complex with β1, β2 and β5 subunits, individually and see if the binding interactions of compound 14 with the active sites were conserved.

The MD of the apo form of β1 subunit demonstrated that the subunit was stable individually with RMSD value of 2.28 Å throughout the 30 ns simulation (Figures 12 and 15). However, the MD simulation of compound **14** in the active site of β1 subunit was stable in the active site but formed different bonds than the proposed binding interactions. It can be speculated that even though the naphthoquinone ring possessed two H-bonds, it was unable to reach Thr1 amino acid which located in the deepest part of the gorge and very important for the inhibitor activity (Figure 12).

MD simulation of compound **14** in complex with β2 subunit was performed for 30 ns, also. The RMSD value obtained from the MD of the apo form of β2 subunit was 11.36 Å. When the simulation images were examined in detail, it is observed that the active site was stable during the simulation but the random coil part of the subunit which is located between Arg187 and Glu220 lead to the high RMSD value (Figures 13 and 16). MD simulation of compound **14** in the active site of β2 subunit demonstrated that the ligand was stable in the active site but the binding interactions were not conserved. In the beginning of the simulation the H-bond formed with Gly47 was broken and

when the ligand reached the stable position, Thr21 and Asp166 bonds were conserved but the naphthoquinone ring was located with a 90° angle compared to its initial pose due to the loss of the H-bond with Gly47 (Figure 13).

RMSD value of the MD simulation obtained from the apo form of β5 subunit had an avarage of 1.16 Å which showed that the enzyme was stable throughout the simulation (Figures 14 and 17). On the other hand, the graphics of compound **14** in complex with β5 subunit indicated that the π-π interaction was lost in the beginning of the simulation and the ligand was leaned towards the deep of the gorge and located between Ala20, Ala22 and Val31 amino acids near the end of the simulation. See supplementary data for figures (12-17) of RMSD plot and ribbon model of the compounds.

3. Conclusion

In this study, novel thirty-three 4-aminobenzensulfonamide/carboxamide derivatives bearing naphthoquinone pharmacophoric group were synthesized and evaluated for their antiproliferative and proteasome inhibitory activities against MCF-7 cell line *in vitro*. The antiproteasomal activity results revealed that most of the compounds exhibited inhibitory activity with varying percentage against the chymotrypsin-like (ChT-L, β5 subunit), caspase-like (C-L, β1 subunit) and trypsin-like (T-L, β2 subunit) activities of proteasome. Among the screened compounds, compound **14** bearing 5-chloro-2-pyridyl ring on the nitrogen atom of sulfonamide group is the most active compound in the series and displayed higher inhibition with IC_{50} values of 9.90±0.61, 44.83±4.23 and 22.27±0.15 µM against ChT-L, C-L and T-L activities of proteasome, respectively compared to the lead compound PI-083 (IC_{50}=12.47±0.21, 53.12±2.56 and 26.37±0.5 µM). According to the antiproliferative activity results, all compounds displayed different ratios of cell growth inhibitory activity with the IC_{50} values varying from 1.72±0.14 to 20.8±0.5 µM against MCF-7 cell line. In particular, compounds **13** and **28** were found to be the most active compounds with IC_{50} values of 1.79±0.21 and 1.72±0.14 µM, respectively. Those results suggested that these derivatives can be starting compounds to obtain new potent proteasome inhibitors.

4. Experimental

4.1. Chemistry

The chemical reagents and solvents in this study were purchased from common commercial suppliers and were used without further purification. Reactions were checked by thin-layer chromatography (TLC) on precoated silica gel plates (Kieselgel 60, F254, E. Merck, Germany). Column chromatography was performed using silica gel (200-300 mesh). All yields were unoptimized and generally represented. Melting points were determined on a Stuart SMP30 (Staffordshire, ST15 OSA, United Kingdom) melting point apparatus and are not corrected. IR spectra of the compounds were recorded on a Perkin Elmer 100 FT-IR (ATR) spectrophotometer (Perkin Elmer Inc., MA) and the representative absorption bands were reported. NMR spectra were recorded on a Varian AS 400 Mercury Plus NMR (Varian Inc., Palo Alto, CA, USA) at 400 MHz for ^{1}H and 101 MHz for ^{13}C using DMSO-d_6 as a solvent. The chemical shifts (δ) of ^{1}H and ^{13}C NMR were reported in parts per million (ppm) relative to internal tetramethylsilane (TMS), and coupling constants (*J*) were reported in hertz (Hz). Splitting patterns were indicated as follows: s (singlet); bs (broad singlet); d (doublet); dd (doublet-doublet); dt (doublet-triplet); t (triplet); td (triplet-dublet); tt (triplet-triplet) and m (multiplet). Molecular masses were determined with atomic pressure chemical ionization (APCI-MS) and electron spray ionization mass spectra (ESI-MS) on a Thermo MSQ Plus LC/MS instrument (Thermoscientific Inc., San Jose, CA). Elemental analyses (C, H, N and S) were performed by Leco TruSpec Micro (Leco, St. Joseph, MI) and are within ±0.4% of the theoretical values.

4.1.1. General procedure for the synthesis of sulfonamide derivatives (1-17)

4.1.1.1. Synthesis of 4-acetamidobenzenesulfonyl chloride

The chlorosulfonic acid (0.08 mol, 5.2 ml) was slowly added to the *N*-phenylacetamide (0.015 mol, 2 g) and stirred at 60 °C for 30 minutes. After cooling to room temperature, the mixture was poured into the ice water, then precipitated 4-acetamidobenzenesulfonyl chloride was filtered and washed with water. The compound was obtained without further purification. Yield 66%, mp: 145 °C.

4.1.1.2. Synthesis of 4-amino-N-substitutedbenzenesulfonamide intermediates

4-Acetamidobenzenesulfonyl chloride (0.016 mol, 3.72 g) was added in portion to a solution of appropriate amine (0.02 mol) in pyridine (0.129 mol, 10 ml) at 0 °C, then stirred for 1 h. The reaction mixture was poured into the ice water, after stirring another 3 h at room temperature. The resulting solid was filtered and dried followed by dissolved in 10% NaOH aqueous solution. Impurities were removed by filtration. The filtrate was acidified with HCl (5 M) to pH 3-4. The precipitate formed in reaction flask was filtered and dried. After precititate was dissolved in 20 ml of NaOH (5 M) solution, 12 ml of methanol was added and heated at 70 °C for 3h. Then, 2 M HCl solution was added to the reaction mixture until the pH was 6. The crude product formed by acid addition was recrystallized from ethanol/ water.

4.1.1.3. Synthesis of sulfonamide derivative final compounds (1-17)

2,3-Dichloro-1,4-naphthoquinone (0.03 mol, 0.6 g) and appropriate 4-amino-*N*-substitutedbenzenesulfonamide (0.03 mol) were suspended in 15 ml of absolute ethanol and refluxed at 115 °C for 3 days. The resultant precipitate

colored with orange/red was filtered and washed with ethanol. The crude product was rinsed with EtOAc (5 ml), DCM (5–10 ml), methanol (5–10 ml) and the mixture of DCM: aceton (1:1; 5 ml) to remove the impurities, respectively and recrystallized from ethanol.

*4.1.1.4. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(2-ethylphenyl) benzenesulfonamide (**1**)*

Yield 13%; mp 258 ^{o}C; IR υ_{maks} (FT/ATR): 3245 (NH), 3227 (NH), 3020 (CH-arom), 2971 (CH-aliph), 1673 (C=O), 1591 (C=C), 1329 (SO_2-asym), 1158 (SO_2-sym) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.52 (1H, bs, SO_2NH), 9.43 (1H, bs, NH-Phenyl), 8.03 (2H, dd, *J*=7.6; 1.2 Hz, Naphthoquinone H-5 and H-8), 7.86 (1H, td, *J*=7.6; 1.2 Hz, Naphthoquinone H-6 or H-7), 7.81 (1H, td, *J*=7.6; 1.2 Hz, Naphthoquinone H-6 or H-7), 7.52 (2H, d, *J*=8.4 Hz, Benzene H-2 and H-6), 7.17 (2H, d, *J*=8.4 Hz, Benzene H-3 and H-5), 7.19-7.11 (2H, m, Phenyl H-4 and H-6), 7.04 (1H, td, *J*=7.6; 1.3 Hz, Phenyl H-5), 6.88 (1H, dd, *J*=8.0; 1.2 Hz, Phenyl H-3), 2.46 (2H, q, *J*=7.6 Hz, CH_2), 0.98 (3H, t, *J*=7.6 Hz, CH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.4, 177.4, 143.5, 143.1, 140.8, 135.2, 134.9, 134.6, 134.0, 132.2, 130.9, 129.3, 127.2, 127.2, 127.0, 126.7, 126.5, 122.8, 118.8, 23.5, 14.8 ppm; MS (APCI) m/z: 467 ($M+H^+$), 469 ($M+H+2^+$); Anal. Calcd. for $C_{24}H_{21}ClN_2O_4S$ (468.95): C, 61.73; H, 4.10; N, 6.10; S, 6.87%, Found: C, 61.74; H, 4.18; N, 5.73; S, 7.10%.

*4.1.1.5. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(2-isopropylphenyl)benzenesulfonamide(**2**)*

Yield 43%; mp 186 ^{o}C; IR υ_{maks} (FT/ATR): 3238 (NH), 3085 (CH-arom), 2968 (CH-aliph), 1672 (C=O), 1590 (C=C), 1327 (SO_2-asym), 1155 (SO_2-sym) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.47 (1H, bs, NH-Phenyl), 8.03 (2H, d, *J*=7.2 Hz, Naphthoquinone H-5 and H-8), 7.88-7.79 (2H, m, Naphthoquinone H-6 and H-7), 7.53 (2H, d, *J*=8.0 Hz, Benzene H-2 and H-6), 7.26 (1H, d, *J*=7.6 Hz, Phenyl H-6), 7.16 (2H, d, *J*=8.4 Hz, Benzene H-3 and H-5), 7.19-7.15 (1H, m, Phenyl H-4), 7.02 (1H, t, *J*=7.6 Hz, Phenyl H-5), 6.82 (1H, d, *J*=7.6 Hz, Phenyl H-3), 3.29-3.23 (1H, m, $CH(CH_3)_2$), 0.98 (6H, d, *J*=6.8 Hz, 2xCH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): 180.3, 177.4, 146.3, 143.4, 143.1, 135.2, 135.1, 134.9, 134.0, 132.2, 131.4, 130.9, 127.4, 127.0, 126.7, 126.2, 122.7, 119.0, 27.1, 24.1 ppm; MS (APCI) m/z: 479 ($M+H^+$), 481 ($M+H+2^+$); Anal. Calcd. for $C_{25}H_{21}ClN_2O_4S$. 0.2 H_2O (484.57): C, 61.97; H, 4.45; N, 5.78; S, 6.62%, Found: C, 61.58; H, 4.07; N, 5.49; S, 6.71%.

