Bioorganic & Medicinal Chemistry 18 (2010) 5576–5592

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of naphthoquinone analogs as a novel class of proteasome inhibitors

Harshani R. Lawrence [a,b,*,†], Aslamuzzaman Kazi [a,†], Yunting Luo [b,†], Robert Kendig [b,†], Yiyu Ge [a,b], Sanjula Jain [b], Kenyon Daniel [b], Daniel Santiago [d], Wayne C. Guida [a,b,c,d], Saïd M. Sebti [a,c]

[a] Drug Discovery Department, Moffitt Cancer Center, 12901 Magnolia Drive, Tampa, FL 33612, USA
[b] High throughput Screening and Chemistry Core, Moffitt Cancer Center, 12901 Magnolia Drive, Tampa, FL 33612, USA
[c] Department of Oncologic Sciences, University of South Florida, Tampa, FL 33620, USA
[d] Department of Chemistry, University of South Florida, Tampa, FL 33620, USA

ARTICLE INFO

Article history:
Received 15 April 2010
Revised 10 June 2010
Accepted 14 June 2010
Available online 18 June 2010

Keywords:
Structure–activity relationship (SAR)
Chymotrypsin-like (CT-L)
Proteasome activity
Naphthoquinone pharmacophore
β5 and β6 subunits

ABSTRACT

Screening of the NCI Diversity Set-1 identified **PI-083** (NSC-45382) a proteasome inhibitor selective for cancer over normal cells. Focused libraries of novel compounds based on **PI-083** chloronaphthoquinone and sulfonamide moieties were synthesized to gain a better understanding of the structure–activity relationship responsible for chymotrypsin-like proteasome inhibitory activity. This led to the demonstration that the chloronaphthoquinone and the sulfonamide moieties are critical for inhibitory activity. The pyridyl group in **PI-083** can be replaced with other heterocyclic groups without significant loss of activity. Molecular modeling studies were also performed to explore the detailed interactions of **PI-083** and its derivatives with the β5 and β6 subunits of the 20S proteasome. The refined model showed an H-bond interaction between the Asp-114 and the sulfonamide moiety of the **PI-083** in the β6 subunit.

1. Introduction

Cancer is associated with increased proliferation and/or decreased apoptosis. Both of these processes are regulated by a complex interplay of transcription, protein synthesis, protein–protein interactions, protein phosphorylation, and protein degradation. The 26S proteasome, a large protein complex that consists of the catalytic 20S proteasome[1] (molecular weight 700 kDa, also called 20S core particle [CP]) and 19S regulatory particle (molecular weight 900 kDa), has several proteolytic activities defined by different substrate specificities and is responsible for the degradation of more than 80% of intracellular proteins.[2]

The molecular and functional characteristics of the ubiquitin-proteasome system (UPS) have been studied by several groups[3–5] and it has been shown that the 26S proteasome is responsible for the degradation of proteins involved in a diverse array of biological processes including cell cycle progression, apoptosis, DNA repair, immune response, signal transduction, transcription, metabolism, and developmental processes. Furthermore, the UPS has been reported to play a crucial role in tumorigenesis,[3] inflammation[4] and autoimmunity.[5] Much effort has therefore been dedicated to the discovery of proteasome inhibitors. Indeed, several proteasome inhibitors elicit apoptosis in malignant cells and represent a new class of antineoplastic agents.[6] Subsequently, the proteasome has emerged as a promising molecular target for new cancer therapeutics.[7] In 2003, Bortezomib (Velcade™, PS-341),[8] a covalent but reversible peptidomimetic with a boronic acid moiety (Fig. 1), was approved by the FDA for the treatment of multiple myeloma and mantle cell lymphoma. However, side effects and tumor cell resistance against Bortezomib demand the development of improved and selective proteasome inhibitors.[9] Several new proteasome inhibitors, such as carfilzomib (PR-171, a synthetic peptide),[10] CEP-18770[11] (a boronic acid derivative), and the natural product salinosporamide A (NPI-0052, monochlorinated compound)[12] are in phase I and II clinical trials (Fig. 1). Recent preclinical trials with the irreversible proteasome inhibitor salinosporamide A suggested a significantly stronger and prolonged effect on the chymotrypsin-like (CT-L) and trypsin-like (T-L) activities of the proteasome compared to Bortezomib.[12] Other examples of proteasome inhibitors include natural products[13] that possess reactive functional groups such as aldehydes (tyropeptin A), β-lactones (omuralide), epoxyketones (epoxomicin, eponemycin), cyclic peptides (TMC-95A), macrocyclic vinylketones (syringolin A, glidobactin A), and synthetic peptides (MG-132, PS-519 and TMC-95 analogs). Other natural products, such as (−)-epigallocatechin 3-gallate [(−)-EGCG)], the most abundant catechin (in green tea)

* Corresponding author. Tel.: +1 813 745 6076.
E-mail address: Harshani.Lawrence@moffitt.org (H.R. Lawrence).
† These authors contributed equally.

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.06.038

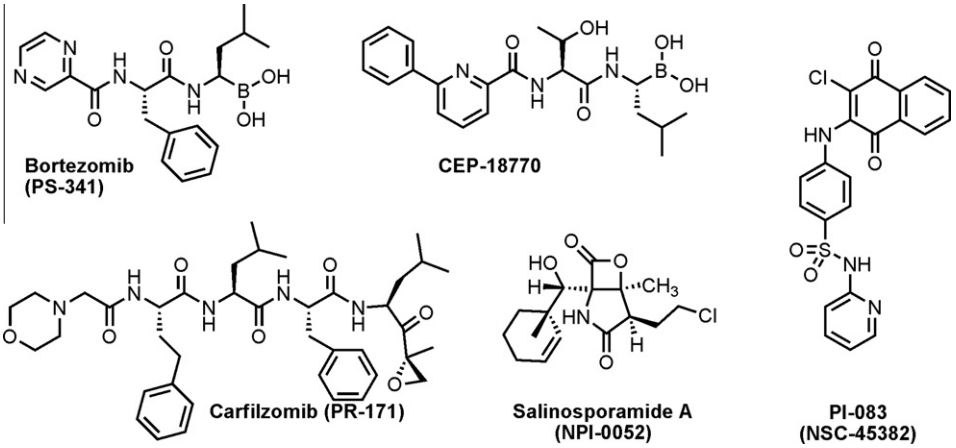


Figure 1. Proteasome inhibitors; Bortezomib (clinically approved covalent and reversible inhibitor). CEP-18770 (reversible inhibitor), carfilzomib and salinosporamide A (irreversible inhibitors) currently in phase I and II clinical trials. **PI-083** (reversible inhibitor identified from the NCI diversity set-1).

and genistein (a soy isoflavone) has been suggested to act as a chemoprotective and anti-cancer agent by inhibiting the CT-L activity of the proteasome in vitro.[14] Small, drug-like synthetic proteasome inhibitors that are selective for cancer over normal cells are rare, but clearly would have a potential advantage over the existing inhibitors listed above.

As part of our many efforts to identify proteasome inhibitors, we screened the NCI diversity set-1 and other NCI libraries against CT-L activity of the 20S of the proteasome and identified **PI-083**[15] (Fig. 1). **PI-083** has a unique skeleton, demonstrates good inhibition of CT-L activity of the proteasome and is selective for malignant over normal cells in vitro and in vivo.[15] Interestingly, similar naphthoquinone scaffolds have been previously studied as potent anti-diabetic agents[16] and inhibitors of protein tyrosine phosphatases such as CD45[17] and CDC25B.[18] In this study we investigated possible approaches to modulate the **PI-083** template through the design of focused compound libraries to gain a better understanding of the structure–activity relationships (SAR) responsible for proteasome inhibitory activity.

2. Chemistry

First, in-house synthesis of **PI-083** (NSC-45382) was carried out using a literature protocol[19] to provide the material with >95% purity (as determined by HPLC) to confirm the inhibitory activity (IC_{50} = 1.0 ± 0.6 μM) and the structure (shown in Fig. 1). The structure of **PI-083** was confirmed using ^1H and ^{13}C NMR and high resolution mass spectrometry. The synthesis of the initial **PI-083** compound library involved modification of the sulfonamide moiety, where functionality is present for rapid analog synthesis. Commercially available 'sulfapyridine-like' building blocks with diverse electronic properties, for example, hydrogen-bond donor/acceptor, charge-transfer, dipolar interactions and steric properties were employed to explore the CT-L inhibitory activities (Fig. 2). Based on molecular docking, the predicted binding interactions of **PI-083** in the β5 and β6 subunits of the proteasome (Fig. 2) suggest favorable interactions with Thr-21, Asp-114, Ala-49, Gly-47 and Thr-1. We were able to introduce diverse chemical and electronic properties to target compound libraries **2**, **3**, **6** and **13** (Schemes 1–3) to exploit these interactions. Further diversity was also introduced via naphthoquinone ring substituents.

The library **2** was synthesized from commercially available aniline sulfonamide building blocks and 2,3-dichloro-1,4-naphthoquinone, 1,4-naphthoquinone or 2-methyl-1,4-naphthoquinone using the protocol employed for **PI-083** (Scheme 1). The synthetic protocol for compounds **2k–2o** (Scheme 1) was validated using commercially available building blocks with 2-methyl-1,4-naphthoquinone and ytterbium trifluoromethanesulfonate in anhydrous dioxane under reflux. The crude reaction mixtures were purified by SiO_2 chromatography to obtain the desired compounds with low to moderate yields. To study the effects of hydrophobic and hydrophilic substitutions at the 3-position of the naphthoquinone ring in **PI-083** (R^4, Scheme 1), a set of analogs was generated via a two-step synthesis. These final compounds with amine groups at the 3-position (**3**, Scheme 1) were prepared in moderate yields with microwave-assisted heating reactions of **PI-083** with appropriate secondary amines.

Intermediates **5a** and **5b** were generated via reductive amination of the commercially available sulfapyridine **4** with requisite aldehydes as shown in Scheme 2. Attempts to react **5a** and **5b** with commercially available 2,3-dichloronaphthoquinone to obtain alkylated amine analogs of **PI-083** were not successful. In an alternative approach, **PI-083** was reacted with either an alkyl bromide or an alkyl iodide in DMF (at room temperature or with microwave heating). Under these conditions, we observed the alkylation of the sulfonamide group of **PI-083** (R^2 in library **6**, Scheme 2). The library **6** was purified using flash chromatography to obtain the desired compounds with greater than 95% purity as assessed by ^1H NMR and LC–MS analysis. Intermediates **7** were synthesized in good yields via coupling commercially available 5- and 6-nitro-2,3-dichloro-1,4-naphthoquinone with the sulfapyridine **4** in refluxing ethanol. Compounds **7a** and **7b** were obtained as mixtures of regio-isomers approximately 1:2 ratios (assessed by ^1H NMR). The 5-nitro-2,3-dichloronaphthoquinone is reported to be more reactive towards amines affording regio-isomeric mixtures of mono-substituted products.[20] Reduced products of **8** (a mixture of regio-isomers approximately 1:5 ratio after purification using SiO_2 chromatography) were obtained from **7a** using the hydrogenation conditions described in the Scheme 2. Attempts to separate the pure isomers of compounds **7** or **8** by chromatography were not successful.

The possible binding interactions of the sulfonamide moiety of **PI-083** with the proteasome were further investigated via the synthetic modifications outlined in Scheme 3. A series of nitrosulfonamide building blocks **11** were generated using standard reagents in good yield by coupling (microwave-assisted heating or room temperature) commercially available sulfonyl chlorides and anilines. The corresponding amine intermediates **12** were obtained in good yields via $NiCl_2/NaBH_4$ mediated reduction.[21] The final library **13**

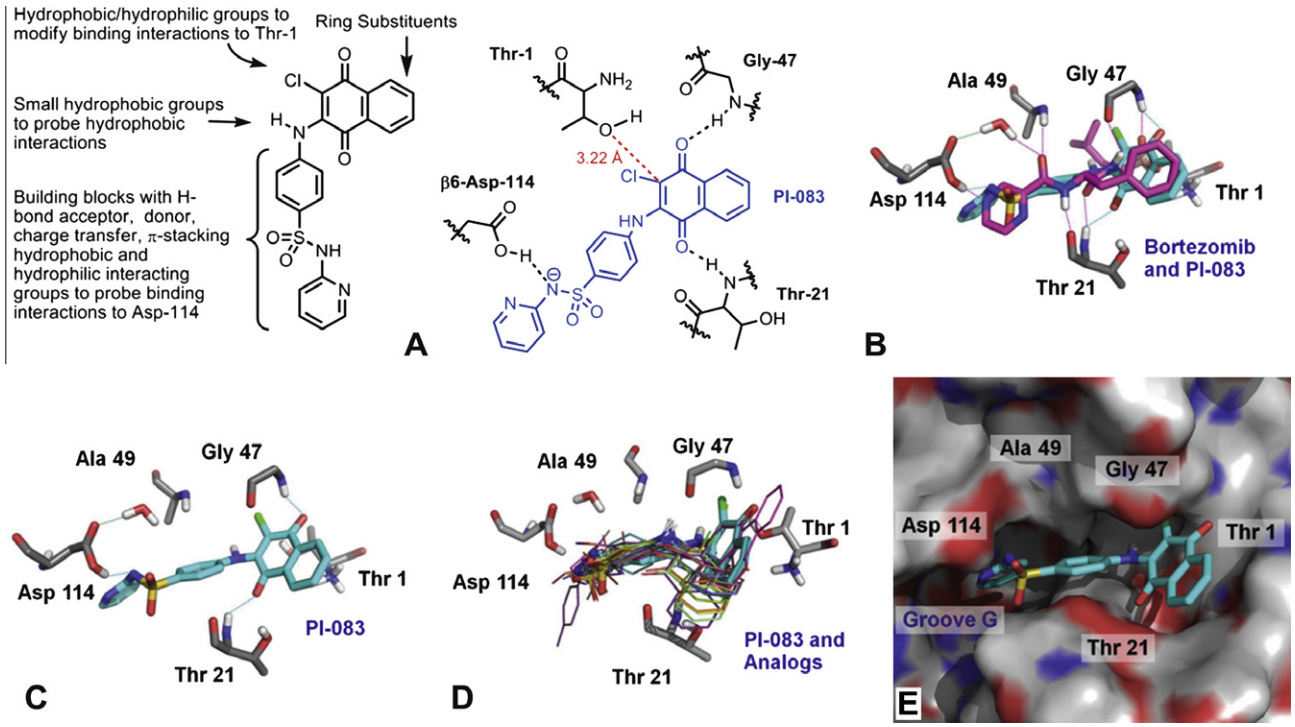


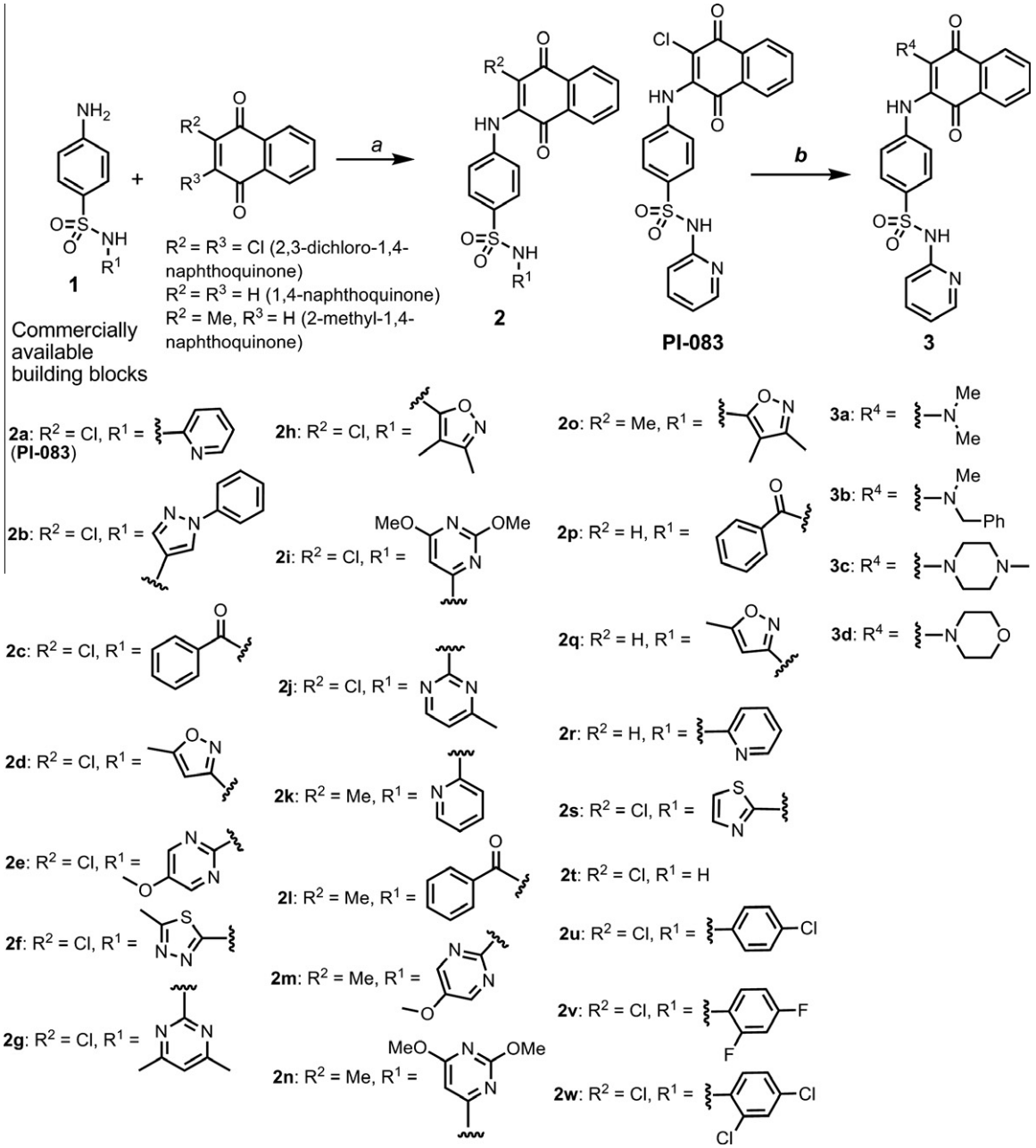
Figure 2. (A) Modifications around **PI-083** for library synthesis and predicted binding interactions of **PI-083** in the β5 and β6 subunits of the 20S proteasome. (B) Overlay of **PI-083** (cyan, docked pose) with the Bortezomib (magenta, X-ray crystal pose) in the β5 and β6 units of the 20S proteasome. (C) **PI-083** overlaid in the β5 and β6 subunits of the 20S proteasome. (D) **PI-083** (cyan, stick representation) and analogs (line representations) shown in Table 1 overlaid in the β5 and β6 subunits of the proteasome. (E) Surface model of the 20S proteasome with **PI-083**. The protein surface of the proteasome is colored according to electrostatic potential; positively charged areas are colored in blue, and negatively charged areas are colored in red. For **PI-083**, carbon atoms are colored in cyan, oxygen in red, nitrogen in blue, hydrogen in white, and sulfur in yellow. Images were created by PyMol.[41]