*4.1.1.6. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(3,5-dimethoxyphenyl)benzenesulfonamide(**3**)*

Yield 49%; mp 254 ^{o}C; IR υ_{maks} (FT/ATR): 3231 (NH), 2981 (CH-aliph), 1674 (C=O), 1606 (C=C), 1591 (C=C), 1340 (SO_2-asym), 1290 (=C-O-C), 1161 (SO_2-sym) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.11 (1H, s, SO_2NH), 9.47 (1H, s, NH-Phenyl), 8.03-8.00 (2H, m, Naphthoquinone H-5 and H-8), 7.86-7.77(2H, m, Naphthoquinone H-6 and H-7), 7.67 (2H, d, *J*=8.4 Hz, Benzene H-2 and H-6), 7.18 (2H, d, *J*=8.4 Hz, Benzene H-3 and H-5), 6.26 (2H, s, Phenyl H-2 and H-6), 6.15 (1H, t, *J*=2.2 Hz, Phenyl H-4), 3.63 (6H, s, 2xOCH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.2, 177.4, 161.1, 143.8, 143.0, 140.1, 135.1, 133.9, 133.6, 132.1, 130.9, 127.4, 127.0, 126.6, 122.7, 119.4, 98.6, 95.8, 56.5, 55.5 ppm; MS (APCI) m/z: 497 ($M-H^-$), 499 ($M-H+2^-$); Anal. Calcd. for $C_{24}H_{19}ClN_2O_6S$. 3.41 H_2O (560.37): C, 51.44; H, 4.64; N, 5.00; S, 5.72%, Found: C, 51.00; H, 4.37; N, 5.15; S, 6.11%.

*4.1.1.7. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(2-nitrophenyl)benzenesulfonamide(**4**)*

Yield 23%; mp 244 ^{o}C; IR υ_{maks} (FT/ATR): 3308 (NH), 3276 (NH), 3100 (CH-arom), 2925 (CH-aliph), 2854 (CH-aliph), 1672 (C=O), 1647 (C=O), 1591 (C=C), 1567 (C=C), 1514 (NO_2-asym), 1350 (SO_2-asym), 1328 (NO_2-sym), 1160 (SO_2-sym) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.12 (1H, s, SO_2NH), 9.52 (1H, s, NH-Phenyl), 8.03 (2H, dd, *J*=7.6; 1.5 Hz, Naphthoquinone H-5 and H-8), 7.92 (1H, dd, *J*=8.0; 1.5 Hz, Phenyl H-5), 7.86 (1H, td, *J*=7.6, 1.6 Hz, Naphthoquinone H-6 or H-7), 7.81 (1H, td, *J*= 7.6; 1.6 Hz, Naphthoquinone H-6 or H-7), 7.63-7.59 (1H, m, Phenyl H-3 or H-4), 7.60 (2H, d, *J*=8.6 Hz, Benzene H-2 and H-6), 7.36 (1H, td, *J*=7.1; 1.2 Hz, Phenyl H-3 or H-4), 7.28 (1H, dd, *J*=8.2; 1.3 Hz, Phenyl H-2), 7.16 (2H, d, *J*=8.8 Hz, Benzene H-3 and H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.3, 177.5, 144.2, 143.4, 142.9, 135.2, 134.6, 134.0, 133.0, 132.1, 130.9, 130.9, 127.5, 127.0, 126.7, 126.7, 126.0, 125.9, 122.6, 119.7 ppm; MS (ESI) m/z: 482 ($M-H^-$), 484 ($M-H+2^-$); Anal. Calcd. for $C_{23}H_{19}ClN_3O_6S$. 0.9 C_2H_6O (525.34): C, 54.41; H, 3.72; N, 8.00; S, 6.10%, Found: C, 54.60; H, 4.08; N, 8.38; S, 6.57%.

*4.1.1.8. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(3-nitrophenyl)benzenesulfonamide(**5**)*

Yield 59%, mp 268 ^{o}C; IR υ_{maks} (FT/ATR): 3260 (NH), 3257 (NH), 3100 (CH-arom), 1677 (C=O), 1590 (C=C), 1527 (NO_2-asym), 1350 (NO_2-sym), 1331 (SO_2-asym), 1158 (SO_2-sym) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.76 (1H, s, SO_2NH), 9.50 (1H, s, NH-Phenyl), 8.02 (2H, dd, *J*=8.0; 1.6 Hz, Naphthoquinone H-5 and H-8), 7.90-7.78 (4H, m, Naphthoquinone H-6 and H-7, Phenyl H-2 and H-4), 7.67 (2H, d, *J*=8.8 Hz, Benzene H-2 and H-6), 7.55-7.50 (2H, m, Phenyl H-5 and H-6), 7.16 (2H, d, *J*=8.8 Hz, Benzene H-3 and H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.2, 177.4, 176.3, 148.6, 144.2, 142.9, 142.9, 139.7, 135.1, 135.1, 134.0, 132.7, 132.1, 131.4, 131.2, 130.9, 127.5, 127.5, 127.0, 126.6, 126.1, 122.7, 119.8, 118.9, 114.1 ppm; MS (APCI) m/z: 484 ($M+H^+$), 486 ($M+H+2^+$); Anal. Calcd. for $C_{22}H_{14}ClN_3O_6S$ (483.88): C, 54.61; H, 2.92; N, 8.68; S, 6.63%, Found: C, 54.59; H, 3.16; N, 8.29; S, 6.86%.

*4.1.1.9. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(4-nitrophenyl)benzenesulfonamide(**6**)*

Yield 21%; mp 271 ^{o}C; IR υ_{maks} (FT/ATR): 3300 (NH), 3252 (NH), 2990 (CH-arom), 1676 (C=O), 1592 (C=C), 1508 (NO_2-asym), 1332 (SO_2-asym), 1155 (SO_2-sym) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.10 (1H, bs, SO_2NH), 9.49 (1H, bs, NH-Phenyl), 8.12 (2H, d, *J*=9.1 Hz, Phenyl H-3 and H-5), 8.02 (2H, dd, *J*=8.0; 1.6 Hz, Naphthoquinone H-5 and H-8), 7.86 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.81 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.74 (2H, d, *J*=8.8 Hz, Benzene H-2 and H-6), 7.30 (2H, d, *J*=9.1 Hz, Phenyl H-2 and H-6), 7.18 (2H, d, *J*=8.8 Hz, Benzene H-3 and H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.2, 177.4, 144.8,

144.5, 142.9, 142.9, 135.1, 134.0, 132.8, 132.1, 131.0, 127.5, 127.0, 126.7, 125.7, 122.5, 120.2, 118.5 ppm; MS (APCI) m/z: 484 ($M+H^+$), 486 ($M+H+2^+$); Anal. Calcd. for $C_{22}H_{14}ClN_3O_6S$ (483.88): C, 54.61; H, 2.92; N, 8.68; S, 6.63%, Found: C, 54.52; H, 3.11; N, 8.54; S, 6.48%.

*4.1.1.10. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(2-chlorophenyl)benzenesulfonamide(**7**)*

Yield 8%; mp 254 °C; IR υ_{maks} (FT/ATR): 3262 (NH), 3047 (CH-arom), 1671 (C=O), 1591 (C=C), 1337 (SO_2-asym), 1166 (SO_2-sym) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.80 (1H, bs, SO_2NH), 9.51 (1H, bs, NH-Phenyl), 8.03 (2H, dd, *J*=7.6; 1.2 Hz, Naphthoquinone H-5 and H-8), 7.88-7.79 (2H, m, Naphthoquinone H-6 and H-7), 7.56 (2H, d, *J*=7.6 Hz, Benzene H-2 and H-6), 7.37 (1H, d, *J*=7.6 Hz, Phenyl H-3), 7.26-7.25 (2H, m, Phenyl H-5 and H-6), 7.16-7.14 (3H, m, Phenyl H-4, Benzene H-3 and H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.3, 177.5, 143.7, 143.0, 135.2, 134.5, 134.1, 134.0, 132.2, 130.9, 130.3, 129.6, 128.1, 128.0, 128.0, 127.3, 127.0, 126.7, 122.7, 119.0 ppm; MS (APCI) m/z: 473 ($M+H^+$), 475 ($M+H+2^+$), 477 ($M+H+4^+$); Anal. Calcd. for $C_{22}H_{14}Cl_2N_2O_4S$. 0.3 H_2O (478.73): C, 55.19; H, 3.07; N, 5.85; S, 6.70%, C, 55.56; H, 3.25; N, 5.51; S, 6.70%.

*4.1.1.11. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(3-chlorophenyl)benzenesulfonamide(**8**)*

Yield 9%; mp 266 °C; IR υ_{maks} (FT/ATR): 3254 (NH), 3085 (CH-arom), 1677 (C=O), 1592 (C=C), 1329 (SO_2-asym), 1156 (SO_2-sym) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.38 (1H, bs, SO_2NH), 9.48 (1H, bs, NH-Phenyl), 8.02 (2H, dd, *J*=7.6; 1.6 Hz, Naphthoquinone H-5 and H-8), 7.87-7.80 (2H, m, Naphthoquinone H-6 and H-7), 7.63 (2H, d, *J*=8.0 Hz, Benzene H-2 and H-6), 7.24 (1H, t, *J*=8.4 Hz, Phenyl H-5), 7.16 (2H, d, *J*=8.0 Hz, Benzene H-3 and H-5), 7.07-7.04 (3H, m, Phenyl H-2, H-4 and H-6) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.3, 177.4, 144.0, 143.0, 139.9, 135.2, 134.0, 133.8, 133.1, 132.1, 131.3, 130.9, 127.4, 127.0, 126.7, 124.2, 122.7, 119.8, 119.5, 118.8 ppm; MS (APCI) m/z: 473 ($M+H^+$), 475 ($M+H+2^+$), 477 ($M+H+4^+$); Anal. Calcd. for $C_{22}H_{14}Cl_2N_2O_4S$ (473.32): C, 55.82; H, 2.98; N, 5.92; S, 6.7%, Found: C, 55.70; H, 3.23; N, 5.57; S, 6.41%.

*4.1.1.12. N-(4-bromophenyl)-4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)benzenesulfonamide(**9**)*

Yield 59%; mp 276 °C; IR υ_{maks} (FT/ATR): 3296 (NH), 3229 (NH), 3085 (CH-arom), 1670 (C=O), 1588 (C=C), 1341 (SO_2-asym), 1152 (SO_2-sym) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.29 (1H, s, SO_2NH), 9.49 (1H, s, NH-Phenyl), 8.03 (1H, dd, *J*=7.6; 1.6 Hz, Naphthoquinone H-5 or H-8), 8.02 (1H, dd, *J*=7.6; 1.6 Hz, Naphthoquinone H-5 or H-8), 7.86 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.81 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.61 (2H, d, *J*=8.8 Hz, Benzene H-2 and H-6), 7.40 (2H, d, *J*=8.8 Hz, Phenyl H-3 and H-5), 7.15 (2H, d, *J*=8.4 Hz, Benzene H-3 and H-5), 7.02 (2H, d, *J*=8.8 Hz, Phenyl H-2 and H-6) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): 180.2, 177.4, 143.9, 143.0, 137.7, 135.1, 134.0, 133.3, 132.4, 132.1, 130.9, 127.4, 127.0, 126.6, 122.7, 122.5, 119.5, 116.6 ppm; MS (APCI) m/z: 517 ($M+H^+$), 519 ($M+H+2^+$), 521 ($M+H+4^+$); Anal. Calcd. for $C_{22}H_{14}BrClN_2O_4S$. 0.1 C_2H_6O (522.38): C, 51.04; H, 2.82; N, 5.36; S, 6.14%, Found: C, 50.79; H, 2.86; N, 4.99; S, 5.74%.

*4.1.1.13. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(naphthalen-1-yl)benzenesulfonamide(**10**)*

Yield 47%; mp 291°C; IR υ_{maks} (FT/ATR): 3278 (NH), 3225 (NH), 2990 (CH-arom), 1670 (C=O), 1588 (C=C), 1574 (C=C), 1341 (SO_2-asym), 1151 (SO_2-sym) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.08 (1H, bs, SO_2NH), 9.46 (1H, bs, NH- Phenyl), 8.02 (2H, dd, *J*=7.6; 1.2 Hz, Naphthoquinone H-5 and H-8), 7.99 (1H, d, *J*=8.0 Hz, Naphthalene H-4), 7.86 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.80 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.76 (2H, d, *J*=8.0 Hz, Naphthalene H-5 and H-8), 7.53 (2H, d, *J*=8.4 Hz, Benzene H-2 and H-6), 7.43 (2H, td, *J*=8.0; 1.2 Hz; Naphthalene H-6 and H-7), 7.39 (1H, t, *J*=7.6 Hz, Naphthalene H-3), 7.17 (1H, d, *J*=6.8 Hz, Naphthalene H-2), 7.09 (2H, d, *J*=8.4 Hz, Benzene H-3 and H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.3, 177.4, 143.4, 143.0, 135.2, 134.4, 134.3, 133.9, 132.9, 132.2, 130.8, 129.9, 128.4, 127.3, 127.2, 127.0, 126.6, 126.6, 126.4, 125.8, 124.0, 123.6, 122.7, 118.8 ppm; MS (APCI) m/z: 489 ($M+H^+$), 491 ($M+H+2^+$); Anal. Calcd. for $C_{26}H_{17}ClN_2O_4S$ (488.94): C, 63.87; H, 3.50; N, 5.73; S, 6.56%, Found: C, 63.74; H, 3.66; N, 5.37; S, 6.39%.

*4.1.1.14. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(3-methylpyridin-2-yl)benzenesulfonamide(**11**)*

Yield 54%; mp 284°C; IR υ_{maks} (FT/ATR): 3329 (NH), 3259 (NH), 2972 (CH-arom), 2884 (CH-aliph), 1671 (C=O), 1607 (C=C), 1592 (C=C), 1329 (SO_2-asym), 1163 (SO_2-sym) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.44 (1H, bs, NH- Phenyl), 8.03 (1H, dd, *J*=7.6; 1.2 Hz, Naphthoquinone H-5 or H-8), 8.02 (1H, dd, *J*=7.6; 1.2 Hz, Naphthoquinone H-5 or H-8), 7.86 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.83-7.78 (1H, m, Naphthoquinone H-6 or H-7), 7.79 (2H, d, *J*=8.0 Hz, Benzene H-2 and H-6), 7.61-7.59 (2H, m, Pyridine H-4 and H-6), 7.17 (2H, d, *J*=8.0 Hz, Benzene H-3 and H-5), 6.83-6.75 (1H, m, Pyridine H-5), 2.11 (3H, s, CH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): 180.3, 177.3, 143.2, 142.8, 140.5, 135.2, 133.9, 132.2, 130.9, 127.5, 127.0, 126.9, 126.6, 122.6, 122.2, 118.5, 17.7 ppm; MS (APCI) m/z: 454 ($M+H^+$), 456 ($M+H+2^+$); Anal. Calcd. for $C_{22}H_{16}ClN_3O_4S$. 0.55 H_2O (463.81): C, 56.97; H, 3.72; N, 9.06; S, 6.91%, Found: C, 56.53; H, 3.68; N, 8.70; S, 7.38%.

*4.1.1.15. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(4-methylpyridin-2-yl)benzenesulfonamide(**12**)*

Yield 51%; mp 283 °C; IR υ_{maks} (FT/ATR): 3270 (NH), 3047 (CH-arom), 2917 (CH-aliph), 1681 (C=O), 1595 (C=C), 1332 (SO_2-asym), 1145 (SO_2-sym) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.46 (1H, bs, NH-Phenyl), 8.02 (1H, dd, *J*=7.6; 1.2 Hz, Naphthoquinone H-5 or H-8), 8.01 (1H, dd, *J*=7.6; 1.2 Hz, Naphthoquinone H-5 or H-8), 7.85 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.82-7.78 (2H, m, Naphthoquinone H-6 or H-7, Pyridine H-6), 7.74 (2H, d, J=8.4 Hz, Benzene H-2 and H-6), 7.16 (2H, d, *J*=8.8 Hz, Benzene H-3 and H-5), 6.97 (1H, s, Pyridine H-3), 6.66 (1H, d, *J*=4.8 Hz, Pyridine H-5), 2.22 (3H, s, CH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.3, 177.3, 153.9, 143.2, 142.8, 141.5, 137.2, 135.2, 133.9, 132.2, 130.9, 127.0, 126.6, 122.8, 118.4, 114.4, 21.6 ppm; MS (APCI)

m/z: 454 ($M+H^+$), 456 ($M+H+2^+$); Anal. Calcd. for $C_{22}H_{16}ClN_3O_4S$. 0.6 H_2O (464.71): C, 56.86; H, 3.73; N, 9.04; S, 6.90%, Found: C, 56.46; H, 3.78; N, 8.61; S, 6.50%.

*4.1.1.16. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(5-methylpyridin-2-yl)benzenesulfonamide(**13**)*

Yield 48%; mp 223 ^oC; IR υ_{maks} (FT/ATR): 3268 (NH), 2985 (CH-arom), 2907 (CH-aliph), 1679 (C=O), 1592 (C=C), 1365 (SO_2-asym), 1133 (SO_2-sym) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.46 (1H, bs, NH-Phenyl), 8.02 (1H, dd *J*=7.6; 1.2 Hz, Naphthoquinone H-5 or H-8), 8.01 (1H, dd, *J*=7.6; 1.2, Naphthoquinone H-5 or H-8), 7.86 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.88-7.86 (1H, m, Pyridine H-6), 7.82 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.72 (2H, d, *J*=8.8 Hz, Benzene H-2 and H-6), 7.53 (1H, dd, *J*=8.8; 2.4 Hz, Pyridine H-4), 7.15 (2H, d, *J*=8.8 Hz, Benzene H-3 and H-5), 7.06 (1H, d, *J*=8.8 Hz, Pyridine H-3), 2.12 (3H, s, CH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.3, 177.4, 151.1, 143.2, 143.1, 141.1, 136.0, 135.1, 133.9, 132.1, 130.9, 127.5, 127.3, 127.0, 126.6, 126.2, 122.6, 118.9, 113.6, 17.3 ppm; MS (APCI) m/z: 454 ($M+H^+$), 456 ($M+H+2^+$); Anal. Calcd. for $C_{22}H_{16}ClN_3O_4S$ (453.90): C, 58.21; H, 3.55; N, 9.26; S, 7.06%, Found: C, 57.99; H, 3.59; N, 9.21; S, 7.31%.

*4.1.1.17. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(5-chloropyridin-2-yl)benzenesulfonamide(**14**)*

Yield 53%; mp 230 ^oC; IR υ_{maks} (FT/ATR): 3336 (NH), 3228 (NH), 3039 (CH-arom), 1667 (C=O), 1588 (C=C), 1335 (SO_2-asym), 1159 (SO_2-sym) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 11.14 (1H, bs, SO_2NH), 9.51 (1H, bs, NH-Phenyl), 8.20 (1H, d, *J*=2.4 Hz, Pyridine H-6), 8.03 (1H, dd, *J*=7.6; 1.2 Hz, Naphthoquinone H-5 or H-8), 8.02 (1H, dd, *J*=7.6; 1.2 Hz, Naphthoquinone H-5 or H-8), 7.86 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.83-7.76 (2H, m, Naphthoquinone H-6 or H-7, Pyridine H-4), 7.77 (2H, d, *J*=8.8 Hz, Benzene H-2 and H-6), 7.17 (2H, d, *J*=8.8 Hz, Benzene H-3 and H-5), 7.08 (1H, d, *J*=8.8 Hz, Pyridine H-3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.2, 177.4, 150.6, 146.4, 144.0, 143.0, 138.7, 135.1, 134.1, 134.0, 132.1, 130.9, 127.8, 127.0, 126.7, 125.6, 125.5, 122.4, 119.6, 113.8 ppm; MS (APCI) m/z: 474 ($M+H^+$), 476 ($M+H+2^+$), 478 ($M+H+4^+$); Anal. Calcd. for $C_{21}H_{13}Cl_2N_3O_4S$. 0.5 C_2H_6O. 0.4 CH_2Cl_2 (535.92): C, 50.65; H, 3.27; N, 7.84; S, 5.98%, Found: C, 50.84; H, 2.91; N, 7.94; S, 5.80%.

*4.1.1.18. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(2-chloropyridin-3-yl)benzenesulfonamide (**15**)*

Yield 51%; mp 282 ^oC; IR υ_{maks} (FT/ATR): 3256 (NH), 3085 (CH-arom), 1670 (C=O), 1591 (C=C), 1331 (SO_2-asym), 1163 (SO_2-sym) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.53 (1H, bs, NH-Phenyl), 8.20 (1H, dd, *J*=4.4; 1.6 Hz, Pyridine H-6), 8.03 (2H, dd, *J*=7.8; 1.6 Hz, Naphthoquinone H-5 and H-8), 7.86 (1H, td, *J*=7.6; 1.2 Hz, Naphthoquinone H-6 or H-7), 7.81 (1H, td, *J*=7.6; 1.2 Hz, Naphthoquinone H-6 or H-7), 7.72 (1H, dd, *J*=8.0; 1.6 Hz, Pyridine H-4), 7.58 (2H, d, *J*=8.0 Hz, Benzene H-2 and H-6), 7.4 (1H, dd, *J*=8.0; 4.8 Hz, Pyridine H-5), 7.17 (2H, d, *J*=8.0 Hz, Benzene H-3 and H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.3, 177.5, 147.2, 146.2, 144.0, 143.0, 136.1, 135.2, 135.1, 134.0, 132.2, 131.3, 130.9, 127.5, 127.3, 127.0, 126.7, 124.1, 122.7, 122.1, 119.4 ppm; MS (APCI) m/z: 474 ($M+H^+$), 476 ($M+H+2^+$), 478 ($M+H+4^+$); Anal. Calcd. for $C_{21}H_{13}ClN_3O_4S$. 2 H_2O (510.34): C, 49.42; H, 3.36; N, 8.23; S, 6.28%, Found: C, 49.47; H, 2.94; N, 7.80; S, 6.24%.

*4.1.1.19. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(4-methylthiazol-2-yl)benzenesulfonamide(**16**)*

Yield 49%; mp 271 ^oC; IR υ_{maks} (FT/ATR): 3328 (NH), 3224 (NH), 3085 (CH-arom), 1677 (C=O), 1571 (C=C), 1386 (SO_2-asym), 1140 (SO_2-sym) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.56 (1H, bs, SO_2NH), 9.45 (1H, s, NH-Phenyl), 8.02 (2H, dd, *J*= 7.2; 1.2, Naphthoquinone H-5 and H-8), 7.86 (1H, td, *J*= 7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.81 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.67 (2H, d, *J*= 8.8 Hz, Benzene H-2 and H-6), 7.17 (2H, d, *J*= 8.8 Hz, Benzene H-3 and H-5), 6.36 (1H, s, Thiazole H-4), 2.06 (3H, s, CH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.3, 177.3, 169.0, 143.1, 142.9, 137.3, 135.2, 134.1, 133.9, 132.2, 130.9, 127.0, 126.6, 126.4, 122.8, 118.5, 102.6, 13.7 ppm; MS (APCI) m/z: 460 ($M+H^+$), 462 ($M+H+2^+$); Anal. Calcd. for $C_{20}H_{14}ClN_3O_4S_2$. 0.5 H_2O (468.93): C, 51.23; H, 3.22; N, 8.96; S, 13.68%, Found: C, 51.20; H, 3.49; N, 8.56; S, 13.40%.