was prepared as described previously by reacting anilines with 2,3-dichloronaphthoquinone with >95% purity. Starting from 4-nitrobenzenesulfonyl chloride and 5-aminotetrazole, intermediate **11l** was obtained as reported in the literature[22] (Scheme 3). The 4-nitrobenzenesulfonylguanyl azide **11l** intermediate was reduced to *N*-(4-aminobenzenesulfonyl) guanidine with $NaBH_4$ in the presence of $NiCl_6$ to obtain **12l** in good yield. The synthesis and the purity of library **13** were confirmed by ^1H NMR and LC–MS analysis. The inhibitory activities of potent compounds from libraries **2**, **3**, **6** and **13** are summarized in Table 1.

3. Results and discussion

PI-083 was identified from the NCI diversity set-1 as a CT-L proteasome inhibitor and confirmed as a hit compound. In this study, we synthesized several focused libraries around the **PI-083** scaffold; pyridine, sulfonamide and the chloronaphthoquinone moieties to gain a better understanding of the SAR responsible for CT-L proteasome inhibitory activity. To assess the ability of the synthesized compounds to inhibit the CT-L proteolytic activity of purified rabbit 20S proteasome, a fluorometric assay was utilized.[23] Commercially available pyridine sulfonamide (sulfapyridine), 2,3-dichloronaphthoquinone, 2-methyl-1,4-naphthoquinone and 1,4-naphthoquinone building blocks themselves showed no proteosome CT-L activity (IC_{50} >100 μM).

Starting from the pyridine end of the **PI-083** scaffold, we have demonstrated replacing pyridine with hydrogen (**2t**, Scheme 2) or amines bearing small hydrophobic units such as methyl, ethyl, and isopropyl (**13m–13r**, Scheme 3) resulted in loss of inhibitory activity (IC_{50} >100 μM). Replacing the pyridine with hydrophobic electron-withdrawing groups such as chloro- or fluorophenyl units (**2u**, **2v** and **2w**, Scheme 2) also resulted in loss of inhibitory activity (IC_{50} >100 μM). Compounds **13m–13r** and **2u–2w** suggest non-aromatic hydrophobic groups and electron-withdrawing aromatic hydrophobic groups are not tolerated in this region. Our docking suggests Asp-114 is able to H-bond with **PI-083** in the β6 subunit (see Section 4). The Asp-114 interactions are also observed crystallographically for Bortezomib (Fig. 2B, PDB$^+$ ID: 2F16).[24] Previously, we reported[15] the pyridine in **PI-083** interacting with Asp-114 in the β6 subunit via a water molecule might be responsible for CT-L activity. In the focused library synthesis, the rationale for replacement of the pyridine with basic units was to probe interactions with Asp-114 in the β6 subunit (Fig. 2). However, since our original report, the modeling software we employ has become more sophisticated allowing examination of different tautomers and ionization states as well as improved calculation of partial charges using the applications Ligprep,[25] Epik[26] and QM Polarized Ligand Docking[27] (QPLD), respectively. Previously these applications were not available to us. This enabled modeling of a form of **PI-083** with an anionic sulfonamide nitrogen. These improvements led to refinement of our previous model by taking better account of the pK_a for the deprotonation of the sulfonamide nitrogen of **PI-083** which was calculated by Epik to be 6.7. As a result, the construct used for modeling (see Section 4) suggests a hydrogen bond between protonated Asp-114 in the β6 subunit and the negatively charged sulfonamide moiety of the **PI-083** scaffold (Fig. 2A). The resonance stabilization of the sulfonamide anion by the adjacent pyridine of the **PI-083** in its bioactive conformation could be leading to inhibition of the CT-L proteasome activity (resonance stabilization should increase the acidity of the sulfonamide group). This evolution of the model provided insights into the SAR of several **PI-083** derivatives. It is possible that compounds possessing heterocyclic moieties shown in Table 1 such as thiazole (**2s**), oxazole (**2d** and **2h**), thiadiazole (**2f**) and pyrazole (**2b**) are able to stabilize

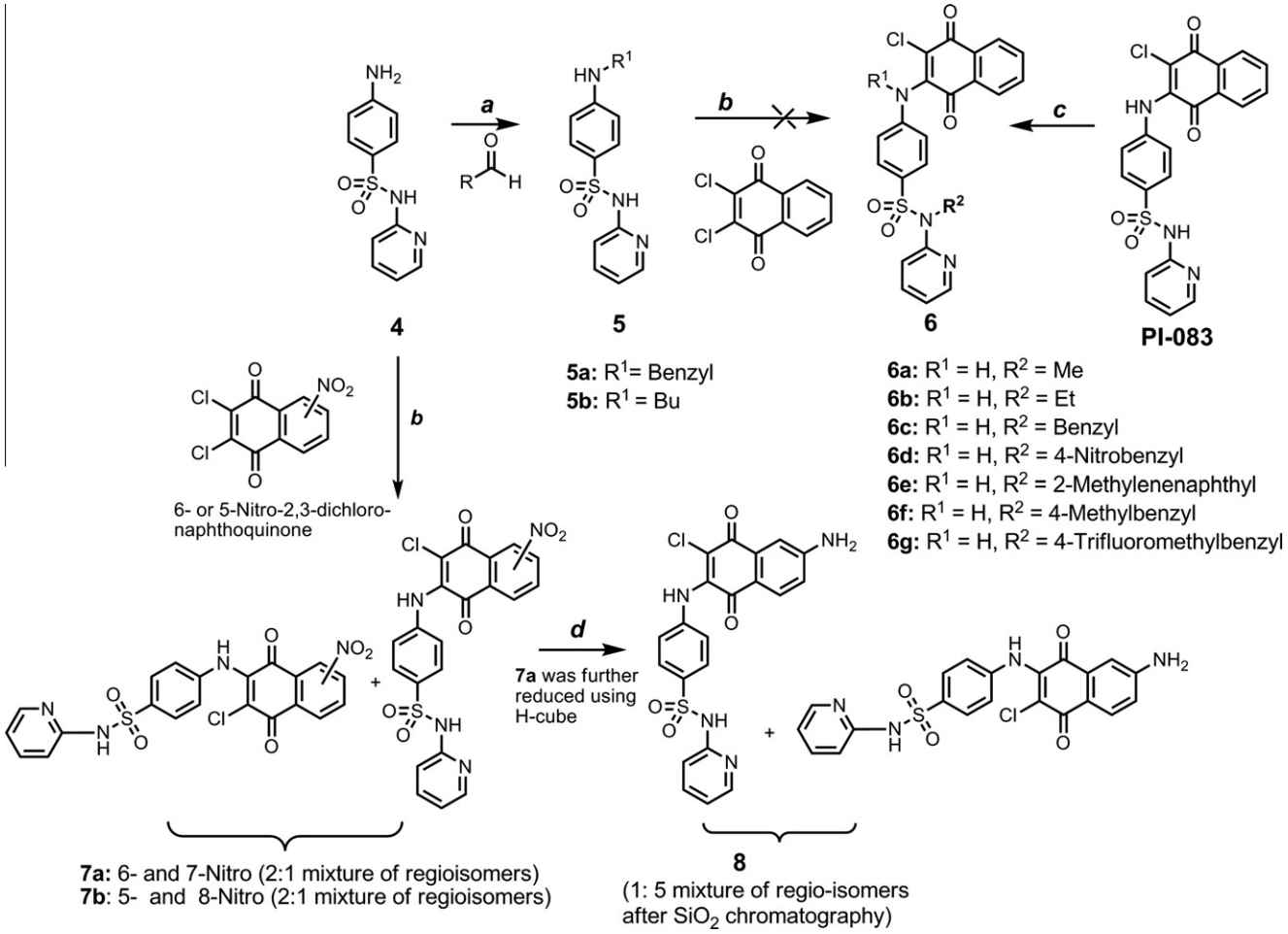


Scheme 1. Synthesis of focused libraries of **PI-083** using commercially available building blocks. Reagents and conditions: (a) (i) when R^2, R^3 = Cl, 95% EtOH, reflux at 115 °C, 3 days; (ii) when R^2 = Me, R^3 = H, anhydrous dioxane, 10% ytterbium triflate, 125 °C, 3 days; (iii) when R^2, R^3 = H, 90% EtOH, reflux, 3 days; (b) appropriate amine, EtOH, microwave, 140 °C, 20 min.

the negative charge on the sulfonamide moiety is contributing to CT-L inhibitory activity (IC_{50} = 3–16 μM). Compounds with pyrimidine moieties (**2e**, **2i**) that contain H-bond acceptor residues showed weak activity (IC_{50} = 100 μM). However, compound **2g**; with methyl pyrimidine showed improved activity (IC_{50} = 19 μM). Compounds with phenyl groups with small hydrophobic residues such as **13d**, **13e**, **13h** and **13j** showed moderate activity (IC_{50} = 13–20 μM) suggesting these compounds might be undergoing non-specific hydrophobic interactions in this area. The region marked as 'Groove G' (Fig. 2E) is a hydrophobic pocket in the β6 subunit, and it is possible compounds **13d**–**13j** are able to partially occupy this region in their binding conformation. The overall binding affinity was not improved when the pyridyl group in **PI-083** was replaced with aromatic hydrophobic groups (compounds **13a**–**13k**, Scheme 3, IC_{50} >100 μM).

Next, we investigated the role of the sulfonamide moiety by replacing the hydrogen with hydrophobic groups. Library **6** (Scheme 2), derived from alkylation of the sulfonamide moiety with methyl (**6a**), ethyl (**6b**), substituted benzyl (**6c**, **6d**, **6e** and **6f**) and naphthyl (**6g**) derivatives lacked inhibitory activities (IC_{50} >100 μM). This observation suggests the H-bond interaction of the sulfonamide hydrogen of the **PI-083** with the β6 Asp-114 subunit of the proteasome is crucial to retain the CT-L inhibitory activity of **PI-083** and further validates the docking results from the refined structural model (Fig. 2).

Finally we assessed the contribution of the chloronaphthoquinone moiety of **PI-083** to its CT-L inhibitory activity. Our dockings resulted in a pose for **PI-083** in which the napthoquinone carbonyl groups hydrogen bond with Gly-47 and Thr-21 creating a hydrogen bond network similar to Bortezomib. Bortezomib forms dual

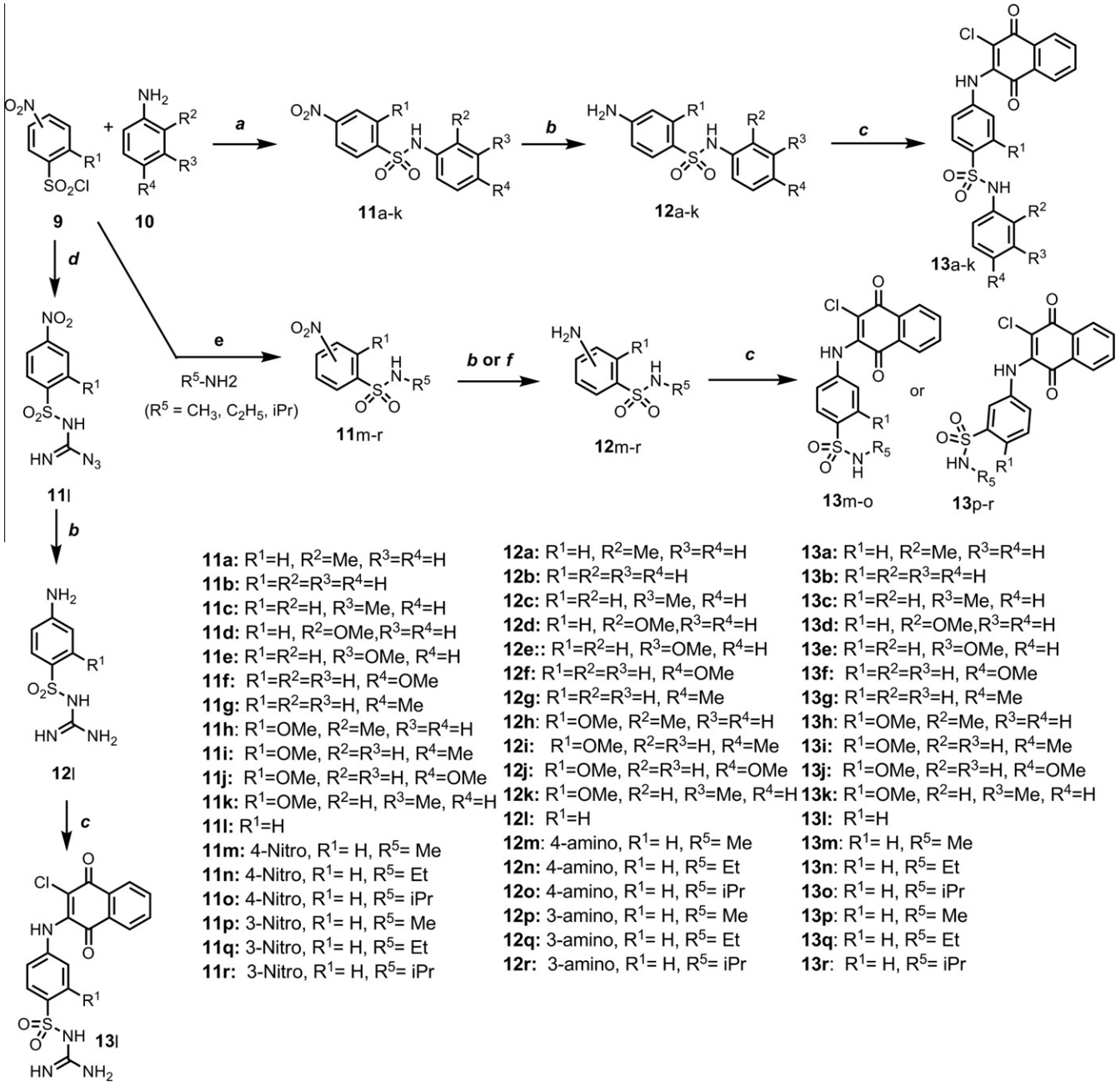


Scheme 2. Reagents and conditions: (a) $NaBH_3CN$, AcOH, MeOH, 0 °C to rt, 1–2 h; (b) 95% EtOH, sealed tube, reflux, 115 °C, 3 days; (c) (i) RBr (or RI), DIPEA, DMF, microwave 160 °C, 15 min or (ii) RI, DIPEA, DMF, Ar, rt, 4–24 h; (d) DMF/MeOH (4:1, 0.2 g in 30 mL), H-cube H_2, 10% Pd/C, 40 bar, rt, flow rate = 1.0 mL/min.

hydrogen bonds with both Gly-47 and Thr-21 where hydrogen bonds are formed with backbone nitrogens and carbonyl groups (Fig. 2B). We found the chlorine at the 2-position of the naphthoquinone moiety is essential for activity. Replacement of the chloride in **PI-083** with methyl (**2k**, **2l**, **2m**, **2n**, **2o**) or hydrogen (**2p**, **2q**, **2r**) is detrimental to in vitro CT-L inhibitory activity (IC_{50} >100). It is conceivable that **PI-083** undergoes Michael type nucleophilic attack with Thr-1 in the β5 subunit and inhibits proteasome via a covalent modification. This is consistent with the docking results (Fig. 2) that place the chlorinated electrophilic carbon of **PI-083** 3.22 Å away from the Thr-1 hydroxyl group in the β5 subunit (Fig. 2A). Attempts to generate hydronaphthoquinone of **PI-083** via reduction of the naphthoquinone moiety were not successful. The hydronaphthoquinone oxidizes back to naphthoquinone instantaneously (experimental details not reported here). The nitro and amino derivatives of **PI-083** (**7** and **8**, respectively, mixtures of isomers shown in Scheme 2) did not lead to any appreciable inhibitory activities in the in vitro enzymatic assay, suggesting that the nitro and amino groups on 5- and 6-position of the chloronaphthoquinone are not tolerable.