*4.1.1.20. 2-chloro-3-((4-(morpholinosulfonyl)phenyl)amino)naphthalene-1,4-dione(**17**)*

Yield 55%; mp 230 ^oC; IR υ_{maks} (FT/ATR): 3281 (NH), 3085 (CH-arom), 2990 (CH-aliph), 1682 (C=O), 1607 (C=C), 1344 (SO_2-asym), 1159 (SO_2-sym), 1134 (C-O-C) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.57 (1H, s, NH-Phenyl), 8.04 (2H, dd, *J*=7.6; 1.2 Hz, Naphthoquinone H-5 and H-8), 7.86 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.82 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.62 (2H, d, *J*=8.0 Hz, Benzene H-2 and H-6), 7.28 (2H, d, *J*=8.0 Hz, Benzene H-3 and H-5), 3.63.3.60 (4H, m, Morpholine-H), 2.86-2.83 (4H, m, Morpholine-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 194.4, 180.2, 177.4, 144.4, 143.0, 135.1, 134.0, 132.1, 131.0, 128.5, 128.3, 127.0, 126.7, 122.6, 119.9, 65.7, 46.4 ppm; MS (APCI) m/z: 433 ($M+H^+$), 435 ($M+H+2^+$); Anal. Calcd. for $C_{20}H_{17}ClN_2O_5S$. 0.9 H_2O (449.09): C, 53.49; H, 3.87; N, 6.11; S, 7.00%, Found: C, 53.14; H, 3.96; N, 5.82; S, 7.02%.

*4.1.1.21. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(pyridin-2-yl)benzenesulfonamide (**PI-083**)*

Yield 44%; mp 272 ^oC; 3221 (NH), 3085 (CH-arom), 1683 (C=O), 1605 (C=C), 1352 (SO_2-asym), 1135 (SO_2-sym) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.44 (1H, bs, NH-Phenyl), 8.04-8.00 (3H, m, Naphthoquinone H-5 and H-8, Pyridine H-6), 7.85 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.80 (1H, td, *J*=7.4; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.75 (2H, d, J=8.0 Hz, Benzene H-2 and H-6), 7.72-7.69 (1H, m, Pyridine H-5), 7.16 (2H, d, *J*=8.0 Hz, Benzene H-3 and H-5), 7.17-7.13 (1H, m, Pyridine H-3), 6.86 (1H, t, *J*=6.4 Hz, Pyridine H-4) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.3, 177.4, 153.4, 143.2, 143.1, 140.5, 136.3, 135.2, 133.9, 132.1, 130.9, 127.5, 127.2, 127.0, 126.6, 122.7, 118.8, 116.2, 114.1 ppm; MS (APCI) m/z: 440 ($M+H^+$), 442 ($M+H+2^+$); Anal. Calcd. for $C_{21}H_{14}ClN_3O_4S$. 0.3 H_2O (445.27): C, 56.64; H, 3.30; N, 9.44; S, 7.20%, Found: C, 56.27; H, 3.43; N, 9.30; S, 6.88%.

*4.1.2. General procedure for the synthesis of carboxamide derivatives (**18-33**)*

4.1.2.1. Synthesis of 4-[(3-chloro-1,4-dioxo-1,4-dihydronaphthalene-2-yl)amino]benzoic acid

2,3-Dichloro-1,4-naphthoquinone (0.002 mol, 0.5 g) and 4-aminobenzoic acid (0.002 mol, 0.3 g) were refluxed in the presence of anhydrous K_2CO_3 (0.0007 mol, 0.1 g) in 20 ml of ethanol for 6 h. The reaction mixture was filtered off and the solid was washed with ethanol and water. The crude product was recrystallized from ethanol. Yield 33%, mp: 307 ^oC.

*4.1.2.2. Synthesis of carboxamide derivative final compounds (**18**, **20-25**, **27**, **32** and **33**)*

The previously synthesized 4-[(3-chloro-1,4-dioxo-1,4-dihydronaphthtalen-2-yl)amino]benzoic acid was refluxed in $SOCl_2$ for 3 h. Then, excess $SOCl_2$ was removed from the reaction mixture by rotary evoporation, the residue was dissolved in 15 ml of dichloromethane (DCM) and stirred under argon atmosphere. Appropriate amine was added to reaction mixture in an ice bath, and it was allowed to stir for 3 h, gradually warming up to room temperature. The resulting precipitate was filtered off and washed with DCM. The crude product was purified by recrystallization from ethanol or methanol. Compound **32** and **33** were purified via coloumn chromatography utilizing a 4:4:1 hexane: chloroform/dichloromethane: methanol.

*4.1.2.3. Synthesis of carboxamide derivative final compounds (**19**, **26**, **28-31**)*

To a stirring solution of 4-[(3-chloro-1,4-dioxo-1,4-dihydronaphthtalen-2-yl)amino]benzoic acid (0.00053 mol) suspended in 10 ml of tetrahydrofuran (THF) cooled to 0 ^oC, N-methylmorpholine (NMM) (0.00107 mol) and isobutyl chloroformate (IBCF) (0.00114 mol) were added under argon atmosphere and stirred for 4h at 0 ^oC. Then, appropriate amine (0.00053 mol) was added and the reaction mixture was refluxed up to 36-72 h. After completion of the reaction was monitered by TLC, the precipitated NMM.HCl was removed by filtration. The organic phase was evaporated under reduced pressure, the obtained solid was purified via column chromatography utilizing a 4:4:1 hexane: chloroform/dichloromethane:methanol.

*4.1.2.4. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-phenylbenzamide(**18**)*

Yield 40%; mp 304 ^oC; IR υ_{maks} (FT/ATR): 3239 (NH), 3066 (CH-arom), 1673 (C=O), 1644 (Amide I), 1595 (C=C), 1569 (Amide II), 1288 (=C-N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.12 (1H, s, CONH), 9.46 (1H, s, NH-Phenyl), 8.04 (2H, dt, *J*=8.0; 1.6 Hz, Naphthoquinone H-5 and H-8), 7.90 (2H, d, *J*=8.0 Hz, Benzene H-2 and H-6), 7.86-7.79 (2H, m, Naphthoquinone H-6 and H-7), 7.75 (2H, d, *J*=8.0 Hz, Phenyl H-2 and H-6), 7.32 (2H, t, *J*=8.0 Hz, Phenyl H-3 and H-5), 7.20 (2H, d, *J*=8.0 Hz, Benzene H-3 and H-5), 7.07 (1H, t, *J*=7.6 Hz, Phenyl H-4) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.6, 177.3, 165.6, 156.5, 143.2, 139.7, 138.9, 135.2, 133.8, 132.3, 130.9, 130.1, 129.0, 128.1, 127.0, 126.6, 123.9, 122.7, 120.8 ppm; MS (APCI) m/z: 403 ($M+H^+$), 405 ($M+H+2^+$); Anal. Calcd. for $C_{23}H_{15}ClN_2O.0.5\ C_2H_6O$ (425.87): C, 68.02; H, 3.99; N, 6.84%, Found: C, 67.75; H, 4.39; N, 6.88%.

*4.1.2.5. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(2-isopropylphenyl)benzamide(**19**)*

Yield 17%; mp 245 ^oC; IR υ_{maks} (FT/ATR): 3213 (NH), 2959 (CH-arom), 2925 (CH-aliph), 2854 (CH-aliph), 1676 (C=O), 1638 (Amide I), 1596 (C=C), 1568 (Amide II), 1285 (=C-N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.82 (1H, s, CONH), 9.47 (1H, s, NH-Phenyl), 8.08-8.05 (2H, m, Naphthoquinone H-5 and H-8), 7.93 (2H, d, *J*=8.1 Hz, Benzene H-2 and H-6), 7.89 (1H, td, *J*=7.5; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.83 (1H, td, *J*=7.5; 1.3 Hz, Naphthoquinone H-6 or H-7), 7.35 (1H, d, *J*=7.5 Phenyl H-6), 7.28-7.23 (1H, m, Phenyl), 7.22 (2H, d, *J*=7.3 Hz, Benzene H-3 and H-5), 7.22-7.18 (2H, m, Phenyl H), 3.18 (1H, m, CH), 1.14 (6H, d, *J*=6.7 Hz, 2xCH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.5, 177.3, 165.9, 145.3, 143.2, 142.5, 135.6, 135.2, 133.8, 132.3, 130.8, 129.7, 128.6, 128.0, 127.3, 127.0, 126.6, 126.2, 126.0, 122.8, 117.5, 28.0, 23.6 ppm; MS (ESI) m/z: 445 ($M+H^+$), 447 ($M+H+2^+$); Anal. Calcd. for $C_{26}H_{21}ClN_2O_3.0.1\ CHCl_3$ (456.85): C, 68.62; H, 4.66; N, 6.13%, Found: C, 68.32; H, 4.58; N, 9.60%.

*4.1.2.6. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(m-tolyl)benzamide(**20**)*

Yield 46%; mp 261 ^oC; IR υ_{maks} (FT/ATR): 3227 (NH), 3066 (CH-arom), 2971 (CH-aliph), 1674 (C=O), 1641 (Amide I), 1593 (C=C), 1567 (Amide II), 1288 (=C-N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.08 (1H, s, CONH), 9.45 (1H, s, NH-Phenyl), 8.04 (2H, dd, *J*=8.0; 1.2 Hz, Naphthoquinone H-5 and H-8), 7.90 (2H, d, *J*=8.0 Hz, Benzene H-2 and H-6), 7.87-7.79 (2H, m, Naphthoquinone H-6 and H-7), 7.61 (1H, s, Phenyl H-2), 7.56 (1H, d, *J*=8.0 Hz, Phenyl H-6), 7.21-7.17 (1H, m, Phenyl H-5), 7.19 (2H, d, *J*=8.0 Hz, Benzene H-3 and H-5), 6.89 (1H, d, *J*=8.0 Hz, Phenyl H-4), 2.28 (3H, s, CH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.4, 177.3, 165.3, 143.2, 142.5, 139.8, 138.1, 135.2, 133.9, 132.4, 130.8, 129.9, 128.8, 128.8, 126.6, 124.6, 123.9, 122.7, 121.4, 120.6, 117.6, 21.7 ppm; MS (APCI) m/z: 417 ($M+H^+$), 419 ($M+H+2^+$); Anal. Calcd. for $C_{24}H_{17}ClN_2O_3.1.1\ C_2H_6O$ (467.54): C, 67.31; H, 5.09; N, 5.99%, Found: C, 67.07; H, 5.49; N, 6.33%.