Additionally, we determined the ability of **PI-083** and its analogs to inhibit T-L and peptidyl glutamyl peptide hydrolase (PGPH) activities in vitro[15] (Table 1). Bortezomib was used as a control and none of the compounds shown in Table 1 demonstrated higher potency towards T-L or PGPH than the CT-L activity. Our data also showed (not reported here) that **PI-083** does not inhibit (IC_{50} >30 μM) purified Calpain, (calcium-dependent non-lysosomal cysteine protease), but inhibited all three proteasomal (CT-L, T-L and PGPH) activities with similar potency.[15] As reported previously, we have shown that **PI-083** inhibits proteasome activity in vitro and in vivo.[15] Treatment of MCF-7 cells with **PI-083** resulted in inhibition of the CT-L activity of the proteasome with an IC_{50} value of 6 μM and **PI-083** also inhibited cell viability with an IC_{50} value of 2.31 ± 0.10 μM.[15] We found **PI-083** inhibits proliferation and induces cell death in three different human tumor cell lines (breast, pancreatic and ovarian), but not in their normal/immortalized counterparts.[15] Our studies indicated that **PI-083** induces apoptosis in cancer cell lines derived from prostate, lung and multiple myeloma, in addition to the human tumor cells lines mentioned above.[15] Furthermore, **PI-083** suppresses the growth of human breast and lung tumors implanted as xenografts into nude mice, and is efficient in inhibiting proliferation and survival of primary cells derived from patients with multiple myeloma.[15]

To investigate whether **PI-083**-mediated proteasome inhibition is reversible, we performed a dialysis[28] experiment with **PI-083** and Bortezomib, a covalent reversible proteasome inhibitor that was used as an internal control. Figure 3 shows that in the absence of dialysis, **PI-083** and Bortezomib were able to inhibit the CT-L activity of the 20S proteasome by 88% and 99%, respectively. During dialysis, the CT-L activity started to recover at the 1 h mark in the **PI-083** treated sample. By contrast, in the Bortezomib treated samples, CT-L activity recovery did not begin until 4 h. These results suggest that both **PI-083** and Bortezomib behave similarly, but that **PI-083** appears to be more rapidly released and/or is



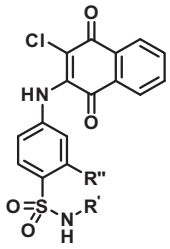
Scheme 3. Reagents and conditions: (a) pyridine, DCE, microwave 150 °C, 10 min; (b) $NiCl_2{\cdot}6H_2O$, MeOH/THF 1:1, $NaBH_4$, 0 °C, 15–30 min; (c) 2,3-dichloronaphthoquinone, 95% EtOH, sealed tube, reflux, 115 °C, 3 days; (d) 5-aminotetrazole, Na_2CO_3, H_2O, rt, 24 h; (e) THF, 0 °C, 30 min; (f) MeOH (0.3 g in 10 mL), H-cube, H_2, 40 bar, 10% Pd/C, 30 °C, flow rate = 1 mL/min.

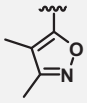
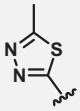
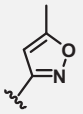
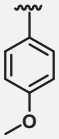
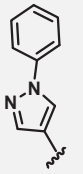
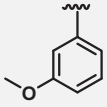
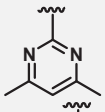
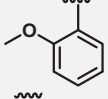
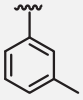
slower to attach. It is likely that **PI-083** behaves as a covalent reversible CT-L inhibitor.

4. Molecular modeling

GLIDE 5.0[29] was employed for docking of the ligands described herein into a structure of the β5,β6 subunits of the 20S yeast proteasome with Bortezomib bound obtained from the Protein Data Bank[30] (PDB ID: 2F16) and appropriately prepared for docking calculations (hydrogen atoms added, appropriate histidine tautomer and protonation state, restrained energy minimization, etc.). To obtain a reasonable sampling of poses, 100 top ranking poses were kept for each structure in the ligand set, which had been docked using GLIDE in standard precision (SP) mode. Poses with the smallest distance between Thr-1 oxygen and the chloro-carbon of the naphthoquinone moiety were chosen for subsequent docking with GLIDE Extra Precision[31] (XP), which allows for more precise calculations of binding energy, poses, hydrophobic interactions, and expulsion of water from pockets. GLIDE XP was employed with QPLD (QM Polarized Ligand Docking) for calculation of partial charges 'on the fly' using the B3LYP density functional method in order to adequately account for charge delocalization of the sulfonamide moiety. Poses with lowest energy that resulted in Thr-1 oxygen and chloro-carbon in naphthoquinone distances less than 5 Å were considered. All structures were viewed, created, and modified with Schrödinger's Maestro 8.5.[32] **PI-083** and its analogs were processed using LigPrep 2.2 and tautomers and structures with ionization states appropriate for a pH range of 5.0–9.0 were generated.

Table 1
PI-083 derivatives with moderate activities



Compound	R′	R″	In vitro IC_{50} (μM)[a]		
			CT-L activity	T-L activity	PGPH activity
Bortezomib (Fig. 1)	—	—	0.009 ± 0.006	7.0 ± 0.24	0.48 ± 0.021
PI-083		H	1.0 ± 0.63	4.5 ± 1.4	4.5 ± 1.2
2s		H	3.3 ± 0.30	8.15 ± 0.84	14.3 ± 1.60
2h		H	3.9 ± 0.50	9.25 ± 0.45	21.2 ± 0.79
2f		H	6.4 ± 0.5	10.7 ± 0.5	13.4 ± 1.1
2d		H	11.2 ± 3.4	13.2 ± 0.2	16.7 ± 0.5
13h		OMe	13.7 ± 0.35	24.3 ± 0.04	30 ± 1.5
13j		OMe	15.3 ± 0.89	23.3 ± 0.94	35 ± 1.90
2b		H	16.3 ± 6.5	20.65 ± 1.45	25.6 ± 1.39
13e		H	17 ± 1	24.55 ± 0.55	43.5 ± 2.5
2g		H	19 ± 0.39	20.9 ± 0.9	50.25 ± 2.25
13d		H	20.8 ± 0.25	24.65 ± 1.0	49.25 ± 1.75
13c		H	20.8 ± 0.3	55 ± 2	>100

[a] Tested in triplicate at least twice.

The pK_a values for the sulfonamide nitrogen of the compounds **2b**–**2s** shown in Table 1 were less than 7.91 (data not shown) as calculated by Epik. Thus, at physiological pH substantial fraction of sulfonamides **2b–2s** will exist in the anionic deprotonated from.

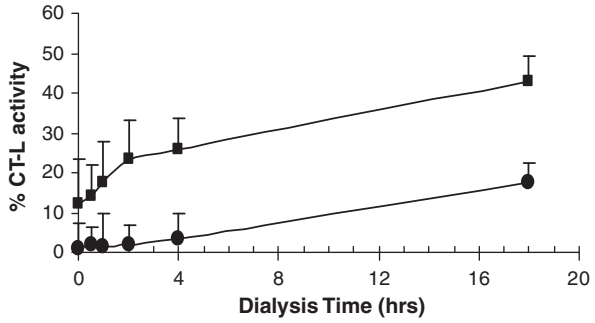


Figure 3. Recovery of CT-L activity upon dialysis of the 20S proteasome-compound complexes after pre-incubation with **PI-083** (■) and Bortezomib (●).

Interestingly, the pK_a calculated by Epik for the sulfonamide substituent in **2t** is 9.5, which means that the compound would predominately exist in the protonated (conjugate acid) form at pH 7.6 that may explain its lack of activity. When **2t** in its protonated form was docked to the B5/B6 subunits of the proteasome, no poses were observed that met our distance criterion of a 5 Å separation between the oxygen atom of the Thr-1 hydroxyl group and the carbon atom to which the chlorine is attached in the naphthoquinone ring.

A low energy pose of **PI-083** is depicted in Figure 2B with interactions modified from the ones we previously reported.[15] Former docking studies were performed with an earlier version of the GLIDE docking software[33] and prior to the availability of LigPrep in our lab. LigPrep 2.2 along with Epik was used to generate tautomers and alternative protonation states for **PI-083**, which includes the anionic form of the sulfonamide nitrogen. Consequently, a low energy pose was generated where the anionic sulfonamide nitrogen formed a hydrogen bond (N–O distance: 2.8 Å) with protonated Asp-114. (Note: the X-ray structure of the proteasome–Bortezomib complex is consistent with protonated Asp-114, and it is reasonable to assume that it may well be protonated in the proteasome–**PI-083** complex.) In the previous model, the pyridyl nitrogen[15] of **PI-083** is 3.3 Å from one of the oxygens of Asp-114 suggesting an electrostatic interaction. The interactions of the refined model slightly shift the pose of **PI-083** allowing for a better angle of nucleophilic attack by Thr-1 on the chloronaphthoquinone group. Furthermore, **PI-083** does not interact with the nearby water in the refined model but does form hydrogen bonds between the carbonyl groups of the naphthoquinone and Thr-21 and Gly-47. In order to determine whether the water molecule near Asp-114 (crystallographically determined for the proteasome–Bortezomib complex) was in an energetically favorable position in our docked proteasome–**PI-083** model, MacroModel[34] was used to sample possible alternative configurations of this water molecule. With the β5 and β6 coordinates held frozen, the water molecule close to Asp-114 and a crystallographically determined water molecule hydrogen bonded to the first one were allowed to freely rotate and translate during a Monte Carlo simulation performed on our docked model of **PI-083** bound to the β5, β6 subunits of the 20S proteasome. Out of 100 low energy configurations generated, 76 retained a water molecule in the location closest to Asp-114 observed crystallographically. The XP pose of **PI-083** places the electrophilic carbon, to which chlorine is attached, 3.22 Å away from the oxygen of Thr-1 with reasonable positioning for nucleophilic attack (Fig. 2A). Low energy XP poses of the active analogs of **PI-083** (Table 1) are observed to have similar binding modes to **PI-083** (Fig. 2D).

As previously stated, our dialysis experiments suggest that **PI-083** behaves as a covalent but reversible proteasome inhibitor. Our docking results suggest that all of the active compounds, which contain a chloro substituent at the 2-position of the naphthoquinone ring, can adopt a low energy docking pose, that is, poised for covalent bond formation with Thr-1. All of the active molecules may, indeed, form a covalent bond with Thr-1 but we have not yet shown this to be the case experimentally. Our modeling studies suggest that it is possible for all of the active compounds to be involved in formation of a pre-organized complex that subsequently leads to covalent bond formation. We have also performed covalent docking of **PI-083** to the β5, β6 subunits of the 20S proteasome using GOLD 4.1 (not shown here). Three poses were obtained that are all qualitatively similar to the pose presented in Figure 2B; however **PI-083** has been translated by ~1.5 Å and rotated slightly due to presence of the covalent bond between carbon-2 of the naphthoquinone ring and the hydroxyl-oxygen atom of Thr-1. The hydrogen bond between Asp-114 and the sulfonamide nitrogen atom is no longer present but the pyridine ring is still located in Groove G (Fig. 2E) in the S3 pocket.

5. Conclusions

In summary, novel naphthoquinone derivatives of **PI-083** were prepared via several routes. The SAR indicates that the inhibitory activity appears very sensitive to changes around the molecule. The chlorine and sulfonamide groups of **PI-083** appear to be essential for activity. The pyridyl group can be replaced with heterocyclic moieties without significant reduction of activity in in vitro. The replacement of the pyridyl unit with aromatic groups (with hydrophobic and hydrophilic characteristics, **13a–13k**) or small hydrophobic units (**13m–13r**) were not tolerable. **PI-083** has been shown to be more selective in inhibiting proliferation, inducing cell death and apoptosis for breast, ovarian and pancreatic cancer cells over their normal counterparts.[15] In nude mice, **PI-083** was efficient in inhibiting the growth of human tumor xenografts derived from breast and lung cancer cells.[15] Altogether our data suggest **PI-083** has potential for further development as an anti-cancer agent.

6. Experimental

6.1. General

All reagents were purchased from commercial suppliers and used without further purification. Melting points were determined using a Barnstead international melting point apparatus and remain uncorrected. ^{1}H NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer with acetone-d_6, $CDCl_3$ or DMSO-d_6 as the solvent. ^{13}C NMR spectra are recorded at 100 MHz. All coupling constants are measured in hertz (Hz) and the chemical shifts (δ_H and δ_C) are quoted in parts per million (ppm) relative to TMS (δ 0), which was used as the internal standard. Liquid chromatography mass spectroscopy (LC–MS) and High resolution mass spectroscopy (HRMS) were carried out on an Agilent 6210 LC–MS (ESI-TOF). For LC–MS and HRMS the compounds were eluted between 2 and 5 min using Rapid Resolution Cartridge (2.1 × 30 mm, particle size 3.5 μm) from Agilent Technologies. LC–MS was used to detect ions of mass 100–1000 Da, and single peak was observed in the chromatogram after purification. Low resolution mass spectroscopy (LRMS) was carried out using Agilent single quad G1956A. HPLC was carried out using Jasco UV-2075 plus UV–vis detector (column: ultra C18, 5 μm, 150 mm × 4.6 mm). H-Cube® (ThalesNano) continuous-flow hydrogenation reactor was used for hydrogenation reactions. Microwave reactions were performed in CEM Discover 908005 model and Biotage initiator 8 machines. Thin layer chromatography was performed using Silica Gel 60 F254 plates (Fisher), with observation under UV when necessary. Anhydrous solvents were used as purchased: dichloromethane (anhydrous, 99.8% contains 50–150 ppm hydrocarbon as stabilizer from Aldrich),

dimethyl formamide (anhydrous, 99.9% from Aldrich), tetrahydrofuran (anhydrous, 99.9%, inhibitor free, Aldrich), acetonitrile (anhydrous, 99.8%, Aldrich), toluene (anhydrous, 99.8%, Aldrich), methanol (anhydrous, 99.8%, Aldrich), ethanol (absolute, 99.5%, Aldrich).

6.2. General procedures for synthesis of library 2 and library 3 Scheme 1

6.2.1. Synthesis of compounds 2a–2j and 2s–2w

The starting material 2,3-dichloronaphthoquinone (700 mg, 3.08 mmol) and appropriate commercially available sulfonamide anilines (0.5 M equiv) were suspended in 95% ethanol (15 mL) and heated at 115 °C for 3 days to obtain mixtures of red/orange precipitates. The reaction mixtures were cooled to room temperature and the resultant precipitates were filtered and washed with ethanol (five times, total volume approximately 15–20 mL). The crude products obtained were rinsed with EtOAc (5 mL), DCM (5–10 mL), MeOH (5–10 mL) to remove remaining starting materials and quick acetone in DCM (50:50 mix, 5 mL) rinse was able to remove the impurities when ethanol wash was not sufficient to remove the impurities. The required pure compounds (95% by ^{1}H NMR) in the library **2** were isolated as red or orange solids between 5% and 98% yields.

6.2.1.1. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(pyridin-2-yl)benzenesulfonamide PI-083 (2a). Orange solid (940 mg, 53%); mp: 265–267 °C (lit. 262 °C decomposed).[19] ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H disappeared on D_2O shake), 8.03–8.00 (m, 3H), 7.85 (dt, J = 7.6, 1.6 Hz, 1H), 7.80 (dt, J = 7.6, 1.2 Hz, 1H), 7.74 (d, J = 8.4 Hz, 2H), 7.69 (apparent dt J = 7.6, 1.6 Hz, 1H), 7.15 (d, J = 8.0 Hz, partially overlapped, 2H), 7.12 (m, partially overlapped, 1H), 6.86 (br t, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.5, 177.6, 143.3, 135.4, 134.1, 132.3, 131.1, 127.7, 127.4, 127.2, 126.8, 122.8, 121.9, 118.9; LC–MS (ES+) 440 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{21}H_{15}ClN_3O_4S$ $(M+H)^+$ 440.0466, found 440.0465; HPLC 99% (R_t = 1.80, 10% water in acetonitrile).

6.2.1.2. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(1-phenyl-1H-pyrazol-4-yl)benzenesulfonamide (2b). Orange solid (198 mg, 24%); mp: 230–231 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H disappeared on D_2O shake), 9.60 (s, 1H disappeared on D_2O shake), 8.05 (d, J = 6.8 Hz, 2H), 7.88 (dt, J = 7.2, 1.2 Hz, 1H), 7.84 (dt, J = 7.2, 1.6 Hz, 1H), 7.58–7.56 (m, 3H), 7.48–7.41 (m, 4H), 7.38–7.36 (m, 1H), 7.19 (d, J = 8.8 Hz, 2H), 5.79 (d, J = 2.0 Hz, 1H), LRMS (ES+) 505 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{25}H_{18}ClN_4O_4S$ $(M+H)^+$ 505.0731, found 505.0735.

6.2.1.3. *N*-(4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)phenylsulfonyl)benzamide (2c). Red solid (58 mg, 8%); mp: 216–217 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.44 (br s, 1H disappeared on D_2O shake), 9.60 (s, 1H disappeared on D_2O shake), 8.05–8.03 (m, 2H), 7.88–7.80 (m, 6H), 7.61 (t, J = 7.2 Hz, 1H), 7.47 (t, J = 8.0 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H); LRMS (ES+) 467 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{23}H_{16}ClN_2O_5S$ $(M+H)^+$ 467.0463, found 467.0468.

6.2.1.4. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (2d). Dark orange solid (97 mg, 14%); mp: 215–217 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 9.55 (s, 1H), 8.05–8.02 (m, 2H), 7.88–7.80 (m, 2H), 7.71 (d, J = 8.8 Hz, 2H), 7.20 (d, J = 8.8 Hz, 2H), 6.13 (s, 1H), 2.28 (s, 3H); LRMS (ES+) 444 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{20}H_{15}ClN_3O_5S$ $(M+H)^+$ 444.0415, found 444.0418.

6.2.1.5. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(5-methoxypyrimidin-2-yl)benzenesulfonamide (2e). Red solid (332 mg, 46%); mp: 269–271 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (s, 1H), 9.52 (s, 1H), 8.28 (s, 2H), 8.03 (br t, J = 6.0 Hz, 2H), 7.89–7.81 (m, 4H), 7.18 (d, J = 8.4 Hz, 2H), 3.77 (s, 3H); LRMS (ES+) 471 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{21}H_{16}ClN_4O_5S$ $(M+H)^+$ 471.0524, found 471.0526.

6.2.1.6. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (2f). Red solid (696 mg, 98%); mp: 220 °C decomposed; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.92 (s, 1H disappeared on D_2O shake), 9.53 (s, 1H disappeared on D_2O shake), 8.03 (br d, J = 8.0 Hz, 2H), 7.84 (dt, J = 7.6, 0.8 Hz, 1H), 7.83 (dt, J = 6.4, 1.2 Hz, 1H), 7.66 (d, J = 8.8 Hz, 2H), 7.18 (d, J = 8.8 Hz, 2H), 4.35 (br s, 1H), 2.45 (s, 3H); LRMS (ES+) 461 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{19}H_{14}ClN_4O_4S_2$ $(M+H)^+$ 461.0139, found 461.0131.

6.2.1.7. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide (2g). Reddish-brown solid (326 mg, 45%); mp: 210 °C decomposed; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.04 (m, 2H), 7.89–7.80 (m, 4H), 7.71 (d, J = 12.0 Hz, 1H), 7.29 (s, 1H), 7.21 (d, J = 8.0 Hz, 2H), 2.31 (s, 6H). (Note: approximately 10% base line impurities present between 8.10 and 7.80 ppm.); LRMS (ES+) 469 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{22}H_{18}ClN_4O_4S$ $(M+H)^+$ 469.0731, found 469.0734.

6.2.1.8. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(3,4-dimethylisoxazol-yl)-benzenesulfonamide (2h). Orange solid (133 mg, 19%); mp: 208–210 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (br s, 1H), 9.61 (s, 1H), 8.04 (d, J = 7.6 Hz, 2H), 7.91–7.81 (m, 2H), 7.62 (d, J = 8.8 Hz, 2H), 7.21 (d, J = 8.8 Hz, 2H), 2.07 (s, 3H), 1.60 (s, 3H); LRMS (ES+) 458 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{21}H_{17}ClN_3O_5S$ $(M+H)^+$ 458.0572, found 458.0578.

6.2.1.9. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(2,6-dimethoxypyrimidin-4-yl)-benzenesulfonamide (2i). Orange solid (299 mg, 65%); mp: 220–222 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.48 (br s, 1H), 9.56 (s, 1H), 8.03 (dt, J = 8.0, 1.6 Hz, 2H), 7.91–7.80 (m, 4H), 7.21 (d, J = 8.8 Hz, 2H), 5.93 (s, 1H), 3.78 (s, 3H), 3.74 (s, 3H); LRMS (ES+) 501 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{22}H_{18}ClN_4O_6S$ $(M+H)^+$ 501.0630, found 501.0634.

6.2.1.10. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(4-methylpyrimidin-2-yl)-benzenesulfonamide (2j). Dark red solid (446 mg, 65%); mp: 246–248 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.31 (d, J = 4.4 Hz, 1H), 8.04–8.02 (m, 2H), 7.87–7.81 (m, 4H), 7.18 (d, J = 8.0 Hz, 2H), 6.90 (s, 1H), 2.30 (s, 3H); LRMS (ES+) 455 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{21}H_{16}ClN_4O_4S$ $(M+H)^+$ 455.0575, found 455.0581.

6.2.1.11. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(thiazol-2-yl)benzene-sulfonamide (2s). Orange red solid (145 mg, 65%); mp: 286–288 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (br s, 1H), 9.51 (s, 1H), 8.04–8.02 (m, 2H), 7.86 (dt, J = 7.4, 1.5 Hz, 1H), 7.81 (dt, J = 7.4, 1.5 Hz, 1H), 7.67 (d, J = 8.7 Hz, 2H), 7.24 (d, J = 4.6 Hz, 1H), 7.17 (d, J = 8.7 Hz, 2H), 6.76 (d, J = 4.6 Hz, 1H); LC–MS (ES+) 446 $(M^{35}Cl+H)^+$, 448 $(M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $C_{19}H_{13}ClN_3O_4S_2$ $(M+H)^+$ 446.0030, found 446.0045.

6.2.1.12. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)benzenesulfonamide (2t). Red solid (24 mg, 38.9%); mp: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (br s, 1H), 8.06 (d, 7.4 Hz, 1H), 7.87–7.80 (m, 2H), 7.72 (d, J = 8.5 Hz, 2H), 7.30 (br s,

2H), 7.23 (d, J = 8.5 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.7, 177.7, 143.5, 142.9, 139.2, 135.5, 134.2, 132.4, 131.2, 127.3, 126.9, 126.4, 123.0, 118.6; LC–MS (ES+) 363 ($M^{35}Cl+H)^+$, 365 ($M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $C_{16}H_{12}ClN_2O_4S$ $(M+H)^+$ 363.0201, found 363.0192.

6.2.1.13. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(4-chlorophenyl)benzenesulfonamide (2u). Yellow solid (180 mg, 76%); mp: 272 °C decomposed; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, NH, 1H, disappeared in D_2O shake), 9.51 (s, NH, 1H disappeared in D_2O shake), 8.02 (ddd, J = 7.0, 3.2, 1.3 Hz, 2H), 7.83 (dtd, J = 19.8, 7.4,1.3 Hz, 2H), 7.60 (d, J = 8.8 Hz, 2H), 7.27 (d, J = 8.8 Hz, 2H), 7.14 (d, J = 8.8 Hz, 2H), 7.07 (d, J = 8.8 Hz, 2H); LC–MS (ES−) 471 $(M-H)^-$; HRMS (ES−) m/z calculated for $C_{22}H_{14}Cl_2N_2O_4S$ $(M-H)^-$ 470.9979, found 470.9991.

6.2.1.14. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(2,4-difluorophenyl)benzenesulfonamide (2v). Gold yellow solid (146 mg, 62%); mp: 250–252 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, NH,1H, disappeared in D_2O shake), 9.55 (s, NH, 1H, disappeared in D_2O shake), 8.03 (dd, J = 7.6, 1.4 Hz, 2H), 7.86 (dt, J = 8.0, 1.2 Hz, 1H), 7.81 (dt, J = 7.6, 1.2 Hz, 1H), 7.53 (d, J = 8.7 Hz, 2H), 7.24–7.20 (m, 2H), 7.15 (d, J = 8.7 Hz, 2H), 7.04–6.95 (m, 1H); LC–MS (ES−) 473 ($M^{35}Cl-H)^-$, 475 ($M^{37}Cl-H)^-$; HRMS (ES−) m/z calculated for $C_{22}H_{13}ClF_2N_2O_4S$ $(M-H)^-$ 473.0180, found 473.0197.

6.2.1.15. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(2,4-dichlorophenyl)benzenesulfonamide (2w). Orange solid (180 mg, 70%); mp: 258 °C decomposed; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.00 (s, NH, 1H, disappeared in D_2O shake), 9.56 (s, NH, 1H, disappeared in D_2O shake), 8.03 (dd, J = 7.3, 1.6 Hz, 2H), 7.87 (dt, J = 7.2, 1.6 Hz, 1H), 7.81 (dt, J = 7.2, 1.6 Hz, 1H), 7.56–7.54 (m, 3H), 7.38 (dd, J = 8.7, 2.4 Hz, 2H), 7.27 (d, J = 8.7 Hz, 2H), 7.16 (d, J = 8.7 Hz, 2H); LC–MS (ES−) 505 ($M^{35}Cl-H)^-$, 507 ($M^{37}Cl-H)^-$, HRMS (ES−) m/z calculated for $C_{22}H_{13}Cl_3N_2O_4S$ $(M-H)^-$ 504.9589, found 504.9600.

6.2.2. General procedure for synthesis of compounds 2k–2o

2-Methyl-1,4-naphthoquinone (100 mg, 0.58 mmol) and ytterbium trifluoromethanesulfonate (15 mg, 0.024 mmol) were added to anhydrous dioxane (5.0 mL) followed by sulfapyridine (144 mg, 0.58 mmol). The reaction mixture was heated under reflux for 24 h and the TLC analysis indicated depletion of starting material. The crude reaction mixtures were purified by SiO_2 chromatography to obtain the desired compounds.

6.2.2.1. 4-(3-Methyl-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(pyridin-2-yl)benzenesulfonamide (2k). Orange solid (74 mg, 20%); mp: 220–222 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (s, 1H), 7.98 (d, J = 7.6 Hz, 2H), 7.82 (dt, J = 7.6, 1.2 Hz, 1H), 7.77 (dt, J = 7.6, 1.2 Hz, 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.09 (d, J = 8.4 Hz, 1H), 6.99 (d, J = 8.8 Hz, 2H), 6.87 (br s, 1H), 1.70 (s, 3H); LC–MS (ES+) 420 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{22}H_{18}N_3O_4S$ $(M+H)^+$ 420.1012, found 420.1019.

6.2.2.2. *N*-(4-(3-Methyl-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)phenylsulfonyl)benzamide (2l). Dark orange solid (12 mg, 26%); mp: 235–237 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.35 (br s, 1H), 9.05 (s, 1H), 8.00 (d, J = 6.8 Hz, 2H), 7.83–7.68 (m, 6H), 7.59 (t, J = 8.0 Hz, 1H), 7.46 (t, J = 7.6 Hz, 2H), 7.05 (d, J = 8.8 Hz, 2H), 1.78 (s, 3H); LC–MS (ES+) 447 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{24}H_{19}N_2O_5S$ $(M+H)^+$ 447.1009, found 447.1014.

6.2.2.3. *N*-(5-Methoxypyrimidin-2-yl)-4-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-benzenesulfonamide (2m). Orange solid (11.2 mg, 15%); mp: 215–217 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 (s, 1H disappeared on D_2O shake), 8.24 (s, 2H disappeared on D_2O shake), 8.00–7.97 (m, 2H), 7.83 (dt, J = 7.6, 1.6 Hz, 1H), 7.80–7.77 (m, 3H), 7.00 (d, J = 8.8 Hz, 2H), 3.75 (s, 3H), 1.70 (s, 3H); LC–MS (ES+) 451 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{22}H_{19}N_4O_5S$ $(M+H)^+$ 451.1071, found 451.1075.

6.2.2.4. *N*-(2,6-Dimethoxypyrimidin-4-yl)-4-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-benzenesulfonamide (2n). Dark orange solid (14 mg, 16%); mp: 255–257 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (br s, 1H), 9.02 (s, 1H), 7.99–7.97 (m, 2H), 7.82 (dt, J = 8.8, 1.2 Hz), 7.79–7.74 (m, 4H), 7.028 (d, J = 8.8 Hz, 2H), 5.91 (s, 1H), 3.76 (s, 3H), 3.73 (s, 3H), 1.72 (s, 3H); LC–MS (ES+) 481 $(M+H)^+$; HRMS (ES+) m/z calculated for $(M+H)^+$ $C_{23}H_{21}N_4O_6S$ 481.1176, found 481.1182.

6.2.2.5. *N*-(3,4-Dimethylisoxazol-5-yl)-4-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-benzenesulfonamide (2o). Orange solid (21 mg, 27%); mp: 235–238 °C decomposed; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (br s, 1H disappeared on D_2O shake), 9.06 (s, 1H disappeared on D_2O shake), 8.00 (d, J = 7.6 Hz, 2H), 7.86–7.77 (m, 2H), 7.57 (d, J = 8.8 Hz, 2H), 7.04 (d, J = 8.8 Hz, 2H), 2.06 (s, 3H), 1.73 (s, 3H), 1.59 (s, 3H); LC–MS (ES+) 438 $(M+H)^+$; HRMS (ES+) m/z calculated for $(M+H)^+$ $C_{22}H_{19}N_3O_5S$ 438.1118, found 438.1116.

6.2.3. General procedure for synthesis of compounds 2p–2r

The 1,4-naphthoquinone (500 mg, 3.16 mmol) was suspended in EtOH/water (90:10, 10.0 mL) followed by addition of the requisite amine (1.58 mmol, 0.5 equiv). The reaction mixture was heated under reflux for 2 days. The reaction mixtures turned orange-brown from bright orange mixture. The precipitates obtained were filtered and rinsed with EtOH (2–5 mL), EtOAc (5 mL) and acetone (2–5 mL). The required products were obtained as orange/brown solids.

6.2.3.1. *N*-(4-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)phenylsulfonyl)benzamide (2p). Brown/orange solid (181 mg, 26%); mp: 294–296 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.57 (br s, 1H), 9.52 (s, 1H), 8.06 (d, J = 7.6 Hz, 1H), 7.99 (d, J = 8.4 Hz, 2H), 7.95 (d, J = 7.2 Hz, 1H), 7.86–7.78 (m, 4H), 7.66–7.59 (m, 3H), 7.47 (t, J = 7.2 Hz, 2H), 6.41 (s, 1H); LC–MS (ES+) 433 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{23}H_{17}N_2O_5S$ $(M+H)^+$ 433.0853, found 433.0857.

6.2.3.2. 4-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (2q). Brown/orange solid (319 mg, 49%); mp: 290–192 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.43 (br s, 1H), 9.47 (s, 1H), 8.06 (d, J = 6.4 Hz, 1H), 7.94 (d, J = 7.6 Hz, 1H), 7.88–7.77 (m, 4H), 7.60 (d, J = 8.8 Hz, 2H), 6.37 (s, 1H), 6.14 (s, 1H), 2.28 (s, 3H); LC–MS (ES+) 410 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{20}H_{16}N_3O_5S$ $(M+H)^+$ 410.0805, found 410.0811.

6.2.3.3. 4-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(pyridin-2-yl)benzenesulfonamide (2r). Orange solid (148 mg, 20%); mp: 286 °C decomposed; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (s, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.99–7.94 (m, 2H), 7.90–7.87 (m, 3H), 7.79 (t, J = 7.6 Hz, 1H), 7.72 (t, J = 7.6 Hz, 1H), 7.53 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.4 Hz, 1H), 6.86 (br s, 1H), 6.30 (s, 1H); LRMS (ES+) 406 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{21}H_{16}N_3O_4S$ $(M+H)^+$ $(M+H)^+$ 406.0856, found 406.0860.

6.2.4. Synthesis of compounds 3a–3d

The starting material **PI-083** (40 mg, 0.040 mmol) was suspended in EtOH (2.0 mL) in a microwave vial and the requisite amine (5 M equiv) was added. The reaction mixture was irradiated in a CEM microwave reactor for 20–25 min at 140 °C (100 W). The resulting crude mixture was purified by SiO_2 chromatography using EtOAc and hexane (gradient elution) to obtain the required compounds.

6.2.4.1. 4-(3-(Dimethylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(pyridin-2-yl)-benzenesulfonamide (3a). Orange solid (55 mg, 33%); mp: 118–120 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 8.03 (br s, 1H), 7.94–7.89 (m, 2H), 7.76–7.71 (m, 2H), 7.66–7.65 (m, 1H), 7.61–7.59 (d, J = 8.0 Hz, 2H), 7.06 (d, J = 8.4 Hz, 1H), 6.87–6.85 (m, 1H), 6.85–6.83 (d, J = 8.0 Hz, 2H, partially overlapped), 2.66 (s, 6H); LRMS (ES+) 449 $(M+H)^+$.

6.2.4.2. 4-(3-(Benzyl(methyl)amino)-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(pyridin-2-yl)-benzenesulfonamide (3b). Dark blue solid (15.8 mg, 79%); mp: 135–140 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, NH, 1H, disappeared in D_2O shake), 8.03 (d, J = 4.0 Hz, 1H), 7.93–7.90 (m, 2H), 7.78–7.72 (m, 2H), 7.67–7.62 (m, 3H), 7.31 (d, J = 4.4 Hz, 1H), 7.22–7.13 (m, 3H), 7.08 (d, J = 8.8 Hz, 1H), 7.03 (d, J = 6.8 Hz, 2H), 6.96 (d, J = 8,8 Hz, 2H), 6.85 (t, J = 6.0 Hz, 1H), 3.85 (s, 2H), 2.75 (s, 3H); LRMS (ES+) 525 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{29}H_{25}N_4O_4S$ $(M+H)^+$ 525.1591, found 525.1588.