*4.1.2.7. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(4-nitrophenyl)benzamide(**21**)*

Yield 13%; mp >300 ^oC; IR υ_{maks} (FT/ATR): 3206 (NH), 3028 (CH-arom), 1673 (C=O), 1645 (Amide I), 1594 (C=C), 1568 (Amide II), 1539 (NO_2-asym), 1331 (NO_2-sym), 1290 (=C-N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.69 (1H, s, CONH), 9.50 (1H, s, NH-Phenyl), 8.25 (2H, d, *J*=8.2 Hz, Phenyl H-3 and H-5), 8.05 (2H, d, *J*=8.4 Hz, Phenyl H-2 and H-6), 8.08-8.02 (2H, m, Naphthoquinone H-5 and H-8), 7.92 (2H, d, *J*=8.4 Hz, Benzene H-2 and H-6), 7.89-7.80 (2H, m, Naphthoquinone H-6 and H-7), 7.22 (2H, d, *J*=8.4 Hz, Benzene H-3 and H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.4, 177.4, 166.1, 146.1, 143.3, 143.2, 142.8, 135.2, 133.9, 132.2, 130.9, 129.1, 128.5, 127.0,

126.6, 125.2, 122.6, 120.2, 118.3 ppm; MS (APCI) m/z: 446 ($M-H^-$), 448 ($M-H+2^-$); Anal. Calcd. for $C_{23}H_{14}ClN_3O_5$.0.5 H_2O (456.84): C, 60.00; H, 3.37; N, 9.02%, Found: C, 60.39; H, 3.46; N, 8.62%.

*4.1.2.8. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(3-chlorophenyl)benzamide(**22**)*

Yield 28%; mp 267 °C; IR υ_{maks} (FT/ATR): 3230 (NH), 3028 (CH-arom), 1672 (C=O), 1644 (Amide I), 1592 (C=C), 1568 (Amide II), 1285 (=C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.36 (1H, s, CONH), 9.47 (1H, s, NH-Phenyl), 8.03 (2H, dd, *J*=7.2; 1.2 Hz, Naphthoquinone H-5 and H-8), 7.97 (1H, d, *J*=1.6 Hz, Phenyl H-6), 7.91 (2H, d, *J*=7.2 Hz, Benzen H-2 and H-6), 7.88-7.79 (2H, m, Naphthoquinone H-6 and H-7), 7.72 (1H, d, *J*=8.0, Phenyl H-2), 7.35 (1H, td, *J*=8.0; 1.2 Hz, Phenyl H-5), 7.20 (2H, d, *J*=7.2 Hz, Benzene H-3 and H-5), 7.13 (1H, td, *J*=8.0; 1.2 Hz, Phenyl H-4) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.4, 177.3, 165.6, 143.2, 142.9, 141.3, 135.2, 133.9, 133.3, 132.2, 130.9, 130.7, 129.8, 129.5, 128.3, 127.0, 126.6, 123.6, 122.7, 122.5, 120.2, 119.1, 117.9 ppm; MS (APCI) m/z: 437 ($M+H^+$), 439 ($M+H+2^+$), 441 ($M+H+4^+$); Anal. Calcd. for $C_{23}H_{14}Cl_2N_2O_3$.0.35 H_2O (479.63): C, 62.28; H, 3.34; N, 6.32%, Found: C, 62.41; H, 3.14; N, 6.29%.

*4.1.2.9. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(4-chlorophenyl)benzamide(**23**)*

Yield 26%; mp 275 °C; IR υ_{maks} (FT/ATR): 3222 (NH), 3047 (CH-arom), 1674 (C=O), 1640 (Amide I), 1595 (C=C), 1568 (Amide II), 1286 (=C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.31 (1H, s, CONH), 9.46 (1H, s, NH-Phenyl), 8.04 (2H, dt, *J*=7.6; 1.6 Hz, Naphthoquinone H-5 and H-8), 7.91 (2H, d, *J*=8.8 Hz, Benzene H-2 and H-6), 7.88-7.83 (2H, m, Naphthoquinone H-6 and H-7), 7.82 (2H, d, *J*=9.2, Phenyl H-2 and H-6), 7.38 (2H, d, *J*=8.8 Hz, Phenyl H-3 and H-5), 7.20 (2H, d, *J*=8.4 Hz, Benzene H-3 and H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.4, 177.3, 165.5, 143.2, 142.7, 138.7, 135.2, 133.9, 132.2, 130.9, 129.7, 128.9, 128.2, 127.5, 127.0, 126.6, 122.7, 122.3, 117.8 ppm; MS (APCI) m/z: 437 ($M+H^+$), 439 ($M+H+2^+$), 441 ($M+H+4^+$); Anal. Calcd. for $C_{23}H_{14}Cl_2N_2O_3$.0.5 C_2H_6O.0.5 H_2O (505.37): C, 61.42; H, 3.87; N, 5.97%, Found: C, 61.66; H, 4.07; N, 5.85%.

*4.1.2.10. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(naphthalen-1-yl)benzamide(**24**)*

Yield 42%; mp 264 °C; IR υ_{maks} (FT/ATR): 3227 (NH), 3047 (CH-arom), 1674 (C=O), 1640 (Amide I), 1597 (C=C), 1569 (Amide II), 1287 (=C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.33 (1H, s, CONH), 9.49 (1H, s, NH-Phenyl), 8.07-7.95 (5H, m, Naphthoquinone H-5 and H-8, Naphthalene-H), 7.91-7.81 (3H, m, Naphthoquinone H-6 and H-7, Naphthalene-H), 7.80-7.71 (1H, m, Naphthalene-H), 7.60-7.52 (3H, m, Benzene H-2 and H-6, Naphthalene-H), 7.45-7.36 (1H, m, Naphthalene-H), 7.24 (2H, d, *J*=8.4 Hz, Benzene H-3 ve H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.5, 177.4, 166.1, 143.3, 142.7, 135.2, 134.4, 134.2, 133.9, 132.3, 130.9, 129.7, 129.6, 128.7, 128.3, 127.2, 127.0, 126.7, 126.6, 126.5, 126.4, 126.2, 126.0, 124.4, 122.8, 122.4, 117.7 ppm; MS (APCI) m/z: 451 ($M-H^-$), 453 ($M-H+2^-$); Anal. Calcd. for $C_{27}H_{17}ClN_2O_3$.0.6 H_2O (463.70): C, 69.94; H, 3.96; N, 6.04%, Found: C, 69.75; H, 4.14; N, 6.29%.

*4.1.2.11. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(pyridin-3-yl)benzamide(**25**)*

Yield 35%; mp 197 °C; IR υ_{maks} (FT/ATR): 3213 (NH), 3100 (CH-arom), 2960 (CH-arom), 1675 (C=O), 1648 (Amide I), 1599 (C=C), 1572 (Amide II), 1294 (=C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.36 (1H, s, CONH), 9.52 (1H, bs, NH-Phenyl), 8.92 (1H, d, *J*=2.4 Hz, Pyridine H-2), 8.29 (1H, dd, *J*=4.4; 1.4 Hz, Pyridine H-6), 8.18 (1H, dt, *J*=8.4; 2.0 Hz, Pyridine H-4), 8.04 (2H, dt, *J*=7.6; 1.6 Hz, Naphthoquinone H-5 and H-8), 7.91 (2H, d, *J*=8.8 Hz, Benzene H-2 and H-6), 7.87 (1H, td, *J*=7.6; 1.6 Hz, Naphthoquinone H-6 or H-7), 7.82 (1H, td, *J*=7.6; 1.6 Hz, Naphthoquinone H-6 and H-7), 7.38 (1H, dd, *J*=8.4; 4.8 Hz, Pyridine H-5), 7.22 (2H, d, *J*=8.8 Hz, Benzene H-3 and H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.4, 177.3, 165.8, 144.8, 143.3, 143.0, 142.4, 136.4, 135.2, 133.9, 132.3, 130.9, 129.8, 129.4, 128.2, 127.7, 127.0, 126.6, 123.9, 122.7, 122.6, 117.9 ppm; MS (APCI) m/z: 404 ($M+H^+$), 406 ($M+H+2^+$); Anal. Calcd. for $C_{22}H_{14}ClN_3O_3$.0.3 H_2O (409.23): C, 64.57; H, 3.60; N, 10.27%, Found: C, 64.38; H, 3.76; N, 10.28%.

*4.1.2.12. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(pyridin-4-yl)benzamide(**26**)*

Yield 17%; mp 233 °C (decomp.); IR υ_{maks} (FT/ATR): 3223 (NH), 3000 (CH-arom), 1673 (C=O), 1639 (Amide I), 1593 (C=C), 1565 (Amide II), 1287 (=C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.49 (1H, s, CONH), 10.07 (1H, bs, NH- Phenyl), 8.47-8.41 (1H, m, Pyridine-H), 8.05-8.00 (2H, m, Pyridine-H), 7.89 (2H, d, *J*=6.9 Hz, Benzene H-2 and H-6), 7.90-7.81 (3H, m, Naphthoquinone H-6 and H-7, Pyridine-H), 7.77 (2H, d, *J*=7.2 Hz, Naphthoquinone H-5 and H-8), 7.16 (2H, d, *J*=6.9 Hz, Benzene H-3 and H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.6, 166.3, 150.7, 147.3, 146.7, 146.6, 135.1, 133.6, 132.6, 131.0, 128.5, 127.0, 126.5, 122.4, 114.5, 114.4 ppm; MS (ESI) m/z: 404 ($M+H^+$), 406 ($M+H+2^+$); Anal. Calcd. for $C_{22}H_{14}ClN_3O_3$.0.78 $CHCl_3$. 0.46 C_6H_{12} (535.64): C, 57.27; H, 3.82; N, 7.84%, Found: C, 57.68; H, 3.85; N, 8.13%.

*4.1.2.13. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(3-methylpyridin-2-yl)benzamide(**27**)*

Yield 7%; mp 257 °C; IR υ_{maks} (FT/ATR): 3228 (NH), 3090 (CH-arom), 2920 (CH-aliph), 1674 (C=O), 1644 (Amide I), 1592 (C=C), 1570 (Amide II), 1283 (=C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.40 (1H, s, CONH), 9.46 (1H, bs, NH-Phenyl), 8.29 (1H, d, *J*=5.1 Hz, Pyridine-H), 8.04 (2H, d, *J*=7.6 Hz, Naphthoquinone H-5 and H-8), 7.92 (2H, d, *J*= 8.7 Hz, Benzene H-2 and H-6), 7.86 (1H, td, *J*=7.6; 1.6 Hz, Naphthoquinone H-6 or H-7), 7.81 (1H, td, *J*=7.4; 1.6 Hz, Naphthoquinone H-6 or H-7), 7.70 (1H, dd, *J*=8.1; 1.6 Hz, Pyridine-H), 7.23 (1H, dd, *J*=7.5; 4.6 Hz, Pyridine-H), 7.18 (2H, d, *J*= 8.6 Hz, Benzene H-3 and H-5), 2.18 (1H, s, CH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.5, 177.4, 165.3, 150.9, 146.3, 143.3, 142.8, 139.7, 135.2, 133.9, 132.3, 130.9, 130.2, 129.0, 128.4, 127.0, 126.6,

122.7, 122.5, 117.9, 18.1 ppm; MS (ESI) m/z: 418 ($M+H^+$), 420 ($M+H+2^+$); Anal. Calcd. for $C_{23}H_{16}ClN_3O_3$ (417.85): C, 66.11; H, 3.86; N, 10.06%, Found: C, 65.75; H, 3.76; N, 9.67%.