6.2.4.3. 4-(3-(4-Methylpiperazin-1-yl)-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N* -(pyridin-2-yl)-benzenesulfonamide (3c). Dark red solid (16.2 mg, 90%); mp: 222–224 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H), 8.02 (d, J = 4.0 Hz, 1H), 7.93–7.90 (m, 2H), 7.78–7.71 (m, 2H), 7.66–7.62 (m, 3H), 7.07 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.8 Hz, 2H), 6.85 (t, J = 6.0 Hz, 1H), 3.09 (br t, 4H), 1.98–1.97 (m, 4H), 1.87 (s, 3H); LRMS (ES+) 504 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{26}H_{26}N_5O_4S$ $(M+H)^+$ 504.1700, found 504.1713.

6.2.4.4. 4-(3-Morpholino-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(pyridin-2-yl)benzenesulfonamide (3d). Dark blue solid (15.0 mg, 77%); mp: 220–222 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (s, 1H), 8.00 (br s, 1H), 7.96–7.91 (m, 2H), 7.67–7.61 (m, 3H), 7.06 (d, J = 8.4 Hz, 1H), 6.95 (d, J = 8.8 Hz, 2H), 6.84 (br s, 1H), 3.14 (d, J = 4.0 Hz, 4 H), 3.06 (d, J = 4.0 Hz, 4H); LRMS (ES+) 491 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{25}H_{23}N_4O_5S$ $(M+H)^+$ 491.1384, found 491.1386.

6.3. General procedure for synthesis of sulfanilamide derivatives 5a and 5b[35]

Sodium cyanoborohydride (0.36 g, 5.65 mmol) was added to a mixture of requisite aldehyde (1.1 equiv), sulfapyridine (1.00 g, 4.01 mmol), and acetic acid (0.67 g, 11.23 mmol) in methanol (13 mL) at 0 °C and the reaction mixture was warmed to rt, stirred for 1 h. The reaction was quenched with $KHSO_4$ (5% aqueous solution, 10 mL), and extracted with ethyl acetate (3 × 20 mL). The organic phase was washed with sat. $NaHCO_3$, brine, dried (Na_2SO_4), and concentrated. The crude product was purified by flash chromatography (SiO_2 MeOH in DCM gradient elution).

6.3.1. 4-(Benzylamino)-*N*-(pyridin-2-yl)benzenesulfonamide (5a)

White solid (246 mg, 27%); mp: 163–165 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.99 (br s, 1H disappeared on D_2O shake), 8.24 (ddd, J = 4.8, 2.0, 0.8 Hz, 1H), 7.67–7.60 (m, 3H), 7.32–7.24 (m, 5H), 7.19–7.17 (m, 1H), 6.93 (ddd, J = 7.2, 4.8, 0.8 Hz, 1H), 6.64 (d, J = 9.2 Hz, 2H), 6.33 (apparent t, J = 5.6 Hz, 1H disappeared on D_2O shake), 4.35 (d, J = 5.6 Hz, 2H, doublet changed to a singlet on D_2O shake); LRMS (ES+) 340 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{18}H_{18}N_3O_2S$ $(M+H)^+$ 340.1114, found 340.1131.

6.3.2. 4-(Butylamino)-*N*-(pyridin-2-yl)benzenesulfonamide (5b)

White solid (658 mg, 54%); mp: 128–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$), 13.15 (br s, 1H), 8.40 (apparent dd, J = 5.6, 0.8 Hz, 1H), 7.62 (d, J = 8.8 Hz, 2H), 7.56 (dt, J = 8.0, 2.0 Hz, 1H), 7.35 (d, J = 8.8 Hz, 1H), 6.75 (t, J = 6.4 Hz, 1H), 6.47 (d, J = 8.8 Hz, 2H), 4.30 (br s, 1H, disappeared on D_2O shake), 3.04 (br t, 2H), 1.52 (p, J = 7.3 Hz, 2H), 1.32 (h, J = 7.3 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H); LRMS (ES+) 306 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{15}H_{20}N_3O_2S$ $(M+H)^+$ 306.1271, found: 306.1311.

6.4. Synthesis of sulfapyridine naphthoquinone derivatives 6a and 6b

N,*N*-Di-isopropylethylamine [DIPEA] (1.2 M equiv) was added to a solution of **PI-083** in anhydrous DMF (10 mL/mmol) under inert conditions. Appropriate alkyl iodide (1.2 M equiv) was added to the reaction mixture after 5 min, and the reaction was stirred at rt for two days. The reaction mixture was concentrated under reduced pressure and purified by flash chromatography (SiO_2, EtOAc in hexane, gradient elution) to obtain the required pure product.

6.4.1. 4-((3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)(methyl)amino)-*N*-(pyridin-2-yl)benzenesulfonamide (6a)

Orange solid (13 mg, 25%); mp: 152–154 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (ddd, J = 4.8, 2.0, 0.8 Hz, 1H), 8.19 (dd, J = 7.6, 1.2 Hz, 1H), 8.13 (dd, J = 7.6, 1.2 Hz, 1H), 7.79 (dt, J = 7.6, 1.6 Hz, 1H), 7.72 (dt, J = 7.6, 1.6 Hz, 1H), 7.73–7.66 (m, 3H), 7.53 (d, J = 8.4 Hz, 2H), 7.13 (ddd, J = 6.8, 4.8, 1.2 Hz, 1H), 7.01 (d, J = 8.4 Hz, 2H), 3.28 (s, 3H); LRMS (ES+) 454 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{22}H_{17}ClN_3O_4S$ $(M+H)^+$ 454.0623, found 454.0638.

6.4.2. 4-((3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)(ethyl)amino)-*N*-(pyridin-2-yl)benzenesulfonamide (6b)

Orange solid, (60 mg, 38%); mp: 180–182 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (ddd, J = 4.8, 2.0, 0.8 Hz, 1H), 8.20 (dd, J = 7.6, 1.2 Hz, 1H), 8.13 (dd, J = 7.6, 1.2 Hz, 1H), 7.80 (dt, J = 7.6, 1.2 Hz, 1H), 7.73 (dt, J = 7.6, 1.2 Hz, 2H), 7.67 (br s, 1H, disappeared on D_2O shake), 7.59–7.55 (m, 3H), 7.16 (ddd, J = 7.2, 4.8, 1.6 Hz, 1H), 7.01 (d, J = 8.4 Hz, 2H), 3.84 (q, J = 7.2 Hz, 2H), 1.12 (t, J = 7.2 Hz, 3H). LRMS (ES+) 468 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{23}H_{19}ClN_3O_4S$ $(M+H)^+$ 468.0779, found 468.0800.

6.5. General procedure for synthesis of sulfapyridine naphthoquinone derivatives (6c–6g)

The **PI-083** (100 mg, 0.23 mmol), appropriate alkyl halide (0.27 mmol, 1.2 M equiv) and DIPEA (35 mg, 0.27 mmol, 1.2 M equiv) were mixed in anhydrous DMF (2 mL). The reaction mixture was heated at 160 °C for 15 min in a microwave reactor (for **6f**, **6g**, the reactions were heated for 30 min at 160 °C). The reaction mixtures were then concentrated under reduced pressure and the products were purified using flash chromatography (SiO_2, EtOAc in hexane, gradient elution).

6.5.1. 4-(Benzyl(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-*N*-(pyridin-2-yl)benzenesulfonamide (6c)

Orange solid (56 mg, 47%); mp: 78–80 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (ddd, J = 4.0, 2.0, 1.2 Hz, 1H), 8.20 (dd, J = 7.6, 1.2 Hz, 1H), 8.14 (td, J = 7.6, 1.6 Hz, 1H), 7.8–7.59 (m, 7H), 7.48 (dd, J = 8.0, 0.8 Hz, 1H), 7.30 (d, J = 7.2 Hz, 2H), 7.24–7.15 (m, 3H), 7.09–7.03 (m, 3H), 5.01 (s, 2H); LRMS (ES+) 530 $(M+H)^+$; HRMS

(ES+) m/z calculated for $C_{28}H_{21}ClN_3O_4S$ $(M+H)^+$ 530.0936, found 530.0936.

6.5.2. 4-((3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)(4-nitrobenzyl)amino)-*N*-(pyridin-2-yl)benzenesulfonamide (6d)

Orange solid (75 mg, 58%); mp: 80–82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (ddd, J = 4.8, 1.6, 0.8 Hz, 1H), 8.20 (dd, J = 7.6, 0.8 Hz, 1H), 8.14 (dd, J = 7.6, 1.2 Hz, 1H), 8.09 (d, J = 8.8 Hz, 2H), 7.81 (dt, J = 7.6, 1.6 Hz, 1H), 7.76–7.60 (m, 4H), 7.57 (d, J = 8.8 Hz, 2H), 7.51 (d, J = 8.8 Hz, 2H), 7.10 (ddd, J = 7.2, 4.8, 1.2 Hz, 1H), 7.03 (d, J = 8.4 Hz, 2H), 5.11 (s, 2H); LRMS (ES+) 575 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{28}H_{20}ClN_4O_6S$ $(M+H)^+$ 575.0787, found 575.0795.

6.5.3. 4-((3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)(naphthalen-2-ylmethyl)amino)-*N*-(pyridin-2-yl)benzenesulfonamide (6e)

Orange solid (60 mg, 45%); mp: 165–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (ddd, J = 4.8, 2.0, 0.8 Hz, 1H), 8.20 (apparent dd, J = 7.6, 1.2 Hz, 1H), 8.13 (apparent dd, J = 7.6, 1.2 Hz, 1H), 7.81 (dt, J = 7.6, 1.2 Hz, 1H), 7.76–7.71 (m, 7H), 7.65–7.57 (m, 3H), 7.51–7.49 (m, 2H), 7.42–7.39 (m, 2H), 7.08–7.03 (m, 2H), 5.15 (s, 2H); LRMS (ES+) 580 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{32}H_{23}ClN_3O_4S$ $(M+H)^+$ 580.1092, found 580.1089.

6.5.4. 4-((3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)(4-methylbenzyl)amino)-*N*-(pyridin-2-yl)benzenesulfonamide (6f)

Orange solid (50 mg, 41%); mp: 99–101 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (ddd, J = 4.8, 2.0, 0.8 Hz, 1H), 8.21 (dd, J = 7.6, 1.2 Hz, 1H), 8.15 (dd, J = 7.6, 1.2 Hz, 1H), 7.80 (dt, J = 7.6, 1.2 Hz, 1H), 7.74 (dt, J = 7.6, 1.2 Hz, 1H), 7.64–7.60 (m, 3H), 7.46 (td, J = 8.4, 0.8 Hz, 1H), 7.19 (d, J = 8.0 Hz, 2H), 7.09–7.01 (m, 5H), 4.96 (s, 2H), 2.25 (s, 3H); LRMS (ES+) 544 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{29}H_{23}ClN_3O_4S$ $(M+H)^+$ 544.1092, found 544.1101.

6.5.5. 4-((3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)(4-(trifluoromethyl)benzyl) amino)-*N*-(pyridin-2-yl)benzenesulfonamide (6g)

Orange solid (62 mg, 46%); mp: 157–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (apparent d, J = 4.8 Hz, 1H), 8.21 (d, J = 7.6 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 7.83–7.64 (m, 4H), 7.59–7.55 (m, 3H), 7.48 (d, J = 8.0 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.11–7.03 (m, 2H), 5.01 (s, 2H); ^{19}F NMR: δ −62.93 (s); LRMS (ES+) 598 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{29}H_{20}ClF_3N_3O_4S$ $(M+H)^+$ 598.0810, found 598.0816.

6.6. Synthesis of compounds 7a and 7b

6.6.1. Mixture of regio-isomers of 4-(3-chloro-6-nitro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(pyridin-2-yl)benzenesulfonamide and 4-(3-chloro-7-nitro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(pyridin-2-yl)benzenesulfonamide (7a)

A well-stirred suspension of sulfapyridine (0.229 g, 0.919 mmol) and 2,3-dichloro-6-nitronaphthalene-1,4-dione (0.5 g, 1.84 mmol) in a mixture of 95% EtOH in water (10.0 mL) was heated in a sealed tube at 115 °C for three days. The resultant orange precipitate was filtered and washed with hot ethanol (5 × 5 mL), acetone (3 × 5 mL), and dried under reduced pressure to afford the title compound as an orange solid (0.393 g, 88%). ^{1}H NMR indicated formation of 2:1 regio-isomers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H, [δ 9.72 minor isomer shown]) 8.64–8.56 (m, 2H), 8.28–8.25 (m, 1H), 8.02 (br s, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.71 (t, J = 7.2 Hz, 1H), 7.22 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.0 Hz, 1H), 6.88 (br t, J = 6.4 Hz, 1H); LRMS (ES+) 485 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{21}H_{14}ClN_4O_6S$ $(M+H)^+$ 485.0317, found 485.0330.

6.6.2. Mixture of regio-isomers of 4-(3-chloro-5-nitro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(pyridin-2-yl) benzenesulfonamide and 4-(3-chloro-8-nitro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(pyridin-2-yl) benzenesulfonamide (7b)

This compound was prepared according to the procedure described for compound **7a**, except using 2,3-dichloro-5-nitronaphthalene-1,4-dione to obtain **7b** as an orange solid (0.178 g, 92%). ^{1}H NMR indicated formation of 2:1 regio-isomers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.67 (s, 1H, [δ 9.73 minor isomer shown]), 8.25 (dd, J = 7.6, 1.2 Hz, 1H, [δ 8.23 minor isomer shown]), 8.13 (d, J = 8.4 Hz, 1H), 8.07–7.97 (m, 2H), 7.77 (dd, J = 8.4, 3.2 Hz, 2H), 7.72 (t, J = 8.4 Hz, 1H), 7.23–7.15 (m, 3H), 6.88 (br t, J = 5.6 Hz, 1H); LC–MS (ES+) 485 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{21}H_{14}ClN_4O_6S$ $(M+H)^+$ 485.0317, found 485.0327.

6.6.3. Mixture of regio-isomers of 4-(6-amino-3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(pyridin-2-yl) benzenesulfonamide and 4-(7-amino-3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-(pyridin-2-yl) benzenesulfonamide (8)

Compound **7a** (0.213 g, 0.44 mmol) was dissolved in a mixture of DMF/MeOH (4:1, 30 mL) and passed through the H-cube apparatus at a rate of 1 mL/min (40 bar pressure, 10% Pd/C as catalyst, room temperature). The resultant solution was evaporated and dried under reduced pressure to obtain a red solid. This product was purified using SiO_2 flash chromatography (MeOH/DCM, 5–10% gradient elution) to obtain the required reduced product as a red solid (50 mg, 25%). ^{1}H NMR indicated 1:5 ratio of regio-isomers after SiO_2 purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H [δ 9.40 minor isomer]), 8.03 (br s, 1H), 7.75 (d, J = 7.6 Hz, 2H), 7.73 (d, J = 6.4 Hz, 2H), 7.17–7.15 (m, 2H), 7.06 (d, J = 8.8 Hz, 2H), 6.86–6.77 (m, 2H), 6.54 (s, 2H disappear on D_2O shake); LC–MS (ES+) 455 $(M+H)^+$; HRMS (ES+) m/z calculated for $C_{21}H_{16}ClN_4O_4S$ $(M+H)^+$ 455.0575, found 455.0588.

6.7. Synthesis of library 11a–11l

6.7.1. 4-Nitro-*N*-*o*-tolyl-benzenesulfonamide (11a)

A solution of 4-nitrobenzenesulfonyl chloride (200 mg, 0.90 mmol), *o*-toluidine (106 mg, 0.99 mmol), and pyridine (79 mg, 0.08 mL, 0.99 mmol) in 1,2-dichloroethane (5.0 mL) was heated to 150 °C for 10 min in the microwave reactor. A 1 M HCl solution was added until the pH was 2, and the acidified aqueous layer was separated and extracted with DCM (3 × 10 mL). The combined organic fractions were washed successively with brine and water then dried over Na_2SO_4 and evaporated to dryness to afford the title compound as a peach colored solid (127 mg, 47%). mp: 157–158 °C (lit. 157–159 °C);[36] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 9.0 Hz, 2H), 7.89 (d, J = 9.0 Hz, 2H), 7.28–7.27 (m, 1H), 7.20–7.12 (m, 3H), 6.37 (br s, 1H), 2.01 (s, 3H).

6.7.2. 4-Nitro-*N*-phenyl-benzenesulfonamide (11b)

This compound was prepared according to the procedure described for compound **11a** except using aniline to obtain required product as an off-white solid, (222 mg, 87%). mp: 170–171 °C (lit. 174–176 °C);[36] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 8.4 Hz, 2H), 7.29 (t, J = 6.8 Hz, 2H), 7.21 (t, J = 7.2 Hz, 1H), 7.22–7.18 (m, 1H), 7.08 (d, J = 8.4 Hz, 2H), 6.57 (br s, 1H).

6.7.3. 4-Nitro-*N*-*m*-tolyl-benzenesulfonamide (11c)

This compound was prepared according to the procedure described for compound **11a** except using *m*-toluidine to obtain required product as a light tan solid, (264 mg, 100%); mp: 136–138 °C (lit. 138–139 °C);[36] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d,

J = 8.8 Hz, 2H), 7.92 (d, J = 8.8 Hz, 2H), 7.15 (t, J = 7.8 Hz, 1H), 7.00 (d, J = 7.6 Hz, 1H), 6.90 (br s, 1H), 6.85 (d, J = 8.4 Hz, 1H), 6.56 (br s, 1H).

6.7.4. 4-Nitro-*N*-*p*-tolyl-benzenesulfonamide (11d)

This compound was prepared according to the procedure described for compound **11a** except using *p*-toluidine to obtain required product as a yellow solid, (256 mg, 97%). mp: 170–172 °C (lit. 184–184.5 °C);[37] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, J = 9.0 Hz, 2H), 7.88 (d, J = 9.0 Hz, 2H), 7.08 (d, J = 8.4 Hz, 2H), 6.94 (d, J = 8.4 Hz, 2H), 6.41 (br s, 1H), 2.30 (s, 3H).

6.7.5. *N*-(2-Methoxyphenyl)-4-nitro-benzenesulfonamide (11e)

This compound was prepared according to the procedure described for compound **11a** except using *o*-anisidine. Recrystallization from DCM/hexanes obtained the required product as white crystals, (164 mg, 59%). mp: 141–143 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 8.8 Hz, 2H), 7.56 (dd, J = 8.0, 1.6 Hz, 1H), 7.11 (dt, J =7.9, 1.5 Hz, 1H), 7.02 (br s, 1H), 6.94 (dt, J = 7.6, 1.2 Hz, 1H), 6.74 (dd, J = 8.0, 1.2 Hz, 1H), 3.62 (s, 3H).

6.7.6. *N*-(3-Methoxyphenyl)-4-nitrobenzenesulfonamide (11f)

This compound was prepared according to the procedure described for compound **11a** except using *m*-anisidine. Recrystallization from DCM/hexanes obtained the required product as brown-yellow needles (202 mg, 73%). mp: 96–98 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, J = 9.0 Hz, 2H), 7.94 (d, J = 9.0 Hz, 2H), 7.16 (t, J = 8.0 Hz, 1H), 6.73–6.70 (m, 2H), 6.60–6.58 (m, 2H), 3.76 (s, 3H).

6.7.7. *N*-(4-Methoxyphenyl)-4-nitrobenzenesulfonamide (11g)

This compound was prepared according to the procedure described for compound **11a** except using *p*-anisidine to obtain the required product as a light brown solid (278 mg, 100%). mp: 173–175 °C (lit. 187–189 °C);[37] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (dd, J = 9.2, 2.4 Hz, 2H), 7.85 (dd, J = 9.2, 2.0 Hz, 2H), 6.97 (dd, J = 8.6, 2.2 Hz, 2H), 6.80 (dd, J = 8.6, 2.2 Hz, 2H), 6.36 (br s, 1H), 3.78 (s, 3H).

6.7.8. 2-Methoxy-4-nitro-*N*-*o*-tolyl-benzenesulfonamide (11h)

This compound was prepared according to the procedure for compound **11a** except using 2-methoxy-4-nitrobenzenesulfonyl chloride. Recrystallization from DCM/hexanes obtained the required product as brown crystals (202 mg, 79%). mp: 143–145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.8 Hz, 1H), 7.85–7.88 (m, 2H), 7.14–7.11 (m, 2H), 7.06–7.04 (m, 2H), 6.76 (br s, 1H), 4.13 (s, 3H), 2.26 (s, 3H).

6.7.9. 2-Methoxy-4-nitro-*N*-*m*-tolyl-benzenesulfonamide (11i)

This compound was prepared according to the procedure described for compound **11a** except using 2-methoxy-4-nitrobenzenesulfonyl chloride and *m*-toluidine as starting materials. Recrystallization from DCM/hexanes obtained the required product as gold-brown needles (236 mg, 92%). mp: 142–145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 9.2 Hz, 1H), 7.84–7.81 (m, 2H), 7.28 (br s, 1H), 7.07 (t, J = 7.6 Hz, 1H), 6.90–6.83 (m, 3H), 4.16 (s, 3H), 2.25 (s, 3H).

6.7.10. 2-Methoxy-4-nitro-*N*-*p*-tolyl-benzenesulfonamide (11j)

This compound was prepared according to the procedure described for compound **11a** except using 2-methoxy-4-nitrobenzenesulfonyl and *p*-toluidine as starting materials. Recrystallization from DCM/hexanes gave the required product as yellow-brown needles (199 mg, 78%). mp: 126–129 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.4 Hz, 1H), 7.84 (d, J = 2.0 Hz, 1 H), 7.81 (dd, J = 8.8, 2.0 Hz, 1H), 7.00 (d, J = 8.2 Hz, 2H), 6.94 (d, J = 8.4 Hz, 2H), 7.10 (br s, 1H), 4.17 (s, 3H), 2.23 (s, 3H).

6.7.11. 2-Methoxy-*N*-(4-methoxyphenyl)-4-nitrobenzenesulfonamide (11k)

This compound was prepared according to the procedure described for compound **11a** except using 2-methoxy-4-nitrobenzenesulfonyl and *p*-anisidine as starting materials. Recrystallization from DCM/hexanes gave the required product as gold-brown needles (213 mg, 79%). mp: 117–119 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 2.0 Hz, 1H), 7.82 (dd, J = 8.4, 2.0 Hz, 1H), 6.97 (d, J = 8.8 Hz, 2H), 7.00 (br s, 1H), 6.72 (d, J = 9.2 Hz, 2H), 4.19 (s, 3H), 3.72 (s, 3H).

6.7.12. 4-Nitro-*N*-(1-azidoformimidoyl)benzenesulfonamide (11l)

This compound was prepared according to the literature procedure[22] to afford the title compound as a yellow solid (682 mg, 51%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (br, 1H), 8.39 (d, J = 8.8 Hz, 2H), 8.15 (d, J = 8.8 Hz, 2H), 8.00 (br s, 1H); LC–MS (ES−) 269 $(M-H)^-$; HRMS (ES−) m/z calculated for $C_7H_5N_6O_4S$ $(M-H)^-$ 269.0099, found 269.0094.

6.8. General procedure for synthesis of 11m–11r

The sulfonyl chloride (1.0 g, 4.51 mmol) was dissolved in THF (10 mL) and appropriate amine (3 M equiv) was added to the reaction mixture. The reaction was stirred 30 min at room temperature until TLC (30% ethyl acetate in hexane) indicated completion of the reaction. The reaction mixture was acidified with 1 M HCl (pH 2) at 0 °C and the solvent was evaporated under reduced pressure and the white solid obtained at this point was triturated in ethyl acetate/hexane and filtered. The product obtained was washed with water and dried under vacuum to afford a white solid. Upon acidification to pH 2, some reactions produced oily mixtures. These oily mixtures were diluted with water (10 mL) and extracted twice with ethyl acetate (20 mL). The organic layer was separated dried (Na_2SO_4), filtered and the filtrate was evaporated and dried under vacuum to afford the desired compound as a solid.

6.8.1. *N*-Methyl-4-nitrobenzenesulfonamide (11m)

White solid (0.67 g, 68%); mp: 105–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (apparent d, J = 8.8 Hz, 2H), 8.06 (apparent d, J = 8.8 Hz, 2H), 4.52 (br s, 1H), 2.74 (d, J = 5.2 Hz, 3H), 1.58 (s, 3H); LC–MS (ES−) m/z 215 $(M-H)^-$; HRMS (ES−) calculated for $C_7H_7N_2O_4S$ $(M-H)^-$ 215.0132, found 215.0148.

6.8.2. *N*-Ethyl-4-nitrobenzenesulfonamide (11n)

Yellow solid (0.406 g, 78%); mp: 99–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, J = 8.8 Hz, 2H), 8.06 (d, J = 8.8 Hz, 2H), 4.53 (br s, 1H), 3.10–3.06 (m, 2H), 1.15 (t, J = 7.2 Hz, 3H); LC–MS (ES−) m/z 229 $(M-H)^-$; HRMS (ES−) m/z calculated for $C_8H_9N_2O4S$ $(M-H)^-$ 229.0289, found 229.0304.

6.8.3. *N*-Isopropyl-4-nitrobenzenesulfonamide (11o)

Yellow solid (1.031 g, 94%); mp: 112–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 8.8 Hz, 2H), 8.07 (d, J = 8.4 Hz, 2H), 4.44 (d, J = 8.0 Hz, 1H), 3.61–3.52 (m, 1H), 1.12 (d, J = 6.8 Hz, 6H); LC–MS (ES−) m/z 243 $(M-H)^-$; HRMS (ES−) calculated for $C_9H_{11}N_2O_4S$ $(M-H)^-$ 243.0445, found 243.0465.

6.8.4. *N*-Methyl-3-nitrobenzenesulfonamide (11p)

White solid (0.67 g, 69%); mp: 120–121 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (t, J = 2.0 Hz, 1H), 8.45 (ddd, J = 8.4, 2.0, 0.8 Hz, 1H),

8.20 (ddd, *J* = 8.0, 1.6, 0.8 Hz, 1H), 7.76 (t, *J* = 8.0 Hz, 1H), 2.74 (d, *J* = 9.2 Hz, 3H); LC–MS (ES−) *m*/*z* 215 (M−H); HRMS (ES−) calculated for $C_7H_7N_2O_4S$ $(M-H)^-$ 215.0132, found 215.0147.

6.8.5. *N*-Ethyl-3-nitrobenzenesulfonamide (11q)

Yellow solid (0.48 g, 92%); mp: 86–87 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (apparent t, *J* = 2.0 Hz, 1H), 8.44 (ddd, *J* = 8.8, 2.0, 1.2 Hz, 1H), 8.21 (ddd, *J* = 7.6, 1.6, 1.2 Hz, 1H), 7.75 (t, *J* = 8.4 Hz, 1H), 4.58 (br s, 1H), 3.11–3.06 (m, 2H), 1.63 (t, *J* = 7.2 Hz, 3H ; LC–MS (ES−) *m*/*z* 229 $(M-H)^-$; HRMS (ES−) *m*/*z* calculated for $C_8H_9N_2O4S$ $(M-H)^-$ 229.0289, found 229.0298.

6.8.6. *N*-Isopropyl-3-nitrobenzenesulfonamide (11r)

White solid (0.962 g, 87%); mp: 67–68 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (t, *J* = 2.0 Hz, 1H), 8.43 (ddd, *J* = 8.4, 2.0, 0.8 Hz, 1H), 8.21 (ddd, *J* = 8.0, 2.0, 1.2 Hz, 1H), 7.74 (t, *J* = 8.0 Hz, 1H), 4.43 (br s, 1H), 3.62–3.53 (m,1H), 1.13 (d, *J* = 6.8 Hz, 6H); LC–MS (ES−) *m*/*z* 243 $(M-H)^-$; HRMS (ES−) calculated for $C_9H_{11}N_2O_4S$ $(M-H)^-$ 243.0445, found 243.0462.

6.9. Synthesis of library 12a–12l

6.9.1. 4-Amino-*N*-*o*-tolyl-benzenesulfonamide (12a)

To a solution of 4-nitro-*N*-*o*-tolyl-benzenesulfonamide **11a** in a mixture of MeOH/THF (1:1, 4.0 mL), was added nickel chloride hexahydrate (163 mg, 0.68 mmol) at 0 °C under constant stirring. Sodium borohydrate (52 mg, 1.37 mmol) was added portionwise and the reaction was monitored by TLC (60% hexanes/40% ethyl acetate). The solvent was removed in vacuo and the remaining black solid was re-suspended in EtOAc and filtered using a pad of Celite and washed with EtOAc until the filtrate, when visualized under UV light, showed no product. The solvent was removed under vacuum affording the title compound as an off-white solid (0.045 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 8.8 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.07 (dt, *J* = 7.2, 2.4 Hz, 1H), 7.01–6.95 (m, 2H), 6.52 (d, *J* = 8.8 Hz, 2H), 6.35 (br s, 1H), 4.05 (br s, 2H), 1.95 (s, 3H).

6.9.2. 4-Amino-*N*-phenyl-benzenesulfonamide (12b)

This compound was prepared according to the procedure described for compound **12a** except using **11b** to obtain the required product as **a** pale yellow solid (109 mg, 70%). mp: 180–182 °C (lit. 260.5–261.5 °C);[37] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.8 Hz, 2H), 7.24–7.21 (m, 2H), 7.10 (t, *J* = 7.6 Hz, 1H), 7.04 (dd, *J* = 8.8, 1.2 Hz, 2H), 6.58 (d, *J* = 8.8 Hz, 2H), 6.32 (br s, 1H), 4.08 (br s, 2H).

6.9.3. 4-Amino-*N*-*m*-tolyl-benzenesulfonamide (12c)

This compound was prepared according to the procedure described for compound **12a** except using **11c** to obtain the required product as a light yellow solid (160 mg, 74%). mp: 117–120 °C (lit. 132.5–133 °C);[37] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.6 Hz, 2H), 7.03 (t, *J* = 7.6 Hz, 1H), 6.84–6.81 (m, 2H), 6.76 (br d, *J* = 8.0 Hz, 1H), 6.52 (d, *J* = 8.6 Hz, 2H), 6.26 (br s, 1H), 4.01 (br s, 2H), 2.20 (s, 3H).

6.9.4. 4-Amino-*N*-*p*-tolyl-benzenesulfonamide (12d)

This compound was prepared according to the procedure for compound **12a** except using **11d** to obtain the required product as an off-white solid (225 mg, 99%). mp: 174–176 °C (lit. 190–190.5 °C);[37] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.8 Hz, 2H), 6.96 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.52 (d, *J* = 8.8 Hz, 2H), 6.14 (br s, 1H), 4.00 (br s, 2H), 2.20 (s, 3H).

6.9.5. 4-Amino-*N*-(2-methoxyphenyl)benzenesulfonamide (12e)

This compound was prepared according to the procedure described for compound **12a** except using **11e** to obtain the required product as a white solid (85 mg, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.6 Hz, 2H), 7.42 (dd, *J* = 7.8, 1.1 Hz, 1H), 6.94 (dt, *J* = 8.0, 1.6 Hz, 1H), 6.92 (br s, 1H), 6.81 (dt, *J* = 7.8, 1.1 Hz, 1H), 6.67 (dd, *J* = 8.2, 1.1 Hz, 1H), 6.49 (d, *J* = 8.6 Hz, 2H), 3.60 (s, 3H).

6.9.6. 4-Amino-*N*-(3-methoxyphenyl)benzenesulfonamide (12f)

This compound was prepared according to the procedure described for compound **12a** except using **11f** to obtain the required product as a pale yellow solid (151 mg, 83%). mp: 142–145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, *J* = 8.8 Hz, 2H), 7.05 (t, *J* = 8.0 Hz, 1H), 6.61–6.50 (m, 5H), 6.32 (br s, 1H), 3.679 (s, 3H).

6.9.7. 4-Amino-*N*-(4-methoxyphenyl)benzenesulfonamide (12g)

This compound was prepared according to the procedure described for compound **12a** except using **11g** to obtain the required product as a light yellow solid, (197 mg, 79%). mp: 150 °C, decomposed; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 8.8 Hz, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 6.76 (d, *J* = 9.2 Hz, 2H), 6.58 (d, *J* = 8.4 Hz, 2H), 6.09 (br s, 1H), 4.07 (br s, 2H), 3.76 (s, 3H).

6.9.8. 4-Amino-2-methoxy-*N*-*m*-tolyl-benzenesulfonamide (12h)

This compound was prepared according to the procedure described for compound **12a** except using **11h** to obtain the required product as an orange-brown solid in (169 mg, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.4 Hz, 1H), 6.99 (t, *J* = 7.8 Hz, 1H), 6.82 (br s, 1H), 6.79–6.73 (m, 2H), 6.65 (br s, 1H), 6.11 (d, *J* = 2.0 Hz, 1H), 6.09 (s, 1H), 3.99 (br s, 2H), 3.86 (s, 3H), 2.18 (s, 3H).

6.9.9. 4-Amino-2-methoxy-*N*-*p*-tolyl-benzenesulfonamide (12i)

This compound was prepared according to the procedure described for compound **12a** except using **11i** to obtain the required product as a light yellow solid (130 mg, 93%). mp: 158–160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.8 Hz, 1H), 6.98 (d, *J* = 8.2 Hz, 2H), 6.93 (d, *J* = 8.2 Hz, 2H), 6.70 (br s, 1H), 6.16 (s, 1H), 6.14 (appd, *J* = 2.0 Hz, 1H), 4.05 (br s, 2H), 3.95 (s, 3H), 2.23 (s, 3H).

6.9.10. 4-Amino-2-methoxy-*N*-*o*-tolyl-benzenesulfonamide (12j)

This compound was prepared according to the procedure described for compound **12a** except using **11j** to obtain the required product as a yellow solid (63 mg, 93%). mp: 189–191 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.30 (d, *J* = 8.8 Hz, 1H), 7.10–7.06 (m, 2H), 7.01–6.97 (m, 2H), 6.31 (d, *J* = 1.6 Hz, 1H), 6.12 (dd, *J* = 8.8, 2.0 Hz, 1H), 3.30 (s, 3H), 2.22 (s, 3H).

6.9.11. 4-Amino-2-methoxy-*N*-(4-methoxyphenyl) benzenesulfonamide (12k)

This compound was prepared according to the procedure described for compound **12a** except using **11k** to obtain the required product as a yellow solid (141 mg, 77%). mp: 51–54 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 8.4 Hz, 1H), 6.97 (d, *J* = 8.8 Hz, 2H), 6.73 (d, *J* = 8.8 Hz, 2H), 6.61 (br s, 1H), 6.19 (d, *J* = 1.6 Hz, 1H), 6.14 (dd, *J* = 8.2, 2.2 Hz, 1H), 4.06 (br s, 2H), 3.98 (s, 3H), 3.72 (s, 3H).

6.9.12. 4-Amino-*N*-carbamimidoylbenzenesulfonamide (12l)

This compound was prepared according to the procedure for compound **12a** except using **11l** to afford the title compound as a gray solid (80 mg, 13.2%). mp: 169–171 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37 (d, *J* = 8.3 Hz, 2H), 6.55 (br, 4H), 6.53 (d, *J* = 8.3 Hz, 2H), 5.69 (br, 2H); LC–MS (ES+) 215 $(M+H)^+$, 237 $(M+Na)^+$, 451 $(2\,M+Na)^+$; HRMS (ES+) *m*/*z* calculated for $C_7H_{11}N_4O_2S$ $(M+H)^+$ 215.0597, found 215.0595.

6.10. General procedure for synthesis of 12m–12r

6.10.1. 4-Amino-*N*-methylbenzenesulfonamide (12m)

N-Methyl-4-nitrobenzenesulfonamide **11m** (0.143 g, 0.66 mmol) was dissolved in MeOH/THF (1:1 solution, 12 mL) and nickel

chloride(11) hexahydrate (0.627 g, 2.64 mmol) was added to the solution at 0 °C under constant stirring. Sodium borohydride (0.199 g, 5.28 mmol) was added portionwise over 15 min. The reaction mixture was stirred for additional 5 min until TLC (50% ethyl acetate in hexane) indicated completion of the reaction and the solvent was evaporated to obtain a black solid. The solid was suspended in ethyl acetate, filtered through a pad of Celite, and washed with ethyl acetate until the filtrate showed no product on TLC (under uv visualization). The filtrate was evaporated and dried to obtain the desired compound as a yellow solid (0.103 g, 84%); mp: 108–109 °C [lit. 98–99 °C];[38] ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.36 (d, J = 8.8 Hz, 2H), 6.90 (q, J = 4.8 Hz, 1H), 6.58 (d, J = 8.8 Hz, 2H), 5.91 (s, 2H), 2.29 (d, J = 4.8 Hz, 3H); LC–MS (ES+) m/z 187 $(M+H)^+$, 156 $(M-CH_3NH)^+$; HRMS (ES+) calculated for $C_7H_{11}N_2O_2S$ $(M+H)^+$ 187.0536, found 187.0527.

6.10.2. 4-Amino-*N*-ethylbenzenesulfonamide (12n)

N-Ethyl-4-nitrobenzenesulfonamide (0.3 g, 1.3 mmol) was dissolved in methanol and passed through H-cube apparatus (full H_2 mode, 30 bar pressure and 10% Pd/C catalyst cartridge at 25 °C, flow rate 1.0 mL/min). The reaction was monitored by TLC (40% ethyl acetate in hexane). After completion, the solvent was evaporated and dried under vacuum to afford a white solid (0.229 g, 88%); mp: 98–99 °C [lit. 107 °C];[39] ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37 (d, J = 6.8 Hz, 2H), 7.00 (t, J = 5.6 Hz, 1H), 6.58 (d, J = 8.8 Hz, 2H), 5.88 (s, 2H), 2.69–2.62 (m, 2H), 0.92 (t, J = 7.2 Hz, 3H); LC–MS (ES+) m/z 201 $(M+H)^+$, 156 $(M-C_2H_5NH)^+$, HRMS (ES+) calculated for $C_8H_{13}N_2O_2S$ $(M+H)^+$ 201.0692, found 201.0694.

6.10.3. 4-Amino-*N*-isopropylbenzenesulfonamide (12o)

This compound was prepared according to the procedure described for **12n** except using *N*-isopropyl-4-nitrobenzenesulfonamide **11o** (0.3 g, 1.23 mmol) and H-cube settings at 30 °C and pressure at 40 bars to obtain a yellowish-orange solid (0.29 g, 97%); mp: 117–118 °C, [lit. 116.5–117 °C];[40] ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (d, J = 8.8 Hz, 2H), 7.19 (d, J = 7.2 Hz, 1H), 6.85 (d, J = 8.8 Hz, 2H), 3.17–3.09 (m,1H), 0.89 (d, J = 6.8 Hz, 6H); LC–MS (ES+) m/z 215 $(M+H)^+$, 156 $(M-C_3H_7NH)^+$; HRMS (ES+) calculated for $C_9H_{15}N_2O_2S$ $(M+H)^+$ 215.0849, found 215.0849.

6.10.4. 3-Amino-*N*-methylbenzenesulfonamide (12p)

This compound was prepared according to the procedure described for **12m** except using **11p** *N*-methyl-3-nitrobenzenesulfonamide (0.04 g, 0.185 mmol). Yellow oil (0.034 g, 99%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (ddd, J = 7.8, 1.6, 1.1 Hz, 1H), 7.60 (t, J = 1.8 Hz, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.31 (ddd, J = 7.9, 2.2, 1.0 Hz, 1H), 4.34 (br s, 2H), 2.70 (d, J = 5.2 Hz, 3H); LC–MS (ES+) m/z 187 $(M+H)^+$; HRMS (ES+) calculated for $C_7H_{11}N_2O_2S$ $(M+H)^+$ 187.0536, found 187.0548.

6.10.5. 3-Amino-*N*-ethylbenzenesulfonamide (12q)

This compound was prepared according to the procedure described for **12n** except using *N*-ethyl-3-nitrobenzenesulfonamide **11q** (0.3 g, 1.3 mmol). White solid (0.229 g, 88%); mp: 98–99 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31 (t, J = 6.0 Hz, 1H), 7.16 (t, J = 8.0 Hz, 1H), 6.95 (t, J = 2.0 Hz, 1H), 6.84 (ddd, J = 7.6, 1.6, 0.8 Hz, 1H), 6.71 (ddd, J = 8.0, 2.2, 0.8 Hz, 1H), 5.54 (s, 2H), 2.69–2.62 (m, 2H), 0.92 (t, J = 7.2 Hz, 3H); LC–MS (ES+) m/z 201 $(M+H)^+$; HRMS (ES+) calculated for $C_8H_{13}N_2O_2S$ $(M+H)^+$ 201.0692, found 201.0695.

6.10.6. 3-Amino-*N*-isopropylbenzenesulfonamide (12r)

This compound was prepared according to the procedure described for **12m**, except using **11r** *N*-isopropyl-3-nitrobenzenesulfonamide (0.3 g, 1.2 mmol). Yellow solid (0.25 g, 96%); mp: 96–97 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.33 (d, J = 7.2 Hz, 1H), 7.15 (t, J = 8.0 Hz, 1H), 6.96 (s, 1H), 6.86 (d, J = 7.6 Hz, 1H), 6.70 (d, J = 9.2 Hz, 1H), 5.52 (s, 2H), 3.22–3.13 (m,1H), 0.92 (d, J = 6.4 Hz, 6H); LC–MS (ES+) m/z 215 $(M+H)^+$; HRMS (ES+) calculated for $C_9H_{15}N_2O_2S$ $(M+H)^+$ 215.0849, found 215.0860.

6.11. Synthesis of library 13a–13l

6.11.1. 4-(3-Chloro-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-*N*-*o*-tolyl-benzenesulfonamide (13a)

A well-stirred suspension of 4-amino-*N*-*o*-tolyl-benzenesulfonamide **12a** (47 mg, 179 mmol) and 2,3-dichloro-1,4-naphthoquinone (41 mg, 179 mmol) in 95% EtOH in water (10.0 mL) was refluxed at 115 °C for three days. The orange precipitate obtained was filtered and washed with hot ethanol (5 × 5 mL), concentrated and dried (under vacuum) to afford the title compound (23 mg, 28%). mp: 265–266 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (br s, 1H), 9.45 (br s, 1H), 8.04 (d, J = 7.6 Hz, 2H), 7.89–7.80 (m, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.17–7.07 (m, 5H), 6.98–6.96 (m, 1H), 1.97 (s, 3H); LRMS (ES+) 453 $(M^{35}Cl+H)^+$, 455 $(M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $C_{23}H_{18}ClN_2O_4S$ $(M+H)^+$ 453.0670, found 453.0665.

6.11.2. 4-(3-Chloro-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-*N*-phenylbenzenesulfonamide (13b)

This compound was prepared according to the procedure described for compound **13a** except using **12b** to obtain the required product as an orange-red solid (79 mg, 41%). mp: 220–223 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (br s, 1H), 9.50 (br s, 1H), 8.03–8.01 (m, 2H), 7.86–7.79 (m, 2H), 7.60 (d, J = 8.8 Hz, 2H), 7.20 (t, J = 7.6 Hz, 2H), 7.14 (d, J = 8.4 Hz, 2H), 7.06 (d, J = 8.4 Hz, 2H), 7.00 (t, J = 7.6 Hz, 1H); LRMS (ES+) 439 $(M^{35}Cl+H)^+$, 441 $(M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $C_{22}H_{16}ClN_2O_4S$ $(M+H)^+$ 439.0514, found 439.0508.

6.11.3. 4-(3-Chloro-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-*N*-*m*-tolylbenzenesulfonamide (13c)

This compound was prepared according to the procedure described for compound **13a** except using **12c** to obtain the required product as an orange-red solid (139 mg, 56%). mp: 234–237 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (br s, 1H, disappeared on D_2O shake), 9.50 (br s, 1H, disappeared on D_2O shake), 8.04–8.01 (m, 2H), 7.88–7.79 (m, 2H), 7.61 (d, J = 8.4 Hz, 2H), 7.14 (d, J = 8.8 Hz, 2H), 7.08 (t, J = 7.6 Hz, 1H), 6.87–6.81 (m, 3H), 2.17 (s, 3H); LRMS (ES+) for 453 $(M^{35}Cl+H)^+$, 455 $(M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $C_{23}H_{18}ClN_2O_4S$ $(M+H)^+$ 453.0670, found 453.0662.

6.11.4. 4-(3-Chloro-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-*N*-(2-methoxyphenyl)-benzenesulfonamide (13d)

This compound was prepared according to the procedure described for compound **13a** except using **12d** to obtain the required product as an orange solid (41 mg, 28%). mp: 198–201 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (br s, 1H), 9.34 (br s, 1H), 8.04–8.02 (m, 2H), 7.88–7.79 (m, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.20 (d, J = 8.0 Hz, 1H), 7.14–7.08 (m, 3H), 6.90–6.83 (m, 2H), 3.52 (s, 3H); LRMS (ES+) 469 $(M^{35}Cl+H)^+$, 471 $(M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $C_{23}H_{18}ClN_2O_5S$ $(M+H)^+$ 469.0620, found 469.0609.

6.11.5. 4-(3-Chloro-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-*N*-(3-methoxyphenyl)-benzenesulfonamide (13e)

This compound was prepared according to the procedure described for compound **13a** except using **12e** to obtain the required product as an orange solid, (135 mg, 53%). mp: 213–216 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (br s, 1H), 9.51 (br s, 1H), 8.04–8.01 (m, 2H), 7.88–7.79 (m, 2H), 7.63 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H), 7.11 (t, J = 8.0 Hz, 1H), 6.65–6.63 (m, 2H),

6.58 (apparent dd, J = 8.4, 2.4 Hz, 1H), 3.64 (s, 3H): LRMS (ES+) 469 ($M^{35}Cl+H)^+$, 471 ($M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $C_{23}H_{18}ClN_2O_5S$ $(M+H)^+$ 469.0620, found 469.0611.

6.11.6. 4-(3-Chloro-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-*N*-(4-methoxyphenyl)-benzenesulfonamide (13f)

This compound was prepared according to the procedure described for compound **13a** except using **12f** to obtain the required product as a yellow-orange solid (295 mg, 78%). mp: 233–234 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (br s, 1H), 9.50 (br s, 1H), 8.03 (d, J = 7.2 Hz, 2H), 7.86–7.79 (m, 2H), 7.53 (d, J = 8.8 Hz, 2H), 7.13 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 8.8 Hz, 2H), 6.78 (d, J = 8.8 Hz, 2H), 3.65 (s, 3H); LRMS (ES+) 469 ($M^{35}Cl+H)^+$, 471 ($M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $C_{23}H_{18}ClN_2O_5S$ $(M+H)^+$ 469.0620, found 469.0612.

6.11.7. 4-(3-Chloro-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-*N*-*p*-tolylbenzenesulfonamide (13g)

This compound was prepared according to the procedure described for compound **13a** except using **12g** to obtain the required product as a yellow-orange solid (245 mg, 63%). mp: 257–260 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (br s, 1H), 9.49 (br s, 1H), 8.04–8.01 (m, 2H), 7.86–7.79 (m, 2H), 7.58 (d, J = 8.4 Hz, 2H), 7.13 (d, J = 8.8 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 6.94 (d, J = 8.4 Hz, 2H), 2.17 (s, 3H); LRMS (ES+) 453 ($M^{35}Cl+H)^+$, 455 ($M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $C_{23}H_{18}ClN_2O_4S$ $(M+H)^+$ 453.0670, found 453.0661.

6.11.8. 4-(3-Chloro-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-2-methoxy-*N*-*o*-tolyl-benzenesulfonamide (13h)

This compound was prepared according to the procedure described for compound **13a** except using **12h** to obtain the required product as an orange-red solid (55 mg, 35%). mp: 197–200 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.46 (br s, 1H), 9.15 (br s, 1H), 8.04 (dd, J = 7.6, 1.2 Hz, 2H), 7.87–7.80 (m, 2H), 7.42 (d, J = 8.4 Hz, 1H), 7.12–7.09 (m, 1H), 7.04–6.97 (m, 3H), 6.89 (d, J = 1.6 Hz, 1H), 6.65 (dd, J = 8.4, 1.6, Hz, 1H), 3.78 (s, 3H), 2.14 (s, 3H); LRMS (ES+) 483 ($M^{35}Cl+H)^+$, 485 ($M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $(M+H)^+$ $C_{24}H_{20}ClN_2O_5S$ 483.0776, found 483.0771.

6.11.9. 4-(3-Chloro-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-2-methoxy-*N*-*p*-tolyl-benzenesulfonamide (13i)

This compound was prepared according to the procedure described for compound **13a** except using **12i** to obtain the required product as a red solid (80 mg, 38%). mp: 186–189 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (br s, 1H), 9.40 (br s, 1H), 8.04–8.01 (m, 2H), 7.86–7.79 (m, 2H), 7.55 (d, J = 8.4 Hz, 1H), 6.97–6.92 (m, 4H), 6.93 (d overlapped, J = 8.8 Hz, 2H), 6.80 (d, J = 1.6 Hz, 1H), 6.65 (dd, J = 8.6, 1.8 Hz, 1H), 3.79 (s, 3H), 2.14 (s, 3H); LRMS (ES+) 483 ($M^{35}Cl+H)^+$, 485 ($M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $C_{24}H_{20}ClN_2O_5S$ $(M+H)^+$ 483.0776, found 483.0769.

6.11.10. 4-(3-Chloro-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-2-methoxy-*N*-(4-methoxyphenyl)-benzenesulfonamide (13j)

This compound was prepared according to the procedure described for compound **13a** except using **12j** to obtain the required product as a red solid (118 mg, 54%). mp: 165–167 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (br s, 1H), 9.40 (br s, 1H), 8.04–8.01 (m, 2H), 7.88–7.79 (m, 2H), 7.48 (d, J = 8.8 Hz, 1H), 6.96 (d, J = 8.8 Hz, 2H), 6.82 (br s, 1H), 6.74 (d, J = 8.8 Hz, 2H), 6.63 (dd, J = 8.6, 1.4 Hz, 1H), 3.82 (s, 3H), 3.63 (s, 3H); LRMS (ES+) 499 ($M^{35}Cl+H)^+$, 501 ($M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $C_{24}H_{20}ClN_2O_6S$ $(M+H)^+$ 499.0725, found 499.0717.

6.11.11. 4-(3-Chloro-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-2-methoxy-*N*-*m*-tolyl-benzenesulfonamide (13k)

This compound was prepared according to the procedure described for compound **13a** except using **12k** to obtain the required product as an orange solid, (91 mg 64%). mp: 264–267 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (br s, 1H), 9.41 (br s, 1H), 8.04–8.01 (m, 2H), 7.83 (dt, J = 7.2, 1.2 Hz, 1H), 7.79 (dt, J = 7.2, 1.2 Hz, 1H), 7.60 (d, J = 8.8 Hz, 1H), 7.03 (t, J = 8.2 Hz, 1H), 6.86–6.84 (m, 2H), 6.80 (d, J = 2.0 Hz, 1H), 6.76 (d, J = 7.6 Hz, 1H), 6.67 (dd, J = 8.6, 1.8 Hz, 1H), 3.78 (s, 3H), 2.15 (s, 3H); LRMS (ES+) 483 ($M^{35}Cl+H)^+$, 485 ($M^{37}Cl+H)^+$; HRMS (ES+) m/z calculated for $C_{24}H_{20}ClN_2O_5S$ $(M+H)^+$ 483.0803, found 483.0809.

6.11.12. *N*-Carbamimidoyl-4-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)benzenesulfonamide (13l)

This compound was prepared according to the procedure for compound **13a** except using compound **12l** to afford the title compound as an orange-red solid (35 mg, 54.2%). mp: 294–295 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (br s, 1H), 8.05 (d, J = 7.2 Hz, 2H), 7.86–7.80 (m, 2H), 7.65 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 8.5 Hz, 2H), 6.70 (br s, 4H); LC–MS (ES+) 405 ($M^{35}Cl+H)^+$, 407 ($M^{37}Cl+H)^+$**;** HRMS (ES+) m/z calculated for $C_{17}H_{14}ClN_4O_4S$ $(M+H)^+$ 405.0419, found 405.0408.

6.12. General procedure for synthesis of 13m–13r

Amino-*N*-alkylbenzenesulfonamides **12m–12r** (0.1 g, 0.499 mmol) and 2,3-dichloronaphthoquinone (0.11 g, 0.499 mmol) were dissolved in 95% ethanol in water (5 mL). The reaction mixture was heated at 120 °C and stirred for 3 days in a sealed tube until TLC (50% ethyl acetate in hexane) indicated completion of the reaction. The solvent was partially evaporated and the solid was filtered and washed with ethanol (20–30 mL). The pure products obtained were dried under vacuum to afford orange or brown solid.

6.12.1. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-methylbenzenesulfonamide (13m)

Orange solid (0.122 g, 61%); mp: 277–278 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H disappeared on D_2O shake), 8.02 (d, J = 7.6 Hz, 2H), 7.87 (dt, J = 7.6, 1.6 Hz, 1H), 7.80 (dt, J = 7.2, 1.2 Hz, 1H), 7.63 (d, J = 8.8 Hz, 2H), 7.32 (apparent q, J = 4.8 Hz, 1H disappeared on D_2O shake), 7.22 (d, J = 8.8 Hz, 2H), 2.37 (d, J = 5.2 Hz, 3H changed to singlet on D_2O shake); LC–MS (ES+) m/z 377 $(M+H)^+$; HRMS (ES+) calculated for $C_{17}H_{14}ClN_2O_4S$ $(M+H)^+$ 377.0357, found 377.0355.

6.12.2. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-ethylbenzenesulfonamide (13n)

Orange solid (0.125 g, 62%); mp: 240–241 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H disappeared on D_2O shake), 8.03 (d, J = 11.6 Hz, 2H), 7.88–7.79 (m, 2H), 7.65 (d, J = 7.6 Hz, 2H), 7.43 (t, J = 8.0 Hz, 1H disappeared on D_2O shake), 7.21 (d, J = 6.4 Hz, 2H), 2.79–2.72 (m, 2H, changed to a quartet on D_2O shake), 0.94 (t, J = 8.0 Hz, 3H); LC–MS (ES+) m/z 391 $(M+H)^+$; HRMS (ES+) calculated for $C_{18}H_{16}ClN_2O_4S$ $(M+H)^+$ 391.0514, found 391.0502.

6.12.3. 4-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-*N*-isopropylbenzenesulfonamide (13o)

Light brown solid (0.32 g, 14%); mp: 244–245 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.03 (dd, J = 7.6, 1.6 Hz, 2H), 7.87 (dt, J = 7.6, 1.6 Hz, 1H), 7.81 (dt, J = 7.2, 2.0 Hz, 1H), 7.68 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 7.2 Hz, 1H), 7.21 (d, J = 8.4 Hz, 2H), 3.24–3.19 (m, 1H), 0.92 (d, J = 6.8 Hz, 6H); LC–MS (ES+) m/z 405

$(M+H)^+$; HRMS (ES+) calculated for $C_{19}H_{18}ClN_2O_4S$ $(M+H)^+$ 405.0670, found 405.0664.

6.12.4. 2-Chloro-3-(3-(methylaminoperoxythio)phenylamino) naphthalene-1,4-dione (13p)

Brown solid (0.28 g, 97%); mp: 229–230 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H disappeared on D_2O shake), 8.04–8.02 (m, 2H), 7.82 (dt, J = 7.6, 1.2 Hz, 1H), 7.76 (dt, J = 7.6, 1.2 Hz, 1H), 7.53–7.45 (m, 4H changed to 3H on D_2O shake), 7.35 (d, J = 4.0 Hz, 1H), 2.79–2.76 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H); LC–MS (ES+) m/z 377.03 $(M+H)^+$; HRMS (ES+) calculated for $C_{17}H_{14}ClN_2O_4S$ $(M+H)^+$ 377.0357, found 377.0370.

6.12.5. 2-Chloro-3-(3-(ethylaminoperoxythio)phenylamino) naphthalene-1,4-dione (13q)

Orange solid (0.193 g, 99%); mp: 239–240 °C decomposed; ^1H NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.04–8.01 (m, 2H), 7.86 (dt, J = 7.6, 1.2 Hz, 1H), 7.81 (dt, J = 7.6, 1.2 Hz, 1H), 7.55 (t, J = 5.6 Hz, 1H), 7.50–7.48 (m, 2H), 7.35–33 (m, 1H), 2.79–2.76 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H); LC–MS (ES−) m/z 389 $(M-H)^-$; HRMS (ES−) calculated for $C_{18}H_{16}ClN_2O_4S$ $(M-H)^-$ 389.0369, found 389.0371.

6.12.6. 2-Chloro-3-(3-(isopropylaminoperoxythio) phenylamino)naphthalene-1,4-dione (13r)

Orange solid (0.64 g, 34%); mp: 211–212 °C; ^1H NMR (400 MHz, MeOH-d_4) δ 8.13 (dd, J = 3.2, 1.2 Hz, 1H), 8.12 (dd, J = 4.8, 1.6 Hz, 1H), 7.83 (dt, J = 9.2, 1.6 Hz, 1H), 7.77 (dt, J = 7.6, 1.2 Hz, 1H), 7.65 (ddd, J = 7.6, 2.0, 1.2 Hz, 1H), 7.56 (t, J = 1.6 Hz, 1H), 7.51 (t, J = 8.0 Hz, 1H), 7.34 (ddd, J = 8.0, 2.4, 1.2 Hz, 1H), 3.32 (m, partially overlapped with residual MeOH signal), 1.04 (d, J = 6.4 Hz, 6H); LC–MS (ES+) m/z 405.07 $(M+H)^+$; HRMS (ES+) calculated for $C_{19}H_{18}ClN_2O_4S$ $(M+H)^+$ 405.0670, found 405.0664.

Acknowledgment

This work was supported by NIH Grant P01CA118210.

References and notes

1. Groll, M.; Ditzel, L.; Loewe, J.; Stock, D.; Bochtler, M.; Bartunik, H. D.; Huber, R. *Nature* **1997**, *386*, 463; Groll, M.; Berkers, C. R.; Ploegh, H. L.; Ovaa, H. *Structure (Cambridge, MA, US)* **2006**, *14*, 451.
2. Burger, A. M.; Seth, A. K. *Eur. J. Cancer* **2004**, *40*, 2217; Hjerpe, R.; Rodriguez, M. S. *Int. J. Biochem. Cell Biol.* **2008**, *40*, 1126.
3. Yamasaki, L.; Pagano, M. *Curr. Opin. Cell Biol.* **2004**, *16*, 623.
4. Ostrowska, H. *Cell. Mol. Biol. Lett.* **2008**, *13*, 353.
5. Bennett, M. K.; Kirk, C. J. *Curr. Opin. Drug Disc. Dev.* **2008**, *11*, 616.
6. Adams, J. *Nat. Rev. Cancer* **2004**, *4*, 349; Orlowski, R. Z.; Kuhn, D. J. *Clin. Cancer Res.* **2008**, *14*, 1649.
7. Voorhees, P. M.; Dees, E. C.; O'Neil, B.; Orlowski, R. Z. *Clin. Cancer Res.* **2003**, *9*, 6316; Nalepa, G.; Rolfe, M.; Harper, J. W. *Nat. Rev. Drug Disc.* **2006**, *5*, 596; Zavrski, I.; Jakob, C.; Schmid, P.; Krebbel, H.; Kaiser, M.; Fleissner, C.; Rosche, M.; Possinger, K.; Sezer, O. *Anti-Cancer Drugs* **2005**, *16*, 475.
8. Jung, L.; Holle, L.; Dalton William, S. *Oncology* **2004**, *18*, 4; Lara, P. N., Jr.; Davies, A. M.; Mack, P. C.; Mortenson, M. M.; Bold, R. J.; Gumerlock, P. H.; Gandara, D. R. *Semin. Oncol.* **2004**, *31*, 40; Adams, J. *Semin. Oncol.* **2001**, *28*, 613.
9. Bang, S.-M.; Lee, J. H.; Yoon, S.-S.; Park, S.; Min, C.-K.; Kim, C.-C.; Suh, C.; Sohn, S. K.; Min, Y.-H.; Lee, J.-J.; Kim, K.; Seong, C.-M.; Yoon, H.-J.; Cho, K. S.; Jo, D.-Y.; Lee, K. H.; Lee, N.-R.; Kim, C. S. *Int. J. Hematol.* **2006**, *83*, 309.
10. Sterz, J.; von Metzler, I.; Hahne, J.-C.; Lamottke, B.; Rademacher, J.; Heider, U.; Terpos, E.; Sezer, O. *Expert Opin. Invest. Drugs* **2008**, *17*, 879; Kuhn, D. J.; Chen, Q.; Voorhees, P. M.; Strader, J. S.; Shenk, K. D.; Sun, C. M.; Demo, S. D.; Bennett, M. K.; van Leeuwen, F. W. B.; Chanan-Khan, A. A.; Orlowski, R. Z. *Blood* **2007**, *110*, 3281.
11. Piva, R.; Ruggeri, B.; Williams, M.; Costa, G.; Tamagno, I.; Ferrero, D.; Giai, V.; Coscia, M.; Peola, S.; Massaia, M.; Pezzoni, G.; Allievi, C.; Pescalli, N.; Cassin, M.; di Giovine, S.; Nicoli, P.; de Feudis, P.; Strepponi, I.; Roato, I.; Ferracini, R.; Bussolati, B.; Camussi, G.; Jones-Bolin, S.; Hunter, K.; Zhao, H.; Neri, A.; Palumbo, A.; Berkers, C.; Ovaa, H.; Bernareggi, A.; Inghirami, G. *Blood* **2008**, *111*, 2765.
12. Fenical, W.; Jensen Paul, R.; Palladino Michael, A.; Lam Kin, S.; Lloyd, G. K.; Potts Barbara, C. *Bioorg. Med. Chem.* **2009**, *17*, 2175; Groll, M.; McArthur, K. A.; Macherla, V. R.; Manam, R. R.; Potts, B. C. *J. Med. Chem.* **2009**, *52*, 5420; Chauhan, D.; Hideshima, T.; Anderson, K. C. *Br. J. Cancer* **2006**, *95*, 961.
13. Piccinini, M.; Mostert, M.; Rinaudo, M. T. *Curr. Drug Targets* **2003**, *4*, 657; Crawford, L. J.-A.; Walker, B.; Irvine, A. E. *Front. Biosci.* **2008**, *13*, 4285.
14. Guedat, P.; Colland, F. *BMC Biochem.* **2007**, *8*, S14; Kazi, A. D. K.; Smith, D. M.; Kumar, N. B.; Dou, Q. P. *Biochem. Pharmacol.* **2003**, *66*, 965.
15. Kazi, A.; Lawrence, H.; Guida, W. C.; McLaughlin, M. L.; Springett, G. M.; Berndt, N.; Yip, R. M. L.; Sebti, S. M. *Cell Cycle* **2009**, *8*, 1940.
16. Ahn, J. H.; Cho, S. Y.; Ha, J. D.; Chu, S. Y.; Jung, S. H.; Jung, Y. S.; Baek, J. Y.; Choi, I. K.; Shin, E. Y.; Kang, S. K.; Kim, S. S.; Cheon, H. G.; Yang, S.-D.; Choi, J.-K. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1941.
17. Urbanek, R. A.; Suchard, S. J.; Steelman, G. B.; Knappenberger, K. S.; Sygowski, L. A.; Veale, C. A.; Chapdelaine, M. J. *J. Med. Chem.* **2001**, *44*, 1777.
18. Brun, M.-P.; Braud, E.; Angotti, D.; Mondesert, O.; Quaranta, M.; Montes, M.; Miteva, M.; Gresh, N.; Ducommun, B.; Garbay, C. *Bioorg. Med. Chem.* **2005**, *13*, 4871.
19. Calandra, J. C.; Adams, E. C., Jr. *J. Am. Chem. Soc.* **1950**, *72*, 4804; Calandra, J. C.; Adams, E. C., Jr. U.S. Patent 2647123, 1953.
20. Blackburn, C. *Tetrahedron Lett.* **2005**, *46*, 1405.
21. Walz, A. J.; Sundberg, R. J. *J. Org. Chem.* **2000**, *65*, 8001.
22. Nagy, H. K.; Tomson, A. J.; Horwitz, J. P. *J. Am. Chem. Soc.* **1960**, *82*, 1609.
23. 70 ng of purified 20S rabbit proteasome was incubated with 20 μM Suc-Leu-Leu-Val-Tyr-AMC for the CT-L activity, Bz-Val-Gly-Arg-AMC for the T-L activity, and benzyloxycarbonyl Z-Leu-Leu-Glu-AMC for the PGPH activity for 1 h at 37 °C in 100 μL of assay buffer (50 mM Tris–HCl, pH 7.6) with or without inhibitors. After incubation, production of hydrolyzed 7-amido-4-methyl-coumarin (AMC) was measured using a WALLAC Victor2 1420 Multilabel Counter with an excitation filter of 355 nm and an emission filter of 460 nm (Perkin Elmer Life Sciences, Turku, Finland). The inhibitory activity of the compounds was calculated based on vehicle control.
24. Groll, M.; Berkers, C. R.; Ploegh, H. L.; Ovaa, H. *Structure (Cambridge, MA, US)* **2006**, *14*, 451.
25. LigPrep, Version 2.2, Schrödinger, LLC, New York, 2005.
26. Shelley, J. C.; Cholleti, A.; Frye, L. L.; Greenwood, J. R.; Timlin, M. R.; Uchimaya, M. *J. Comput. Aided. Mol. Des.* **2007**, *21*, 681.
27. Cho, A. E.; Guallar, V.; Berne, B.; Friesner, R. A. *J. Comput. Chem.* **2005**, *26*, 915.
28. *Dialysis using purified rabbit 20S proteasome*: To measure the effect of dialysis on CT-L activity, compounds (10 μM) or vehicle (0.1% DMSO) were added to rabbit 20S proteasome at a final concentration of 3 nM in proteasome assay buffer (50 mM Tris–HCl, pH 7.6) and incubated at room temperature for 30 min. After 30 min of incubation, proteasome-compound mixtures were added to 10,000 MWCO Thermo Scientific Slide-A-Lyzer Dialysis Cassette (Rockford, IL) and dialyzed against proteasome assay buffer. Immediately (t = 0) and 0.5, 1, 2, 4, and 18 h of dialysis at 4 °C, samples were removed from the dialysis cassette and the CT-L 20S proteasome activity was determined as described in Ref. [23]. Proteasome activity was normalized against proteasome activity of DMSO control.
29. Glide, Version 5.0, Schrödinger, LLC, New York, 2008.
30. Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F., Jr.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. *J. Mol. Biol.* **1977**, *112*, 535.
31. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. *J. Med. Chem.* **2006**, *49*, 6177.
32. Maestro, Version 8.5, Schrödinger, LLC, New York, 2008.
33. Glide, Version 3.0, Schrödinger, LLC, New York, 2004.
34. Mohamadi, F.; Richard, N. G.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
35. Miki, T.; Kori, M.; Mabuchi, H.; Banno, H.; Tozawa, R.-i.; Nakamura, M.; Itokawa, S.; Sugiyama, Y.; Yukimasa, H. *Bioorg. Med. Chem.* **2001**, *10*, 401.
36. Le Pera, A.; Leggio, A.; Liguori, A. *Tetrahedron* **2006**, *62*, 6100.
37. Zheng, X.; Oda, H.; Takamatsu, K.; Sugimoto, Y.; Tai, A.; Akaho, E.; Ali, H. I.; Oshiki, T.; Kakuta, H.; Sasaki, K. *Bioorg. Med. Chem.* **2007**, *15*, 3299.
38. Felt, P. W. *Acta Chem. Scand.* **1962**, *16*, 297.
39. Bezzi, S.; Concilio, C.; Zambon, A. *Farmaco (1946–1952)* **1950**, *5*, 260.
40. Fishwick, B. R. Application: GB, GB Patent 908656, 1962.
41. DeLano, W. L. The PyMol Molecular Graphics System, version 0.99, DeLano Scientific, Palo Alto, CA, 2004.