*4.1.2.14. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(4-methylpyridin-2-yl)benzamide(**28**)*

Yield 2%; mp 259 °C; IR υ_{maks} (FT/ATR): 3217 (NH), 3045 (CH-arom), 2922 (CH-aliph), 2852 (CH-aliph), 1673 (C=O), 1636 (Amide I), 1595 (C=C), 1561 (Amide II), 1286 (=C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.52 (1H, s, CONH), 9.46 (1H, bs, NH-Phenyl), 8.21 (1H, d, *J*=5.1, Pyridine-H), 8.10-8.01 (3H, m, Naphthoquinone H-5 and H-8, Pyridine H), 7.97 (2H, d, *J*= 9.8 Hz, Benzene H-2 and H-6), 7.91-7.80 (2H, m, Naphthoquinone H-6 and H-7), 7.17 (2H, d, *J*=8.3 Hz, Benzene H-3 and H-5), 7.01-6.96 (1H, m, Pyridine-H), 2.34 (1H, s, CH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.5, 177.4, 165.6, 152.8, 149.1, 148.0, 143.2, 143.0, 135.2, 133.9, 132.3, 130.9, 129.1, 128.5, 127.0, 126.6, 122.5, 121.1, 118.1, 115.6, 21.4 ppm; MS (ESI) m/z: 418 ($M+H^+$), 420 ($M+H+2^+$); Anal. Calcd. for $C_{23}H_{16}ClN_3O_3$. 0.07 $CHCl_3$. 0.28 C_6H_{12} (449.77): C, 66.09; H, 4.35; N, 9.34%, Found: C, 66.53; H, 3.91; N, 9.31%.

*4.1.2.15. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(5-methylpyridin-2-yl)benzamide(**29**)*

Yield 8%; mp 241 °C; IR υ_{maks} (FT/ATR): 3213 (NH), 3100 (CH-arom), 2996 (CH-aliph), 1674 (C=O), 1648 (Amide I), 1594 (C=C), 1568 (Amide II), 1289 (=C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.53 (1H, s, CONH), 9.45 (1H, bs, NH-Phenyl), 8.20-8.19 (1H, m, Pyridine-H), 8.07-8.03 (3H, m, Naphthoquinone-H, Pyridine-H), 7.98-7.96 (2H, m, Benzene H-2 and H-6), 7.89-7.85 (1H, m, Naphthoquinone H-6 or H-7), 7.84-7.79 (1H, m, Naphthoquinone H-6 or H-7), 7.64 (1H, dd, *J*=8.5; 2.4 Hz, Pyridine H-4), 7.17 (2H, d, *J*=8.6 Hz, Benzene H-3 and H-5), 2.27 (1H, s, CH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.4, 177.4, 165.5, 150.5, 148.0, 142.9, 138.8, 135.2, 133.9, 132.3, 130.9, 129.1, 128.4, 126.6, 122.5, 118.0, 114.7, 40.6, 17.8 ppm; MS (ESI) m/z: 418 ($M+H^+$), 420 ($M+H+2^+$); Anal. Calcd. for $C_{23}H_{16}ClN_3O_3$. 0.27 C_6H_{12}. 0.4 $CHCl_3$ (488.32): C, 61.54; H, 4.05; N, 8.61%, Found: C, 61.98; H, 3.34; N, 8.54%.

*4.1.2.16. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(5-chloropyridin-2-yl)benzamide(**30**)*

Yield 3%; mp 283 °C (decomp); IR υ_{maks} (FT/ATR): 3388 (NH), 3304 (NH), 3070 (CH-arom) 1676 (C=O), 1665 (Amide I), 1598 (C=C), 1572 (Amide II), 1289 (=C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.83 (1H, s, CONH), 9.50 (1H, bs, NH-Phenyl), 8.42-8.41 (1H, m, Pyridine-H), 8.21 (1H, dd, *J*=8.8; 1.2 Hz, Pyridine H-3), 8.04 (2H, dd, *J*=7.6; 1.6 Hz, Naphthoquinone H-5 and H-8), 8.01-7.92 (1H, m, Pyridine-H), 7.96 (2H, d, *J*=7.2 Hz, Benzene H-2 and H-6), 7.87 (1H, tt, *J*=7.6, 1.5 Hz, Naphthoquinone H-6 or H-7), 7.82 (1H, tt, *J*=7.6, 1.5 Hz, Naphthoquinone H-6 or H-7), 7.18 (2H, d, *J*=7.2 Hz, Benzene H-3 and H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.5, 165.9, 151.4, 146.7, 138.2, 135.2, 133.9, 130.9, 128.6, 127.0, 126.6, 125.8, 122.4, 119.0, 116.3 ppm; MS (ESI) m/z: 436 ($M-H^-$), 438 ($M-H+2^-$); Anal. Calcd. for $C_{22}H_{13}Cl_2N_3O_3$.1.34 C_6H_{12} (551.04): C, 65.48; H, 5.32; N, 7.63%, Found: C, 65.92; H, 4.95 N, 9.38%.

*4.1.2.17. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(thiazol-2-yl)benzamide(**31**)*

Yield 2%; mp 246 °C; IR υ_{maks} (FT/ATR): 3221 (NH), 2960 (CH-arom), 1676 (C=O), 1636 (Amide I), 1596 (C=C), 1560 (Amide II), 1289 (=C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.46 (1H, bs, CONH), 9.48 (1H, s, NH-Phenyl), 8.07-8.02 (2H, m, Naphthoquinone H-5 and H-8), 8.03 (2H, d, *J*=8.4 Hz, Benzene H-2 and H-6), 7.88 (1H, td, *J*=7.6; 1.6 Hz, Naphthoquinone H-6 or H-7), 7.82 (1H, td, *J*=7.6; 1.6 Hz, Naphthoquinone H-6 or H-7), 7.53 (1H, d, *J*=3.6 Hz, Thiazole H-3), 7.24 (1H, d, *J*=3.6 Hz, Thiazole H-4), 7.19 (2H, d, *J*= 8.4 Hz, Benzene H-3 and H-5) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.4, 177.4, 176.4, 143.6, 143.1, 142.9, 135.2, 135.1, 133.9, 132.3, 131.4, 130.9, 128.7, 127.5, 127.0, 126.7, 122.4, 118.8, 114.1, 113.6 ppm; MS (ESI) m/z: 408 ($M-H^-$), 410 ($M-H+2^-$); Anal. Calcd. for $C_{20}H_{12}ClN_3O_3S$.0.64 $CHCl_3$.1.12 C_6H_{12} (580.50): C, 56.61; H, 4.53; N, 7.24; S, 5.52%, Found: C, 56.97; H, 4.11; N, 6.80; S, 5.42%.

*4.1.2.18. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(4-methylthiazol-2-yl)benzamide(**32**)*

Yield 16%; mp 204 °C; IR υ_{maks} (FT/ATR): 3365 (NH), 3269 (NH), 3096 (CH-arom), 3032 (CH-arom), 2918 (CH-aliph), 1665 (C=O), 1646 (Amide I), 1599 (C=C), 1574 (Amide II), 1241 (=C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.48 (1H, bs, NH-Phenyl), 8.06-8.01 (4H, m, Naphthoquinone H-5 and H-8, Benzene H-2 and H-6), 7.87 (1H, td, *J*=7.4; 1.6 Hz, Naphthoquinone H-6 or H-7), 7.82 (1H, td, *J*=7.5; 1.5 Hz, Naphthoquinone H-6 or H-7), 7.18 (2H, d, *J*=8.6 Hz, Benzene H-3 and H-5), 6.78 (1H, s, Thiazole H-5), 2.29 (3H, s, CH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.4, 177.4, 143.5, 143.1, 135.2, 133.9, 132.2, 130.9, 128.6, 127.0, 126.6, 122.4, 118.7, 108.4, 17.3 ppm; MS (ESI) m/z: 422 ($M-H^-$), 424 ($M-H+2^-$); Anal. Calcd. for $C_{21}H_{14}ClN_3O_3S$. 0.15$CHCl_3$. 0.2CH_3OH (448.18): C, 57.22; H, 3.36; N, 9.38; S, 7.15%, Found: C, 57.65; H, 2.44; N, 8.09; S, 6.70%.

*4.1.2.19. 4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-N-(5-methylisoxazol-3-yl)benzamide(**33**)*

Yield 5%, mp 159 °C; IR υ_{maks} (FT/ATR): 3284 (NH), 3215 (NH), 3081 (CH-arom), 2972 (CH-aliph), 2852 (CH-aliph), 1692 (C=O), 1668 (Amide I), 1597 (C=C), 1575 (Amide II), 1280 (=C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.49 (1H, bs, NH-Phenyl), 8.04 (2H, dt *J*=7.6; 1.6 Hz, Naphthoquinone H-5 and H-8), 7.90-7.84 (3H, m, Naphthoquinone H-6 or H-7, Isoxazole-H), 7.86 (2H, d, *J*=8.8 Hz, Benzene H-2 and H-6), 7.82 (1H, td, *J*=7.6; 1.6 Hz, Naphthoquinone H-6 or H-7), 7.16 (2H, d, *J*=8.8 Hz, Benzene H-3 and H-5), 3.81 (3H, s, CH_3) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 180.4, 177.5, 166.4, 144.2, 143.1, 135.2, 134.0, 132.2, 130.9, 129.7, 127.0, 126.7, 124.4, 122.5, 119.0, 52.4 ppm; MS (APCI) m/z: 407 (M^+), 409 ($M+2^+$); Anal. Calcd. for $C_{21}H_{17}Cl_2N_2O_4$.0.9 C_2H_6O (449.27): C, 60.95; H, 4.35; N, 9.35%, Found: C, 61.38; H, 4.76; N, 9.28%.

4.2. Biological evaluation

4.2.1. Cell culture

Human breast cancer cell line (MCF-7) was obtained from American Type Culture Collection (ATCC) and maintained as exponentially growing monolayers by culturing according to the supplier's instructions.

4.2.2. Cell proliferation inhibition assay

The antiproliferative activity of the synthesized compounds was determined on MCF-7 cells. Cells were seeded in 96-well plates at a density of 6000 cells/well. After 24 h incubation, cells were treated for 48 h with desired concentration of compounds according to their solubility ranges. Cell viability was determined by MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) (Sigma Aldrich, UK). After 48 h incubation of compounds, the mixture of MTT and medium (1:9) was replaced with old media in each well and the plates were incubated for 4 h at 37°C. 150 µL of DMSO was added to dissolve violet formazan crystals and the amount of formazan product was measured absorbance at wavelength 570 nm with a microplate reader a (Varioscan, Thermo Fisher Scientific, US). DMSO (dimethyl sulfoxide, VWR, US) was used a solvent control or a negative control. All tests were performed in triplicate. Cytotoxic effects of the compounds were determined as IC_{50} which refers to the concentration of the drug to inhibit 50% of cell viability and calculated by Graph Pad Prism 5 (San Diego, CA, US).

4.2.3. Cell-based proteasome inhibition assay

To determine proteasome inhibitory activity of compounds in protein extracts, MCF-7 cells were harvested with a lysis buffer of 8.56 g sucrose, 0.6 g HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 0.2 g $MgCl_2$, 0.037 g EDTA (ethylenediaminetetraacetic acid) prepared in 100 ml (before lysis, DTT (dithiothreitol) was added freshly to the lysis buffer at a final concentration of 1 mM). The supernatant was used to measure the activity of proteasome subunit activity. Reaction mixture included 225 mM Tris buffer (pH 7.8) containing 7.5 mM MgOAc, 7.5 mM $MgCl_2$, 45 mM KCl, and 1 mM DTT.[37] The protein extracts were incubated with reaction mixture for 10 min. Then, potential proteasome inhibitors with determined doses according to the cell viability assay were added and incubated for 1h at 37 °C. While the fluorogenic peptide Suc-Leu-Leu-Val-Tyr-AMC (Enzo Life Sciences; US) was used as a substrate at a concentration of 200 µM to measure chymotrypsin-like activity of the proteasome, Ac-Gly-Pro-Leu-Asp-AMC (100 µM) (Enzo Life Sciences; US) and Z-Leu-Leu-Glu-AMC (40 µM) (Enzo Life Sciences; US) flourogenic peptides were used to determine caspase-like and trypsin-like activity of the proteasome, respectively. After the incubation, the fluorogenic substrates were added with desired compounds for 1 h at 37 °C. Methyl coumarin (MCA) liberation was measured with a fluorescence reader at 360 nm excitation/460 nm emission (Varioscan, Thermo Fisher Scientific, US). While DMSO was used as a solvent control, MG-132, known proteasome inhibitor, was used as a positive control.

The selected and potential proteasome inhibitors were treated to MCF-7 cells for 12 h to determine IC_{50} value at proteasome subunits activity. Then, proteasome subunit activities were determined as mentioned above. The results were normalized via total protein level performed by BCA (Bicinchonic acid) assay (Thermo Fisher Scientific, US).

4.2.4. Immunoblotting assay

MCF-7 cells were treated with IC_{50} values of compound **14** and PI-083 for 12 hours. Cells then lysed with RIPA buffer (50mM Tris-HCl, pH 8.0, 1% NP-40, 0.1% SDS, 150mM NaCl, 0.1% Triton X-100, 5mM EDTA) with protease inhibitors (Roche, Switzerland) in order to prepare whole cell lysates. Protein concentrations were determined by bicinchoninic acid (BCA) protein assay (Thermo Fisher Scientific, US). 40 µg total cellular proteins were used for immunoblotting studies. Samples were denatured in 4x Laemmli buffer at 95 °C for 5 min and were separated by handcast SDS-PAGE electrophoresis and transferred to PVDF membranes (EMD Milipore, Thermo Fisher Scientific, US). After blocking the membranes with PBS–0.1% Tween-20 with 5% non-fat dry milk, first primary antibodies namely anti-20S β5 subunit (Enzo, BML-PW8895-, US), anti-K48 linkage Specific Polyubiquitin (CST, 8081, US) and anti-actin (Sigma-Aldrich-A5316, UK) and then goat anti-rabbit and anti-mouse HRP-Conjugated secondary antibodies (Thermo Fisher Scientific, US) were used for determination of target proteins. Protein signals were detected using Clarity ECL substrate solution (BIORAD, US) by Fusion-FX7 (Vilber Lourmat, Thermo Fisher Scientific, US). A well known proteasome inhibitor MG-132 was used at 10 µM concentration.

4.3. Molecular modeling studies

4.3.1. Docking studies

4.3.1.1. Compounds 13, 14 and 28 in β1, β2 and β5 subunits of human 20S proteasome

In order to prepare the ligand and the subunits of the protein for docking studies, compounds **13**, **14** and **28** were built, protonated and energy minimized by using MMFF94x in MOE2014.09. On the other hand, the crystal structure of human 20S proteasome in complex with bortezomib (PDB code 5LF3) resolved at 2.1 Å were downloaded from Protein Data Bank. After the ligand and the water molecules were removed, β1, β2 and β5 subunits were saved individually. Then, each subunit was protonated via protonate 3D protocol and subjected to energy minimization using AMBER99 force field in MOE2014.09.[38]

Docking simulations of compounds **13**, **14** and **28** in complex with β1, β2 and β5 subunits were carried out with GOLD 5.2.1 program with default settings.[39,40] The binding site was defined around the backbone carbon of Gly47 with a sphere of 22 Å in each active site of β1, β2 and β5 subunits. Goldscore and Chemscore standard precision were selected as the scoring function and 50 confirmations were allowed. Proposed binding poses of the ligand-subunit complexes were selected by visual inspection using GOLD 5.2.1 program.

4.3.2. Molecular dynamics simulations

Molecular dynamics simulations were carried out for the apo forms of the β1, β2 and β5 subunits of human 20S proteasome and the selected poses of compound **14** in complex with β1, β2 and β5 subunits of human 20S proteasome, individually. AMBER 14 program was used for all the preparation steps of the apo forms of enzymes and the protein-ligand complexes and AMBER 12 program was used for the simulation part.[41]

After the atom types and the partial charges of compound **14** with selected geometries were calculated by AM1-BCC charge model of antechamber module, protein subunits and protein subunit-ligand complexes were prepared for energy minimisation and MD simulations by using the xLeap module of AmberTools14.[41,42] In order to generate the model, compound **14** and the enzyme subunits were parameterized with *Gaff* and AMBERff99SB force field using parmchk module, respectively.[43,44] While compound **14**-β1 subunit and compound **14**-β5 subunit complexes were neutralized by $7K^+$ and $3Cl^-$ counterions, respectively; compound **14**-β2 subunit was already neutral in its current form. The complexes were solvated by water molecules for each complex, respectively in octahedral box using TIP3P leaving at least 10 Å between the solute atoms and the border of the box.[45] The solvated and neutralized complexes were energy minimized by Sander module of AMBER. Then, the systems were heated from 50 to 300K with positional restrains (force constant: 10 kcal/mol/ Å) for 0.3 ns for compound **14**-β1 subunit and compound **14**-β2 subunit; 0.1 ns for compound **14**-β5 subunit allowing the solvent and the counterions to move freely. 9 Å cut off for the short-range nonbonded interactions was used combined with the particle mesh Ewald option.[46] In order to constrain bond vibrations involving the hydrogen atoms, Settle algorithm was used.[47] Then the positional restrains were reduced to allow unrestrained MD simulations for all atoms and were reached the equilibration state.

Apo forms of the three protein subunits and compound **14**-β1 subunit, compound **14**-β2 subunit and compound **14**-β5 subunit complexes were subjected to free MD simulations for 30 ns for compound **14**-β1 subunit, compound **14**-β2 subunit complexes and 10 ns for compound **14**-β5 subunit complex. VMD was used for the visualization of the trajectories and XMGRACE software was used for preparing the plots.[48,49] (See Supplementary Data)

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at…

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Tables 1, 2 and 3

Table 1. *In vitro* antiproliferative activity of final compounds against MCF-7 cell line.

Compound No	IC_{50}(μM)[a]	Compound No	IC_{50}(μM) [a]
1	2.43±0.11	**18**	15.4 ±5.25
2	5.38±1.16	**19**	2.61±0.2
3	2.20±0.14	**20**	14.43±3.29
4	3.99±0.35	**21**	16.15±2.53
5	2.17±0.19	**22**	16.57±2.71
6	2.84±0.13	**23**	4.52±0.78
7	2.56±0.37	**24**	4.80±0.11
8	2.10±0.31	**25**	6.04±0.26
9	3.37±0.48	**26**	20.80±0.5
10	3.32±0.45	**27**	4.32±0.4
11	1.91±0.13	**28**	1.72±0.14
12	2.11±0.15	**29**	4.16±0.4
13	1.79±0.21	**30**	6.19±0.1
14	4.43±0.31	**31**	3.84±0.27
15	4.65±0.63	**32**	2.44±0.19
16	4.23±0.59	**33**	10.15±0.12
17	1.84±0.18		
PI-083	1.96±0.14	**Doxorubicin**	0.93±0.06

[a]The IC_{50} values are shown as an average of three independent determinations.

Table 2. 20S proteasome β5, β1 and β2 subunits inhibition of final compounds

Compound No	Inhibition % (10 μM)[a]		
	ChT-L(β5) activity	C-L (β1) activity	T-L(β2) activity
1	17.36	13.88	21.08
2	20.01	11.5	17.12
3	19.98	12.01	22.66
4	17.31	14.25	21.27
5	23.51	7.44	19.38
6	26.03	14.13	22.70
7	3.25	1.21	6.29
8	28.83	14.20	24.54
9	22.17	10.02	23.81
10	18.44	15.40	21.16
11	14.76	9.01	12.96
12	11.70	3.35	7.26
13	10.06	n.i.	11.56
14	42.31	28.89	35.02
15	22.41	11.17	22.08
16	24.82	15.44	20.96
17	10.79	16.17	2.76
18	4.03	10.72	3.19
19	17.24	9.94	n.i.
20	14.61	15.06	3.39
21	35.34	22.77	30.52
22	21.64	11.71	27.55
23	15.85	n.i.	1.55
24	16.92	16.71	4.41
25	16.28	1.20	n.i.
26	8.96	7.87	2.43
27	11.41	9.52	n.i.
28	16.65	8.37	n.i.
29	22.01	7.42	n.i.
30	19.40	8.55	n.i.
31	19.65	3.59	n.i.
32	22.29	7.76	7.11
33	18.89	8.36	6.55
PI-083	36.76	18.81	29.59
MG132	38.23	18.39	51.42

[a]The values reported are the average of three independent determinations; n.i.: no inhibition

Table 3. IC_{50} values of compounds 14, 21 and PI-083 for antiproteasomal activity

Compound No	IC_{50} (μM)[a]		
	ChT-L(β5) activity	C-L (β1) activity	T-L(β2) activity
14	9.90±0.61	44.83±4.23	22.27±0.15
21	33.14±0.29	>50	>50
PI-083	12.47±0.21	53.12±2.56	26.37±0.5

[a]The values reported are the mean±SEM of three independent experiments.

Figures 1-4

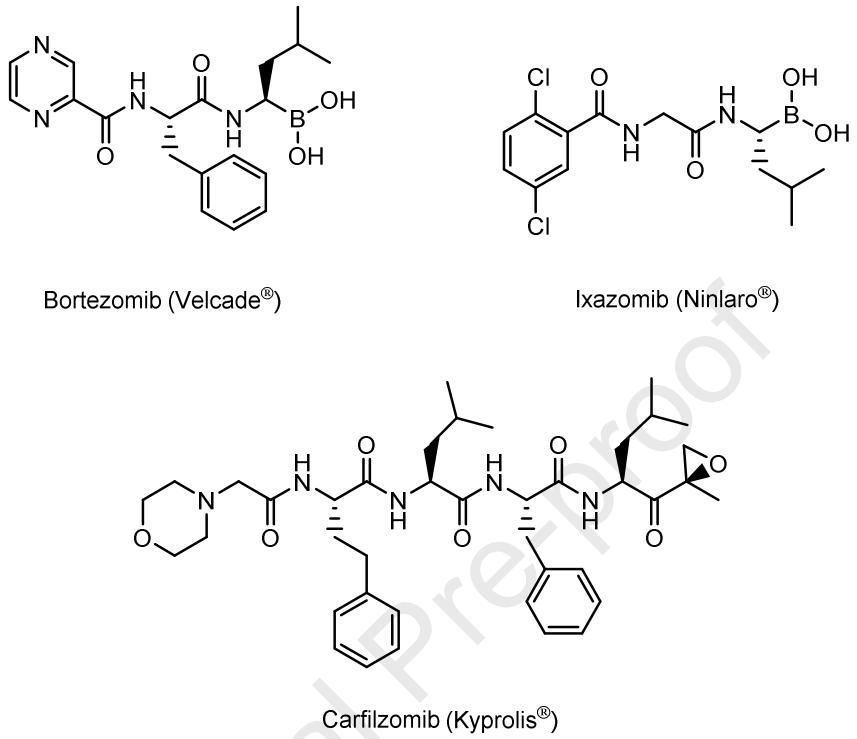


Figure 1. Chemical structures of FDA-approved proteasome inhibitors

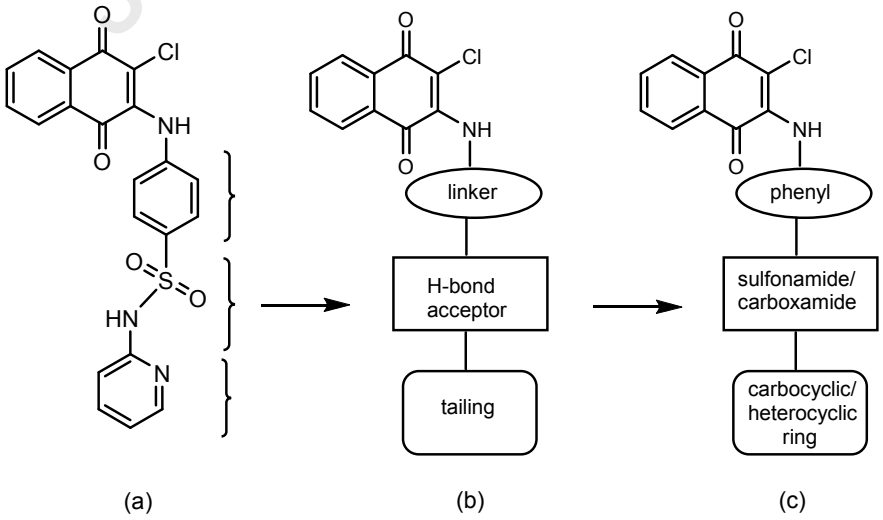


Figure 2. (a) Structure of PI-083 lead compound (b) General schematic structure of proteasome inhibitors bearing 1,4-naphthoquinone pharmacophoric unit (c) General structure of the designed compounds

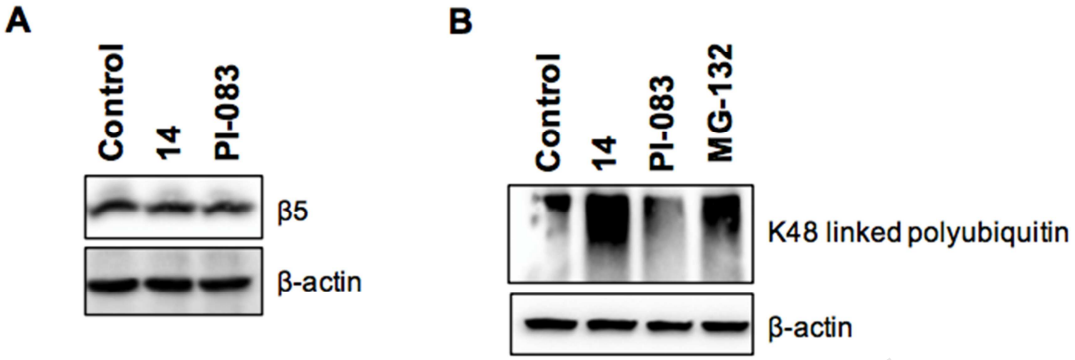
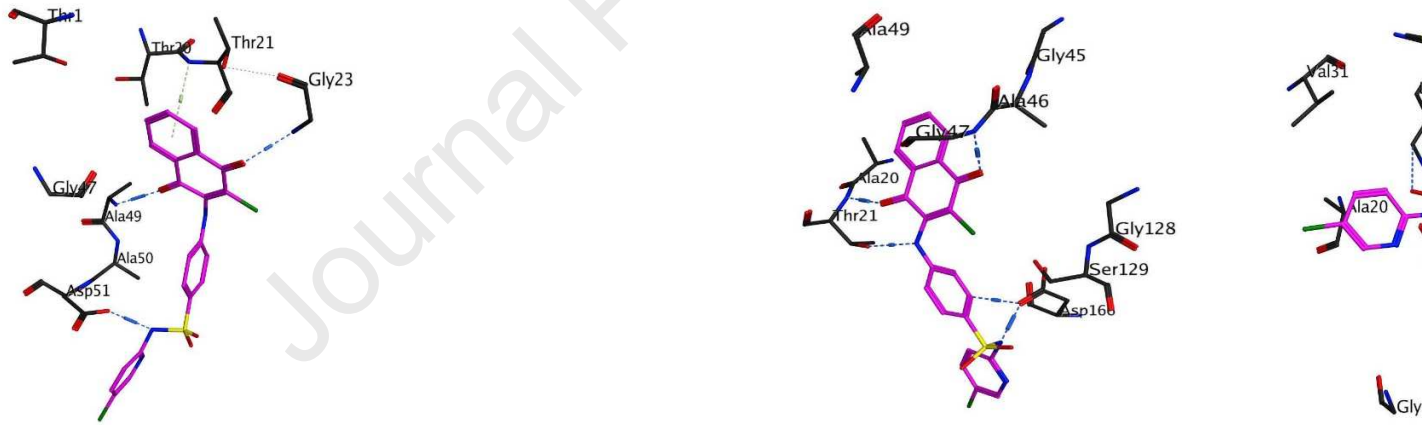


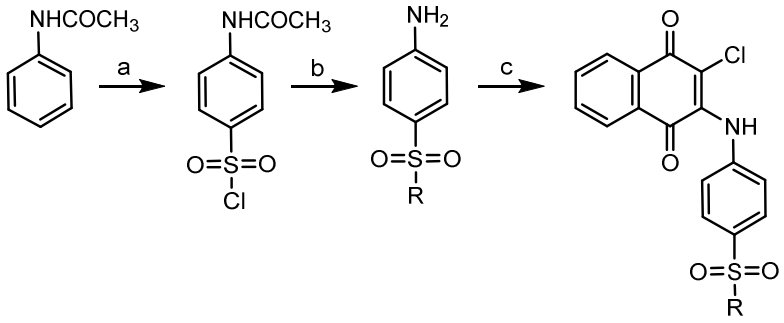
Figure 3. Evaluation of the expression level of 20S proteasome subunit β5 which is related to ChT-L activity (A) and accumulation of K-48 linked polyubiquitinated proteins (B). MCF-7 cells were treated with compound **14** and PI-083 at their IC_{50} concentrations for 12 h (A, B). 20S proteasome subunit β5 protein level and K48-linked polyubiquitinated proteins were detected by immunoblotting with the antibody against β5 and K48 linkage Specific Polyubiquitin, respectively. β-actin was used as loading control. MG-132, well known proteasome inhibitor was used at 10 μM as positive control. The experiments were repeated three times independently; with one representative result shown.



(A) (B) (C)

Figure 4. Proposed binding poses of compound **14** in the active gorge of (A) β1 subunit (B) β2 subunit and (C) β5 subunit of human 20S proteasome. (Compound 14 is colored as pink sticks while the involved residues are named using three letters code and colored as dark gray sticks)

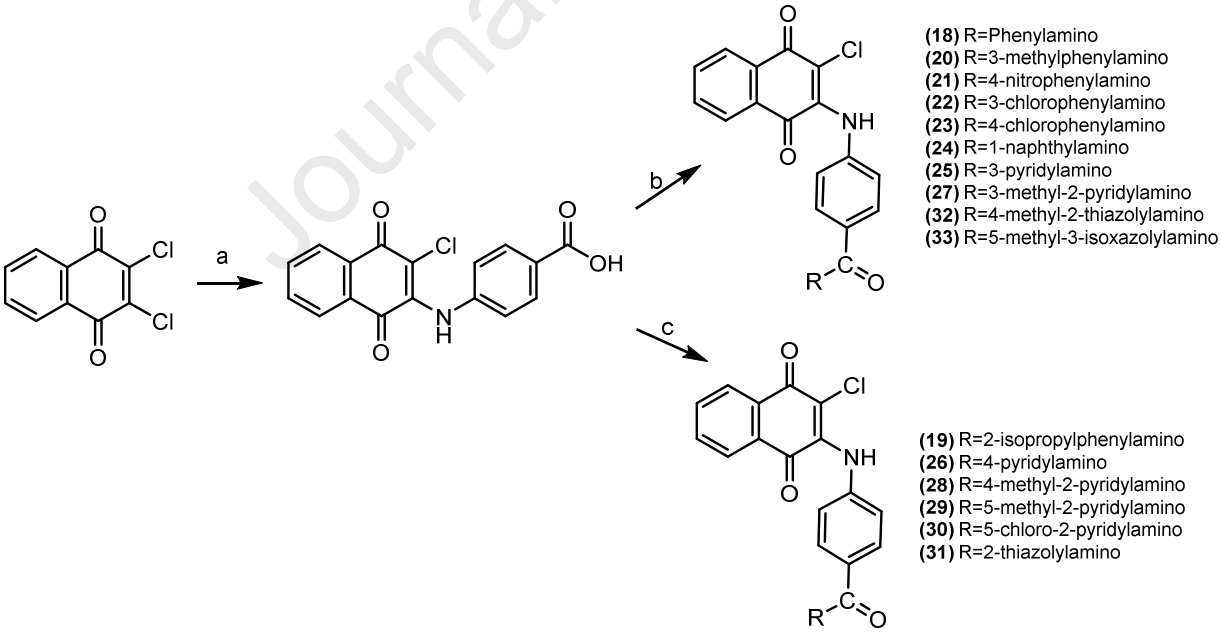
Schemes 1 and 2



(1) R=2-ethylphenylamino
(2) R=2-isopropylphenylamino
(3) R=3,5-dimethoxyphenylamino
(4) R=2-nitrophenylamino
(5) R=3-nitrophenylamino
(6) R=4-nitrophenylamino
(7) R=2-chlorophenylamino
(8) R=3-chlorophenylamino
(9) R=4-bromophenylamino
(10) R=1-naphthylamino
(11) R=3-methyl-2-pyridylamino
(12) R=4-methyl-2-pyridylamino
(13) R=5-methyl-2-pyridylamino
(14) R=5-chloro-2-pyridylamino
(15) R=2-chloro-3-pyridylamino
(16) R=4-methyl-2-thiazolylamino
(17) R=4-morpholino
(PI-083) R=2-pyridylamino

a) Chlorosulfonic acid b) Pyridine, R: appropriate amines; 5 M NaOH, MeOH; 2 M HCl c) 95% EtOH, 2,3-dichloro-1,4-naphthoquinone

Scheme 1. Synthesis of compounds **1-17**



(18) R=Phenylamino
(20) R=3-methylphenylamino
(21) R=4-nitrophenylamino
(22) R=3-chlorophenylamino
(23) R=4-chlorophenylamino
(24) R=1-naphthylamino
(25) R=3-pyridylamino
(27) R=3-methyl-2-pyridylamino
(32) R=4-methyl-2-thiazolylamino
(33) R=5-methyl-3-isoxazolylamino

(19) R=2-isopropylphenylamino
(26) R=4-pyridylamino
(28) R=4-methyl-2-pyridylamino
(29) R=5-methyl-2-pyridylamino
(30) R=5-chloro-2-pyridylamino
(31) R=2-thiazolylamino

a) 95% EtOH, 4-aminobenzoic acid b) $SOCl_2$; DCM, R: appropriate amines c) THF, NMM, IBCF, R:appropriate amines

Scheme 2. Synthesis of compounds **18-33**

Highlights

- A series of novel 4-aminobenzensulfonamide/carboxamide derivatives were explored as proteasome inhibitors
- Compound **14** inhibited ChT-L (IC_{50} =9.90 µM), C-L (IC_{50} =44.83 µM) and T-L (IC_{50} = 22.27 µM) activities of proteasome.
- All of the compounds exhibited good antiproliferative activity in a range of IC_{50}= 1.72- 20.8 µM.
- The binding mechanism of compound **14** was carried out with docking studies.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: