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Article

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Design, synthesis and biological evaluation of sulfonyl acrylonitriles as novel inhibitors of cancer metastasis and spread.

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Abstract

The spread of intra-abdominal cancers is a vexing clinical problem for which there is no widely effective treatment. We discovered previously that (2E)-3-[(4-tert-Butylphenyl)sulfonyl]acrylonitrile (**1**) induced cancer cell apoptosis during adhesion to normal mesothelial cells, which line the peritoneum. We recently demonstrated that the sulfonylacrylonitrile portion of **1** and hydrophobic aryl substitution were essential for pro-apoptotic activity in cancer cells. Here we synthesized a diverse series of analogs of **1** in order to improve the efficacy and pharmaceutical properties. Analogs and **1** were compared in their ability to cause cancer cell death during adhesion to normal mesothelial cell monolayers. Potent analogs identified in the *in vitro* assay were validated and found to exhibit improved inhibition of intra-abdominal cancer spread in two clinically relevant murine models of ovarian and pancreatic cancer spread and metastasis, highlighting their potential clinical use as an adjunct to surgical resection of cancers.

Introduction

Peritoneal carcinomatosis is caused by cancer cell implantation of the peritoneal lining of the abdominal cavity. The peritoneum is a thin tissue that lines the abdominal organs and abdominal wall. The mesothelial cells form a monolayer of cells on the surface of the peritoneum and produce peritoneal fluid that serves to lubricate the peritoneal surfaces, significantly reducing friction between the abdominal organs and cavity. Malignant gastrointestinal and gynecological tumors,

such as advanced pancreatic cancer and ovarian cancer, commonly spread to the peritoneum.[1-6] Approximately 45,220 new cases of and 38,460 deaths from pancreatic cancer occurred in 2013 in the United States, and 22,240 new cases of and 14,030 deaths from ovarian cancer were estimated.[7] As treatment options, surgery, radiation therapy and chemotherapy may extend survival and/or relieve symptoms in many patients, but seldom produce a cure for advanced cases. [8, 9]

During the initial steps of peritoneal carcinomatosis, tumor cells are first shed from intra-abdominal cancers or dislodged during surgical resection, and gain access to the peritoneal cavity.[10-15] The successful implantation of the mesothelium is the key initial step in peritoneal carcinomatosis,[16, 17] and treatments that inhibit this step may significantly decrease recurrence and peritoneal carcinomatosis.

Our previous work showed that the small molecule **1** could inhibit peritoneal carcinomatosis in a murine model.[18] **1** was found to inhibit c-FLIP (FLICE Inhibitory Protein), a key inhibitor of death receptor-mediated apoptosis, which is commonly overexpressed in cancer cells.[19] Many cancer cells express death receptors of the TNFα superfamily yet evade death receptor-mediated apoptosis through the overexpression of survival proteins such as c-FLIP.[19] The over expression of death receptors by cancer cells appears to be conserved as they have been shown to mediate cancer cell proliferation.[20] Many normal tissues contain cells, such as mesothelial cells, that express cognate death receptor ligands.[21] We showed that when cancer cells adhere to mesothelial cells, the death receptor ligands expressed

by mesothelial cells can activate death receptors expressed on cancer cells in a juxtacrine fashion which results in apoptosis if **1** is present.[19] Thus, **1**-mediated inhibition of c-FLIP expression restores death receptor-mediated cancer cell apoptosis during their adhesion to normal human mesothelial cells.[19]

In a recent study, several analogs of **1** were synthesized to determine the role of the sulfonylacrylonitrile moiety and assess the potential of an amide replacement for the *tert*-butyl fragment of **1**.[22] While studies of these analogs pointed out the importance of the functional groups to the pro-apoptotic efficacy of **1**, few of the analogs demonstrated increased efficacy in pancreatic, ovarian, or colon cancer cells compared with the parent compound. In addition, although **1** was effective in inhibiting peritoneal carcinomatosis in preclinical models, its relative insolubility in water required the use of DMSO as a delivery vehicle, which impairs its potential clinical use. The aims of the present study were to improve the efficacy of the parent compound through structural modification strategies outlined (**Figure 1**) and test lead analogs for efficacy in clinically relevant *in vivo* models of intra-abdominal cancers. The incorporation of additional polarity was a priority in the design of new analogs in the hope of increasing potency and drug like character.

Results and Discussion

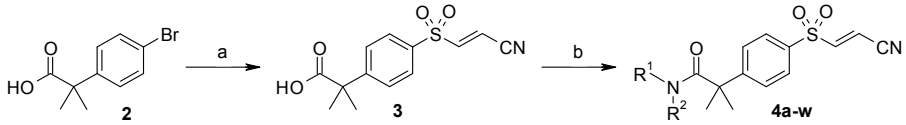
Design. In our preliminary report,[22] analogs of **1** wherein a methyl of the *t*-butyl group is replaced by an amide were designed and evaluated for their ability to induce apoptosis in pancreatic and ovarian cancer cells during adhesion to

mesothelial cell monolayers. Secondary and tertiary amide analogs displayed comparable activity to **1** in co-culture assay across four pancreatic and ovarian cancer cell lines. Any modification to the sulfonyl acrylonitrile fragment resulted in reduced potency in the co-culture assays, highlighting the need of a strong Michael acceptor for activity. To establish a more complete picture of the structure-activity relationship (SAR) and identify compounds with improved in vivo potency versus compound **1**, a systematic approach to modifying compound **1** was undertaken (**Figure 1**). Modification of the *t*-butyl or phenyl ring of **1** resulted in the synthesis of five different classes of compounds. Based on promising in vivo activity demonstrated in a luciferase tagged A2780 ovarian peritoneal carcinomatosis mouse model,[22] additional aromatic, aliphatic and benzylic amides were prepared to expand the Series 1 amide analogs. Bioisosteric replacement of the amide moiety in Series 1 provided the heterocyclic analogs of Series 2. Keeping the amide fragment in place and tethering the remaining geminal methyl groups of **1** into a series of cyclic products furnished Series 3. Appending the amide fragment to the phenylsulfonyl group through an ether linkage, while migrating the *t*-butyl substituent to the *meta*-position afforded the phenoxy amide Series 4. Lastly, the phenyl ring was replaced with either a pyridine or quinoline ring to afford the Series 5 heterocyclic analogs.

Chemistry. 4-Bromophenyl-2-propanoic acid (**2**) served as the starting point for all of the amides of Series 1 (Scheme 1). Deprotonation and metalation, followed by a sulfur dioxide quench furnished the intermediate sulfinic acid, which was converted

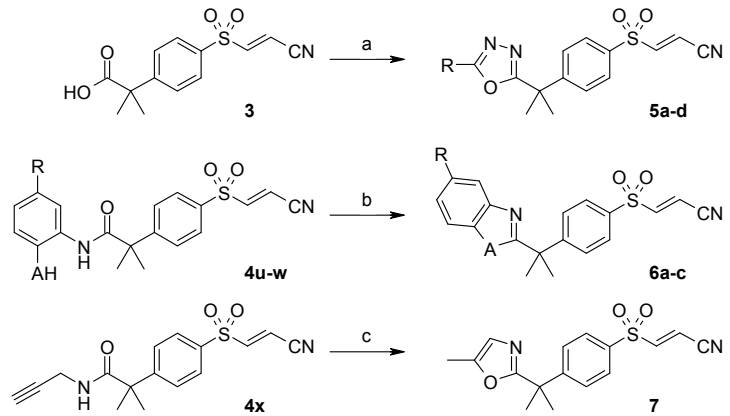
to the arylsulfonyl acrylonitrile in modest yield. Routine coupling of aliphatic and aromatic amines with **3** furnished amides **4a-t** as shown in **Table 1**. The Series 2 heterocyclic analogs also utilized **3** as a starting material (**Scheme 2**); oxadiazole analogs were prepared via a direct coupling[23] with substituted thiosemicarbazides (**5a-b**) or via a two step coupling/dehydration with acyl hydrazines (**5c-d**). Amide analogs **4u-w** were cyclodehydrated under acidic conditions to afford the benzoxazole analogs **6a-b** and benzimidazole **6c**. Propargyl amide **4x** was similarly cyclized, under gold catalysis,[24] to afford the methyl oxazole **7**.

Scheme 1. Synthesis of amide analogs[a]



[a] Reagents and conditions: (a) *i*: PhLi, *n*-BuLi, −80 °C; *ii*: SO_2, −80 °C to rt; *iii*: 2-chloroacrylonitrile, AcOH, MeOH, rt; *iv*: Et_3N, 43% (b) amine, EDCI, HOBT, rt, 22-67%.

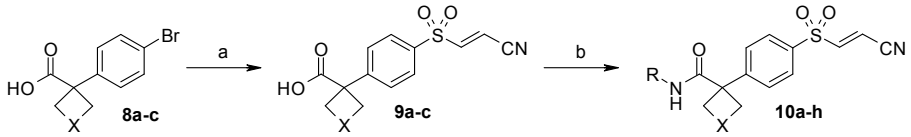
Scheme 2. Synthesis of heterocyclic amide isosteres[a]



[a] Reagents and conditions: (a) $RCSNHNH_2$, 3 equiv EDCI, DCM, rt, 11-19%; or *i*: $RCONHNH_2$, EDCI, HOBT, MeCN, rt, 4h; *ii*: dioxane, $POCl_3$, 90 °C, 2h, 72-76%; (b) MsOH, 1,4-dioxane, 90 °C, 24h, 55% or AcOH, 70-105 °C, 1-3 d, 11-24%; (c) $AuCl_3$, MeCN, 4 d, 30%.

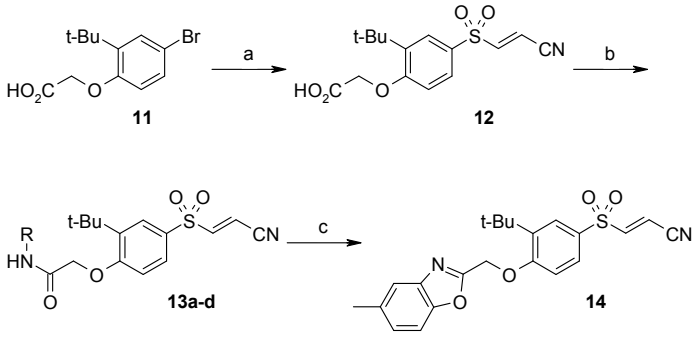
The synthesis of the cyclic amide analogs **10a-h** (Scheme 3) followed the same sequence as the Series 1 amides, simply starting with the appropriately tethered cyclic carboxylic acid **8a-c** (X = direct bond, CH_2 or $–CH_2OCH_2–$). The phenoxy amide analogs of Series 4 utilized bromide **11**[25] as a precursor to the aryl sulfonyl acrylonitrile derivatives **13a-e** (Scheme 4).

Scheme 3. Synthesis of cyclic amide analogs[a]



[a] Reagents and conditions: (a) *i*: PhLi, *n*-BuLi, −80 °C; *ii*: SO_2, −80 °C to rt; *iii*: 2-chloroacrylonitrile, AcOH, MeOH, rt; *iv*: Et_3N, 10-27% (b) amine, EDCI, HOBT, MeCN or DCM, rt, 28-69%.

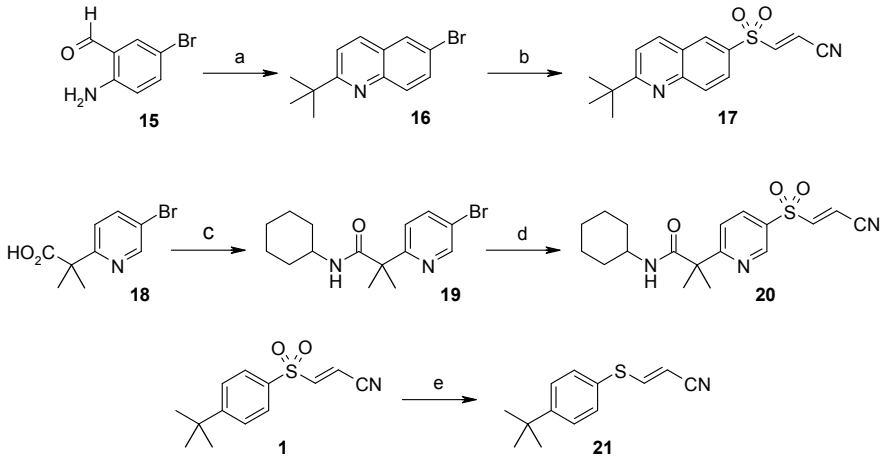
Scheme 4. Synthesis of phenoxy amide analogs[a]



[a] Reagents and conditions: (a) *i*: PhLi, *n*-BuLi, −80 °C; *ii*: SO_2, −80 °C to rt; *iii*: 2-chloroacrylonitrile, AcOH, MeOH, rt; *iv*: Et_3N, 32% (b) amine, EDCI, HOBT, MeCN or DCM, rt, 38-79%; (c) MsOH, 1,4-dioxane, 90 °C, 11 h, 50%.

Lastly, the phenyl ring of **1** was replaced with either a quinoline to afford **17** or a pyridine, with a pendant cyclohexyl amide, to afford **20**. Quinoline **16** was prepared by a Friedländer condensation of bromide **15** with pinacolone; subsequent formation of the sulfonyl acrylonitrile as earlier shown provided **17**. The reversal of the order of the amidation and sulfone formation steps was necessary to prepare pyridine **20** to avoid decomposition observed in reactions of carboxylic acid **18** with *n*-butyllithium. The reduced sulfide **21**, readily prepared from **1**, was used as a negative control, highlighting the need for the sulfone oxidation state in active analogs.

Scheme 5. Synthesis of heteroaryl analogs and a negative control[a]



[a] Reagents and conditions: (a) *t*-$BuCOCH_3$, KOH, water, 100 °C, 3 h, 64%; (b) *i*: PhLi, *n*-BuLi, −80 °C; *ii*: SO_2, −80 °C to rt; *iii*: 2-chloroacrylonitrile, AcOH, MeOH, rt; *iv*: Et_3N, 59%; c) cyclohexylamine, Et3N,

propylphosphonic anhydride, DMF, rt, 51%; d) *i*: PhLi, *n*-BuLi, −80 °C; *ii*: SO_2, −80 °C to rt; *iii*: 2-chloroacrylonitrile, AcOH, MeOH, rt; *iv*: Et_3N, 15%; e) 4-*t*-BuPhSH, Et_3N, $CHCl_3$, 97%.

Biological Evaluation of Inhibitors

Screening was conducted against two pancreatic cancer cell lines (SU.86 and BxPC-3) and two ovarian cancer cell lines (A2780 and SKOV3) that were transduced with a constitutively expressing firefly luciferase plasmid which allowed for specific bioluminescent detection of living cancer cells. The cancer cells were suspended and then allowed to adhere to human mesothelial cell monolayers in the presence of vehicle or drug. Each series of analogs was tested at a single concentration (10 μM) and efficacy was calculated as the fraction of adherent cells which remained alive as compared to vehicle (DMSO). Surviving cancer cells were quantified by firefly luciferase activity in a luminometer. In Series 1 (Table 1), benzyl amide **4b** showed better potency than the phenyl- (**4a**) or phenethyl (**4c**) amides, and **4b** had potency close to that of **1**. Substitution of the benzyl amide was further explored; neither stereoisomer of α-methyl benzylamide (**4d** or **4e**) showed a marked improvement in potency. Aromatic substitutions were investigated and it was found that strongly polar fragments were not tolerated (sulfone **4f** or sulfonamide **4g**). A methoxy group at either the *ortho*- (**4h**) or *meta*- (**4i**) position provided highly potent analogs. In an attempt to aid aqueous solubility, the *para*- methoxy-ethoxy substituent was incorporated (**4j**) but potency against all four cell lines was diminished. Fluorine substitution was tolerated at all three positions (**4k-m**) and resulted in improved efficacy in the ovarian cell lines as compared to **4b**. A weakly

basic heterocyclic substituent showed diminished activity (**4n**) as did the difluoroethyl amide (**4o**). The aminocaprolactam containing analog **4p** was largely inactive, again pointing at the need for non-polar substitution of the amide. The phenylpropargyl amide **4q** exhibited nearly identical activity as the amide without the acetylenic spacer. A pair of *N*-methyl benzyl amides, **4r** and **4s**, as well as the piperidine amide **4t**, showed that disubstituted amides were equally efficacious in the in vitro assay.

The heterocyclic amide bioisosteres prepared in Scheme 2 followed similar polarity based trends in activity (**Table 2**). Phenylamino-oxadiazole **5a** was more potent than the ethyl derivative **5b**, but both were less potent than the aryl/alkyl substituted oxadiazoles **5c**-**d**. Benzoxazoles **6a** and **6b** were among the most potent analogs from all series whereas the benzimidazole **6c** lacked potency in all cell lines. The smaller methyloxazole **7** was also active, but not as potent as the benzoxazole analogs.

The Series 3 analogs, wherein the gem-dimethyl group of the amide analogs is tied back into a cyclic derivative, were assayed across the four cell lines (Table 3). The cyclopropane (X = direct bond, **10a-b**) and cyclobutane (X = CH_2, **10c-e**) analogs were roughly equipotent with the corresponding amide analogs from Series 1. A significant drop in activity was observed for the tetrahydropyran analogs **10f-h** (X = CH_2OCH_2).

For the Series 4 analogs, a more dramatic change in structure was implemented (**Table 4**). Shifting of the *tert*-butyl group from *para* to *meta* on the aryl sulfone was paired with a glycolic ether linkage. The glycolic acid derivative **12** was inactive as expected. The amide derivatives **13a-d** were potent in all four cell lines, particularly the BxPC-3 and A2780 cells. Taking a cue from the highly potent benzoxazole analogs of Series 2, the benzoxazole derivative **14** was prepared and found to be slightly less potent than the corresponding phenolic amide **13d**. For a direct comparison to **1**, *meta* derivative **13e** was synthesized and found to be equipotent.

Lastly, the heterocyclic derivatives **17** and **20** were assessed in the co-culture assay (**Table 5**). Quinoline replacement for phenyl (**17** vs **1**) showed equivalent potency in the ovarian cell lines, but a drop in activity in the pancreatic cell lines was observed. A pyridyl-for-phenyl replacement showed mid-range activity in the four cell lines and was comparable with the cyclohexyl amide analog previously disclosed.[22]

For active analogs, **4b**, **5d**, **6a**, **6b**, **10e**, **13a** and **13d**, in addition to **1**, a series of concentrations were used to determine half-maximal effective concentration (EC_{50}). All of these analogs decreased cancer cell viability in the co-culture assay in the micromolar range (**Table 6**).

As we previously showed, compound **1** rapidly and strongly induces apoptosis of cancer cells during adhesion.[19] The most active analogs in the co-culture assay were

tested for their ability to induce apoptosis of A2780 ovarian cancer cells by demonstrating increased cellular levels of cleaved PARP and cleaved caspase 3, which are indicators of apoptosis. While all of the analogs induced apoptosis to a degree, analogs **6b** and **13a** showed similar levels of apoptotic induction as compound **1** (**Figure 2**). The reduced sulfide **21** served as a negative control and showed no apoptotic effect.

Due to our previous studies showing that both the sulfone and nitrile groups are essential for the apoptotic activity of **1** on cancer cells, the ability of **1** and its analogs to bind glutathione (GSH) was examined. Active analogs **4k**, **4r**, along with **1,** strongly reduced detectable GSH levels, while two inactive analogs,, **22** and **23** (**Figure 3F**), that were previously reported to be inactive compared to **1**[22], did not (**Figure 3A**). To determine if the compounds could diminish GSH in cancer cells *in vivo*, **1**, **4k**, and **4r** and inactive analogs (**22**, **23**), were tested in HT29 cells. Only **1**, **4k**, and **4r** could decrease cellular GSH levels *in vivo* while **22** and **23** could not (**Figure 3B**). The assay used to measure cellular glutathione levels *in vivo* could not distinguish if **1** and its active analogs diminished cellular glutathione levels by inhibiting its synthesis or through covalent modification. Therefore, transiently suspended HT-29 cancer cells were exposed to **1,** in the absence or presence of *N*-acetylcysteine (NAC), during co-culture with mesothelial cell monolayers. NAC was able to completely inhibit the apoptotic activity of **1** on adhering HT-29 cells (**Figure 3C**). Furthermore, mixing of NAC and **1** before exposure to cancer cells completely inactivated the apoptotic activity of **1** on cancer cells (not shown) indicating that **1**

is more than likely binding covalently to glutathione and perhaps other thiol-containing molecules. When amide analog **4b** was incubated with glutathione, the formation of the sulfinic acid elimination product as well as glutathione-conjugated acrylonitrile was observed. Unfortunately, attempts to identify acrylonitrile-conjugated protein from cell studies failed to identify a similar conjugate. These and our previous studies show the importance of the Michael acceptor functional acrylonitrile group to the anti-cancer activity of **1** and its active analogs.

To determine if glutathione depletion may be involved in the apoptotic mechanism of **1** and its active analogs, the apoptotic activity of buthionine-sulfoximine (BSO), an irreversible inhibitor of γ-glutamylcysteine synthetase, which is the rate-limiting enzyme for glutathione (GSH) synthesis, was tested. BSO did decrease GSH levels in HT29 cells within 4 hours (**Figure 3D**), but did not affect cancer cell viability when cultured on mesothelial cell monolayers (**Figure 3E**). These results suggested that diminution of intracellular GSH levels per se was unlikely to be involved in the apoptotic activity of **1** and its active analogs during cell adhesion.

In order to validate the *in vitro* co-culture assay results, an *in vivo* murine peritoneal carcinomatosis model was performed using A2780 and SKOV3 ovarian cancer cells. Athymic 5-week-old female nu/nu mice were randomized to intraperitoneal injection with 5 mg/kg of test compound or an equal volume of vehicle (4:1 PEG400 : EtOH) four hours before IP injection of 1.5 million A2780-luc or 2 million SKOV3-luc ovarian cancer cells (n = 7 for each arm). The strategy to dose prior to injection of cancer cells was designed to mimic the shedding of tumor cells during intraperitoneal surgery with concomitant use of a chemotherapeutic lavage. Dosing

was continued every three days for 14 days total. The peritoneal tumor implants were examined weekly using a bioluminescence detection system that detects luciferase activity in the tumors (**Figure 4**).

For A2780 (**Figure 4A**) and SKOV3 (**Figure 4B**) ovarian cancer cells, significant inhibition of intraperitoneal implantation was observed in the groups treated with **1** and analogs **4b**, **6b, 13a** and **10e** compared with the control mice ($p<0.05$) which received vehicle only. Benzoxazole **6b** showed significantly greater inhibition of peritoneal carcinomatosis than compound **1** for A2780 but not SKOV-3 ovarian cancer cells. None of the other analogs showed significantly greater inhibition of peritoneal carcinomatosis than compound **1**. Some degree of weight loss was initially observed in all mice but this corrected over the 14-day treatment period.

A selection of the active analogs was assayed in a second *in vivo* model of pancreatic cancer metastasis. MIA PaCa-2 cells that stably expressed red fluorescent protein (RFP) were surgically implanted in the pancreatic tail. Surgical resection of the tumor 6-8 weeks post implantation was combined with IP treatment with analogs **4b**, **5d**, **6a**, **10e**, **1** or vehicle (4:1 PEG400:EtOH). Animals were treated at the time of surgery then on days 3, 5 and 7. After 8 weeks, local and metastatic recurrence was assessed via fluorescent imaging of the mice. Control mice treated with vehicle alone exhibited 67% recurrence (local in the remaining pancreas or metastatic to the peritoneum and mesenteric lymph nodes). Mice treated with **1** displayed a reduction in total recurrence to 38% of the study animals (Table 7). Benzyl amide

4b showed a strong reduction in recurrence, with only one of eighteen subjects exhibiting both local and metastatic recurrence. The remaining three analogs, phenyl oxadiazole **5d**, benzoxazole **6a** and the cyclohexyl-cyclobutane amide **10e**, displayed *in vivo* activity comparable or slightly less effective than **1**, with 40-50% recurrence either locally or metastatic.

CONCLUSIONS:

In these studies, we have shown that a variety of structural modifications to **1** are permitted while maintaining *in vitro* co-culture efficacy. *In vivo* efficacy in two models of cancer spread can be improved with incorporation of a benzyl amide or benzoxazole in place of a methyl group present in **1**. Activity in the co-culture assays is correlated with the overall polarity of the test compounds and compounds sufficiently more polar than **1** begin to lose activity. The requirement for more hydrophobic group substitutions for efficacy is likely related to cell penetration. Overall potency of active compounds in the co-culture assay as measured by EC_{50} ranged between 2 and 8 μM and doses of 5 mg/kg were found efficacious in two *in vivo* models of peritoneal carcinomatosis. Importantly, the requirement for DMSO for solubility of **1** and the active analogs of **1** was obviated by the use of polyethylene glycol and ethanol, which makes the clinical use of these drugs in humans more tenable.

Based on the structure and presumed covalent nature of our active analogs, we performed additional glutathione binding studies. Our studies show that the activity of **1** and its active analogs against cancer cells correlates directly with their ability to bind glutathione thus making it likely that these drugs work on cancer cells through covalent binding. While glutathione depletion has been shown to

cause apoptosis in cancer cells, treatment of HT-29 cells with various concentrations of buthionine sulphoximine did not induce apoptosis when they were allowed to adhere to mesothelial cell monolayers. However, pre-incubation of cancer cells with N-acetylcysteine inhibited the apoptotic activity of **1**, suggesting that **1** may bind other thiol-containing molecules.

Traditionally, covalent inhibitors have been shunned due to their broad reactivity (and subsequent off-target effects) and perceived toxicity in humans. However, an increasing proportion of clinically effective drugs, including carfilzomib, dimethyl fumarate, afatinib, and omeprazole, act through covalent binding of cellular targets and with acceptable safety profiles.[28] Covalent inhibitors allow sustained inhibition of cellular targets affording them potential advantages vital to cancer treatment: 1) lower and less frequent dosing, 2) dissociation of pharmacokinetics and pharmacodynamics since the latter is dependent on protein resynthesis, and 3) prevention of drug resistance due to longer target suppression.[30]

We previously showed that **1** and its active analogs rapidly diminish cellular c-FLIP protein, an important inhibitor of death receptor-mediated caspase activation.[19] Previous studies showed that c-FLIP protein is regulated by JNK-dependent activation of ITCH, a ubiquitin ligase, that targets c-FLIP for proteasomal degradation.[30] Activation of the JNK pathway by **1** suggests a potential mechanism for the inhibition of c-FLIP and a focus for future studies.

The first step leading to peritoneal carcinomatosis is the shedding or dislodgement of cancer cells from intra-abdominal cancers. Peritoneal seeding by cancer cells occurs in up to 35% of patients following curative resections of pancreatic cancers.[26] Iatrogenic seeding is a paradoxical event in that resection is the mainstay of treatment of localized intra-abdominal cancers. In fact, the detection of intraperitoneal cancer cells during the resection of intra-abdominal cancers portends a poor survival.[27-32]

While it can be difficult to know if occult peritoneal carcinomatosis is already present by the time some patients undergo curative resection of intra-abdominal cancers, there is evidence that this condition may be due to seeding following medical interventions. The most obvious examples are cancer seeding of port-sites used to remove cancers laparoscopically from the abdominal cavity.[33, 34] In one study, patients with nonmetastatic pancreatic cancer who all underwent neoadjuvant chemoradiation had either percutaneous radiologically guided or endoscopic ultrasound guided final needle aspirates of their pancreatic cancers. [35] At the end of treatment, all patients underwent restaging with computed tomography followed by attempted surgical resection. At the time of surgery, 16.3% and 2.2% of patients who underwent percutaneous or endoscopic needle biopsies, respectively, developed peritoneal carcinomatosis. However, other studies have not reflected an increased risk of peritoneal carcinomatosis in pancreatic cancer patients who underwent or did not undergo percutaneous of their cancers. [36] However, in a large randomized controlled trial, intraperitoneal chemotherapy was

shown to significantly increase patient survival following curative resection of ovarian cancer[34] suggesting that inhibition of peritoneal cancer spread may improve survival. The problem with most of these and other studies regarding this controversy is that the majority of studies is retrospective and small, and, show modest benefit making their clinical import questionable. A drug that specifically and rapidly inhibited implantation of cancer cells in the peritoneum at the time of curative resection would permit randomized controlled trials to resolve the controversy of whether or not biopsies or surgical resection of intra-abdominal cancers results in peritoneal seeding.

The two pre-clinical murine models used for these studies measured different outcomes and clinical scenarios. The ovarian cancer model simply tested the ability of the drugs to inhibit ovarian cancer cells from seeding the peritoneum. The pancreatic cancer model tested the ability of the drugs to inhibit local recurrence of pancreatic cancer at the site of resection as well as distant metastasis to lymph nodes and the liver following pancreatic cancer resection. For certain cancers such as pancreatic cancer, loco-regional recurrence after surgical cancer resection is a large clinical problem for which there is no effective therapy.

All lead analogs and **1** showed significant reductions in peritoneal carcinomatosis in the ovarian cancer mouse model. One analog, **6b**, was significantly more effective at inhibiting peritoneal carcinomatosis by A2780 ovarian cancer cells than **1**, thus

achieving the primary endpoint of designing and synthesizing an analog with greater potency than the parent compound.

For the pancreatic cancer mouse model, **4b** emerged as the most efficacious analog compared with vehicle and **1**. Although **4b** was significantly better at inhibiting peritoneal carcinomatosis from ovarian cancer cells than vehicle, it was not significantly more effective than **1**. These results may reflect tissue-specific differences for the various lead analogs.

The mechanism by which these drugs work appears be due to the ability of **1** to rapidly inhibit the expression of survival proteins that are overexpressed in cancer cells.[18, 19] One key intracellular protein target identified in our previous studies is c-FLIP, which is commonly overexpressed in cancer cells and capable of inhibiting death receptor-mediated apoptosis in cancer cells. [19] Investigations on how **1** and its active analogs inhibit c-FLIP are presently underway.

In summary, these results suggest that these unique agents could be administered in the immediate peri-operative period to inhibit the spread of intra-abdominal cancers following surgical resection of ovarian and pancreatic cancers. The pre-clinical cancer assays developed and utilized for this study may be useful in developing compounds that prevent the spread of cancer cells.

EXPERIMENTAL PROCEDURES

Synthetic Procedures and Analytical Data:

All reagents and solvents were obtained from commercial sources and used as received. All final products were purified to >95% purity as determined by HPLC and LCMS. Analytical HPLC was run using a Zorbax Eclipse XDB-C8 3.5 μm 4.6x75 mm column eluting with a mixture of acetonitrile and water containing 0.1% trifluoroacetic acid with a 5 minute gradient of 10-100%. LCMS results were obtained on either of two instruments. A Waters Acquity Ultra Performance LC with a Waters Aquity UPLC BEH C18 1.7 μm 2.1x50 mm column was paired with a Micromass-ZQ 2000 quadrupole mass spectrometer with electrospray ionization. Alternatively, an Agilent 1100 series HPLC with a Zorbax Eclipse XDB-C8 3.5 μm 2.1x30mm column was paired with a Bruker Esquire 2000 mass spectrometer with electrospray ionization. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Bruker Avance at 400 MHz and 100 MHz, respectively, in the solvent indicated with tetramethylsilane as an internal standard. High resolution LCMS was performed on a Waters Acquity UPLC using a Phenomenex Kinetex C18 1.7 μm 2.1x50 mm column and interfaced to a Waters Synapt G2 Q-TOF mass spectrometer using positive electrospray with Leu-enkephalin as a lock-mass standard. The mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) using a gradient of 2%B to 95%B in 3 minutes to elute the compounds. Preparative HPLC was performed on a Gilson HPLC using a Phenomenex Gemini NX 5 μm C18, 21.2x100 mm column with UV detection or a Waters HPLC using a Waters XBridge

PrepC8 5μm OBD 30x75 mm column with MS detection on a Micromass-ZQ 2000 quadrupole mass spectrometer with electrospray ionization. Gradient silica gel column chromatography was performed on a CombiFlash Companion or Rf instrument (Teledyne Isco Inc.).

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-phenyl-isobutyramide (**4a**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (300.0 mg, 1.074 mmol), aniline (0.098 mL, 1.07 mmol), EDCI (206 mg, 1.07 mmol) and HOBt (72.6 mg, 0.537 mmol) were dissolved in tetrahydrofuran (30 mL) and the reaction mixture was allowed to stir overnight at room temperature. The reaction mixture was poured over saturated sodium bicarbonate and organics were extracted with dichloromethane/ethyl acetate. Combined extracts were then dried over sodium sulfate, filtered and concentrated. The crude reaction mixture was purified on silica gel, eluting with hexanes/ethyl acetate to afford 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-phenyl-isobutyramide as a foam (171 mg, 45%). LCMS (ESI): 355 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.22 (s, 1H), 8.23 (d, 1H, J = 15.7 Hz), 7.90 (d, 2H, J = 7.8 Hz), 7.68 (d, 2H, J = 7.8 Hz), 7.57 (d, 2H, J = 7.7 Hz), 7.27 (m, 2H), 7.05 (m, 1H), 6.90 (d, 1H, J = 15.7 Hz), 1.60 (s, 6H); ^{13}C-NMR: (DMSO-d_6, 100 MHz) δ 173.7, 153.2, 149.2, 139.0, 135.5, 128.4, 128.2, 127.6, 123.5, 120.4, 114.6, 112.0, 47.9, 26.5.

N-Benzyl-2-[4-((E)-2-cyano-ethenesulfonyl)-phenyl]-isobutyramide (**4b**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (300.0 mg, 1.074 mmol), benzylamine (0.115 g, 1.07 mmol), EDCI (206 mg, 1.07 mmol) and

HOBt (72.6 mg, 0.537 mmol) were dissolved in tetrahydrofuran (30 mL) and the reaction mixture was allowed to stir overnight at room temperature. The reaction mixture was poured over saturated sodium bicarbonate and organics were extracted with dichloromethane/ethyl acetate. Combined extracts were then dried over sodium sulfate, filtered and concentrated. The crude reaction mixture was purified on silica gel, eluting with hexanes/ethyl acetate to afford N-Benzyl-2-[4-((E)-2-cyano-ethenesulfonyl)-phenyl]-isobutyramide as a white powder (173 mg, 44%). LCMS (ESI): 369 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.24 (d, 1H, J = 15.6 Hz), 8.06 (t, 1H, J = 5.6 Hz), 7.86 (d, 2H, J = 7.6 Hz), 7.63 (d, 2H, J = 7.6 Hz), 7.27 (m, 2H), 7.21 (m, 1H), 7.11 (d, 2H, J = 7.3 Hz), 6.90 (d, 1H, J = 15.6 Hz), 4.24 (d, 2H, J = 5.6 Hz), 1.52 (s, 6H); ^{13}C-NMR: (DMSO-d_6, 100 MHz) δ 174.6, 153.4, 149.2, 139.6, 135.3, 128.0, 127.9, 127.6, 126.7, 126.5, 114.5, 111.9, 46.6, 42.3, 26.3; HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{21}N_2O_3S$, 369.1273; found, 369.1274.

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-phenethyl-isobutyramide (**4c**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), phenylethylamine (33.7 µL, 0.268 mmol), EDCI (51.5 mg, 0.268 mmol) and HOBt (18.1 mg, 0.134 mmol) were dissolved in methylene chloride (7.5 mL) and the reaction mixture was allowed to stir overnight at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.2 mL of acetonitrile. Purification by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent afforded 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-phenethyl-isobutyramide as a white foam (39 mg, 38%). LCMS (ESI): 383 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.79 (d,

2H, J = 8.6 Hz), 7.49 (d, 2H, J = 8.6 Hz), 7.29-7.21 (m, 3H), 7.19 (d, 1H, J = 15.7 Hz), 7.07-7.02 (m, 2H), 6.55 (d, 1H, J = 15.7 Hz), 5.20-5.08 (m, 1H), 3.53-3.46 (m, 2H), 2.76 (t, 2H, J = 6.7 Hz), 1.54 (s, 6H); ^{13}C-NMR: ($CDCl_3$, 100 MHz) δ 175.2, 153.3, 148.9, 138.4, 135.6, 128.8, 128.67, 128.65, 127.9, 126.7, 113.3, 110.7, 47.4, 40.7, 35.3, 26.8; HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{23}N_2O_3S$, 383.1429; found, 383.1425.

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-((S)-1-phenyl-ethyl)-isobutyramide (**4d**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), (S)-1-phenylethylamine (34.3 μL, 0.268 mmol), EDCI (51.5 mg, 0.268 mmol) and HOBt (18.1 mg, 0.134 mmol) were dissolved in methylene chloride (7.5 mL) and the reaction mixture was allowed to stir overnight at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.3 mL of acetonitrile. Purification by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent afforded 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-((S)-1-phenyl-ethyl)-isobutyramide as an amorphous white solid (66 mg, 64%). LCMS (ESI): 383 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.84 (d, 2H, J = 7.6 Hz), 7.55 (d, 2H, J = 7.6 Hz), 7.37-7.25 (m, 3H), 7.24-7.14 (m, 3H), 6.53 (d, 1H, J = 15.7 Hz), 5.59 (d, 1H, J = 7.1 Hz), 5.16-5.06 (m, 1H), 1.61 (s, 3H), 1.60 (s, 3H), 1.43 (d, 3H, J = 6.8 Hz); HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{23}N_2O_3S$, 383.1429; found, 383.1430.

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-((R)-1-phenyl-ethyl)-isobutyramide (**4e**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), (R)-1-phenylethylamine (34.2 μL, 0.268 mmol), EDCI (51.5 mg, 0.268 mmol) and HOBt (18.1 mg, 0.134 mmol) were dissolved in methylene chloride (7.5 mL) and the reaction mixture was allowed to stir overnight at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.3 mL of acetonitrile. Purification by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent afforded 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-((R)-1-phenyl-ethyl)-isobutyramide as an amorphous white solid (69 mg, 67%). LCMS (ESI): 383 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.84 (d, 2H, J = 7.3 Hz), 7.54 (d, 2H, J = 7.3 Hz), 7.37-7.26 (m, 3H), 7.23-7.15 (m, 3H), 6.54 (d, 1H, J = 15.6 Hz), 5.68 (d, 1H, J = 6.8 Hz), 5.16-5.06 (m, 1H), 1.62 (s, 3H), 1.60 (s, 3H), 1.44 (d, 3H, J = 6.8 Hz); ^{13}C-NMR: ($CDCl_3$, 100 MHz) δ 174.4, 153.5, 148.9, 142.7, 135.7, 128.9, 128.7, 127.8, 127.6, 126.0, 113.3, 110.8, 49.1, 47.4, 26.9, 21.4; HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{23}N_2O_3S$, 383.1429; found, 383.1429.

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(4-methanesulfonyl-benzyl)-isobutyramide (**4f**)

4-Methanesulfonyl-benzylamine hydrochloride (59.5 mg, 0.268 mmol) was placed in methylene chloride (10 mL) and macroporous carbonate resin (3.16 mmol/g loading, 255 mg, 0.806 mmol) was added. The reaction was stirred at room temperature for 30 minutes and then filtered to remove the resin. The solution was

added to a vial containing 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), HOBt (18.1 mg, 0.134 mmol), and EDCI (51.5 mg, 0.268 mmol). The reaction was stirred at 40 °C overnight and was then concentrated under reduced pressure. The residue was taken up in 1.6 mL of DMSO and purified by preparative HPLC using a gradient of 10-45% acetonitrile:water both containing 0.1% TFA as the eluting solvent to afford 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(4-methanesulfonyl-benzyl)-isobutyramide as a white foam (33 mg, 28%). LCMS (ESI): 447 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.87 (d, 2H, J = 8.4 Hz), 7.80 (d, 2H, J = 8.2 Hz), 7.62 (d, 2H, J = 8.4 Hz), 7.32 (d, 2H, J = 8.2 Hz), 7.22 (d, 1H, J = 15.6 Hz), 6.57 (d, 1H, J = 15. 6 Hz), 5.99 (t, 1H, J = 5.6 Hz), 4.49 (d, 2H, J = 6.0 Hz), 3.03 (s, 3H), 1.66 (s, 6H).

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(4-sulfamoyl-benzyl)-isobutyramide (**4g**)

4-Aminomethyl-benzenesulfonamide hydrochloride (59.8 mg, 0.268 mmol) was placed in methylene chloride (10 mL) and macroporous carbonate resin (3.16 mmol/g loading, 255 mg, 0.806 mmol) was added. The reaction was stirred at room temperature for 30 minutes and then filtered to remove the resin. The solution was added to a vial containing 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), HOBt (18.1 mg, 0.134 mmol), and EDCI (51.5 mg, 0.268 mmol). The reaction was stirred at 40 °C overnight and then concentrated under reduced pressure. The residue was taken up in 1.6 mL of DMSO and purified by preparative HPLC using a gradient of 10-45% acetonitrile:water both containing 0.1% TFA as the eluting solvent to afford 2-[4-((E)-2-Cyano-ethenesulfonyl)-

phenyl]-N-(4-sulfamoyl- benzyl)-isobutyramide as a white foam (17 mg, 14%). LCMS = 448 (M+H); HNMR (d6-DMSO, 400 MHz) δ 8.24 (d, 1H, J = 15.6 Hz), 8.16 (t, 1H, J = 5.8 Hz), 7.88 (d, 2H, J = 8.4 Hz), 7.73 (d, 2H, J = 8.1 Hz), 7.64 (d, 2H, J = 8.4 Hz), 7.35-7.26 (m, 4H), 6.91 (d, 1H, J = 15.6 Hz), 4.28 (d, 2H, J = 5.8 Hz), 1.52 (s, 6H)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(2-methoxy-benzyl)-isobutyramide (**4h**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), 2-methoxy-benzylamine (34.7 μL, 0.268 mmol), EDCI (51.5 mg, 0.268 mmol) and HOBt (18.1 mg, 0.134 mmol) were dissolved in acetonitrile (1.0 mL) and the reaction mixture was allowed to stir for 2 hours at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.6 mL of DMSO. Purification by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent afforded 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(2-methoxy-benzyl)-isobutyramide (48 mg, 45%) as a white lyophilate. LCMS (ESI): 399 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.81 (d, 2H, J = 8.7 Hz), 7.53 (d, 2H, J = 8.7 Hz), 7.30-7.23 (m, 1H), 7.23-7.16 (m, 2H), 6.94-6.87 (m, 1H), 6.85-6.80 (m, 1H), 6.54 (d, 1H, J = 15.7 Hz), 5.89-5.80 (m, 1H), 4.38 (d, 2H, J = 5.8 Hz), 3.69 (s, 3H), 1.58 (s, 6H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 175.0, 157.4, 153.7, 148.9, 135.5, 129.9, 129.1, 128.7, 127.9, 125.7, 120.8, 113.2, 110.7, 110.3, 55.2, 47.5, 40.4,26.8; HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{23}N_2O_4S$, 399.1379; found, 399.1381.

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(3-methoxy-benzyl)-isobutyramide (**4i**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), 3-methoxy-benzylamine (34.7 μL, 0.268 mmol), EDCI (51.5 mg, 0.268 mmol) and HOBt (18.1 mg, 0.134 mmol) were dissolved in acetonitrile (1.0 mL) and the reaction mixture was allowed to stir for 2 hours at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.6 mL of DMSO. Purification by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent afforded 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(3-methoxy-benzyl)-isobutyramide (40 mg, 37%) as a white lyophilate. LCMS (ESI): 399 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.85 (d, 2H, J = 8.6 Hz), 7.61 (d, 2H, J = 8.6 Hz), 7.25-7.16 (m, 2H), 6.84-6.78 (m, 1H), 6.77-6.72 (m, 1H), 6.69-6.65 (m, 1H), 6.54 (d, 1H, J = 15.7 Hz), 5.57-5.48 (m, 1H), 4.38 (d, 2H, J = 5.7 Hz), 3.77 (s, 3H), 1.64 (s, 6H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 175.1, 159.9, 153.3, 148.9, 139.5, 135.8, 129.8, 128.9, 127.9, 119.7, 113.4, 113.3, 112.8, 110.7, 55.3, 47.5, 43.9, 26.9; HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{23}N_2O_4S$, 399.1379; found, 399.1378.

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-[4-(2-methoxy-ethoxymethyl)-phenyl]-isobutyramide (**4j**)

a) Potassium hydroxide (0.682 g, 10.3 mmol) was added to a suspension of p-nitrobenzylbromide (2.048 g, 9.480 mmol) in 2-methoxyethanol (20 mL, 254 mmol) and the mixture was heated in a vial at 105 °C. After 90 min, the mixture was cooled to room temperature. The mixture was poured into EtOAc (100 mL) and washed with water (3X10 mL), brine (20 mL) and dried over sodium sulfate. Concentration in vacuo after filtration gave an oil, which was purified on ISCO (25g preload

w/DCM, 80g column, 5-40% EtOAc:Hex). Pure fractions were combined and conc. to afford 1-(2-Methoxy-ethoxymethyl)-4-nitro-benzene (1.245 g, 62%) as an orange oil.

b) 1-(2-Methoxy-ethoxymethyl)-4-nitro-benzene (200 mg, 0.947 mmol was dissolved in methanol (9.5 mL) and 5% platinum on carbon, sulfided (0.5%) (36.9 mg, 0.00947 mmol) was added. The reaction was hydrogenated at 50 psi for 60 minutes and then filtered through celite to remove the catalyst. The filtrate was then concentrated under reduced pressure to afford 4-(2-Methoxy-ethoxymethyl)-phenylamine as a yellow oil.

c) 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), 4-(2-Methoxy-ethoxymethyl)-phenylamine (48.7 mg, 0.268 mmol), EDCI (51.5 mg, 0.268 mmol) and HOBt (18.1 mg, 0.134 mmol) were dissolved in methylene chloride (7.5 mL) and the reaction mixture was allowed to stir overnight at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.6 mL of DMSO. Purification by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent to afford 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-[4-(2-methoxy-ethoxymethyl)-phenyl]-isobutyramide as an orange oil (57 mg, 48%). LCMS = 465 (M+Na); HNMR (d6-DMSO, 400 MHz) δ 9.23 (s, 1H), 8.23 (d, 1H, J = 15.7 Hz), 7.89 (d, 2H, J = 8.6 Hz), 7.67 (d, 2H, J = 8.6 Hz), 7.54 (d, 2H, J = 8.6 Hz), 7.22 (d, 2H, J = 8.6 Hz), 6.0 (d, 1H, J = 15.7 Hz), 4.40 (s, 2H), 3.53-3.43 (m, 4H), 3.24 (s, 3H), 1.60 (s, 6H).

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(4-fluoro-benzyl)-isobutyramide (**4k**) 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), 4-fluoro-benzylamine (30.5 μL, 0.268 mmol), EDCI (51.5 mg, 0.268 mmol) and HOBt (18.1 mg, 0.134 mmol) were dissolved in methylene chloride (7.5 mL) and the reaction mixture was allowed to stir overnight at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.2 mL of acetonitrile. Purification by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent afforded 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(4-fluoro-benzyl)-isobutyramide as a white foam (34 mg, 34%). LCMS (ESI): 387 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.85 (d, 2H, J = 7.4 Hz), 7.59 (d, 2H, J = 7.4 Hz), 7.30-7.24 (m, 1H), 7.20-7.10 (m, 2H), 7.04-6.95 (m, 2H), 6.55 (d, 1H, J = 15.6 Hz), 5.63-5.50 (m, 1H), 4.37 (d, 2H, J = 5.6 Hz), 1.63 (s, 6H); ^{13}C-NMR: (DMSO-d_6, 100 MHz) δ 171.4, 161.2 (d, J = 242 Hz), 149.2, 147.5, 136.0, 135.9, 131.7, 128.9, 128.4 (d, J = 8 Hz), 114.9 (d, J = 21 Hz), 114.6, 112.0, 42.1, 30.4, 15.0.

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(3-fluoro-benzyl)-isobutyramide (**4l**) 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), 3-fluorobenzylamine (30.6 μL, 0.268 mmol), EDCI (51.5 mg, 0.268 mmol) and HOBt (18.1 mg, 0.134 mmol) were dissolved in acetonitrile (1.0 mL) and the reaction mixture was allowed to stir for 2 hours at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.6 mL of DMSO. Purification by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent afforded 2-[4-

((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(3-fluoro-benzyl)-isobutyramide (17 mg, 16%) as a white lyophilate. LCMS = 387 (M+H); HNMR (CDCl3, 400 MHz) δ 7.87 (d, 2H, J = 8.7 Hz), 7.61 (d, 2H, J = 8.7 Hz), 7.32-7.23 (m, 1H), 7.20 (d, 1H, J = 15.6 Hz), 7.00-6.91 (m, 2H), 6.84-6.78 (m, 1H), 6.55 (d, 1H, J = 15.6 Hz), 5.62-5.49 (m, 1H), 4.40 (d, 2H, J = 5.9 Hz), 1.64 (s, 6H).

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(2-fluoro-benzyl)-isobutyramide (**4m**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), 2-fluorobenzylamine (30.6 μL, 0.268 mmol), EDCI (51.5 mg, 0.268 mmol) and HOBt (18.1 mg, 0.134 mmol) were dissolved in acetonitrile (1.0 mL) and the reaction mixture was allowed to stir for 2 hours at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.6 mL of DMSO. Purification by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent afforded 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(2-fluoro-benzyl)-isobutyramide (40 mg, 38%) as a white lyophilate. LCMS = 387 (M+H); HNMR (CDCl3, 400 MHz) δ 7.83 (d, 2H, J = 8.7 Hz), 7.56 (d, 2H, J = 8.7 Hz), 7.31-7.22 (m, 2H), 7.19 (d, 1H, J = 15.6 Hz), 7.12-7.06 (m, 1H), 7.05-6.98 (m, 1H), 6.54 (d, 1H, J = 15.7 Hz), 5.69-5.54 (m, 1H), 4.44 (d, 2H, J = 5.9 Hz), 1.60 (s, 6H).

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(4-pyrazol-1-yl-benzyl)-isobutyramide (**4n**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), 4-pyrazol-1-yl-benzylamine (46.5 mg, 0.268 mmol), EDCI (51.5 mg, 0.268

mmol) and HOBt (18.1 mg, 0.134 mmol) were dissolved in methylene chloride (7.5 mL) and the reaction mixture was allowed to stir overnight at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.2 mL of acetonitrile. Purification by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent afforded 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(4-pyrazol-1-yl-benzyl)-isobutyramide trifluoroacetic acid salt as a pale-yellow solid (33 mg, 28%), mp = 47-53 °C. LCMS = 435 (M+H); HNMR (CDCl3, 400 MHz) δ 7.92-7.83 (m, 3H), 7.80-7.76 (m, 1H), 7.64-7.57 (m, 4H), 7.30-7.25 (m, 2H), 7.21 (d, 1H, J = 15.6 Hz), 6.55 (d, 1H, J = 15.6 Hz), 6.52-6.47 (m, 1H), 5.73-5.63 (m, 1H), 4.44 (d, 2H, J = 5.7 Hz), 1.64 (s, 6H).

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(2,2-difluoro-ethyl)-isobutyramide (**4o**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (100.0 mg, 0.3580 mmol), 2,2-difluoro-ethylamine (0.0290 g, 0.358 mmol), EDCI (68.6 mg, 0.358 mmol) and HOBt (24.2 mg, 0.179 mmol) were dissolved in tetrahydrofuran (10 mL) and the reaction mixture was allowed to stir overnight at room temperature. The reaction mixture was poured over saturated sodium bicarbonate and organics were extracted with dichloromethane/ethyl acetate. Combined extracts were then dried over sodium sulfate, filtered and concentrated. The crude reaction mixture was purified via preparative HPLC to afford 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(2,2-difluoro-ethyl)-isobutyramide as a white foam (40 mg, 32%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.89 (d, 2H, J = 7.6 Hz), 7.60 (d, 2H, J = 7.6 Hz), 7.23 (d, 1H, J = 15.7 Hz), 6.57 (d, 1H, J = 15.7 Hz), 5.84 (tt, 1H, J = 4.0, 56 Hz),

5.46 (m, 1H), 3.61 (m, 2H), 1.62 (s, 6H); ^{13}C-NMR: ($CDCl_3$, 100 MHz) δ 176.1, 152.7, 148.8, 136.1, 129.0, 127.9, 113.24, 113.20 (t, J = 240 Hz), 110.9, 47.6, 42.0 (t, J = 26 Hz), 26.9; HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{17}F_2N_2O_3S$, 343.0928; found, 343.0928.

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-((S)-2-oxo-azepan-3-yl)-isobutyramide (**4p**)

(S)-3-Amino-azepan-2-one hydrochloride (44.2 mg, 0.268 mmol) was placed in methylene chloride (10 mL) and macroporous carbonate resin (3.16 mmol/g loading, 255 mg, 0.806 mmol) was added. The reaction was stirred at room temperature for 30 minutes and then filtered to remove the resin. The solution was added to a vial containing 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), HOBt (18.1 mg, 0.134 mmol), and EDCI (51.5 mg, 0.268 mmol). The reaction was stirred at 40 °C overnight and was then concentrated under reduced pressure. The residue was taken up in 1.6 mL of DMSO and purified by preparative HPLC using a gradient of 10-45% acetonitrile:water both containing 0.1% TFA as the eluting solvent to afford 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-((S)-2-oxo-azepan-3-yl)-isobutyramide (60 mg, 57%) as a white foam. LCMS (ESI): 390 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.23 (d, 1H, J = 15.6 Hz), 7.90-7.84 (m, 1H), 7.86 (d, 2H, J = 8.5 Hz), 7.69 (d, 2H, J = 6.5 Hz), 7.23 (d, 1H, J = 6.2 Hz), 6.90 (d, 1H, J = 15.6 Hz), 4.41-4.30 (m, 1H), 3.23-3.10 (m, 1H), 3.10-2.97 (m, 1H), 1.93-1.82 (m, 1H), 1.82-1.69 (m, 2H), 1.69-1.55 (m, 1H), 1.51 (s, 6H), 1.41-1.26 (m, 1H), 1.25-1.10 (m, 1H).

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(3-phenyl-prop-2-ynyl)-isobutyramide (**4q**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (0.254 g, 0.909 mmol), 3-phenyl-prop-2-ynylamine hydrochloride (0.272 g, 1.62 mmol), EDCI (0.225 g, 1.18 mmol) and HOBt (0.019 g, 0.14 mmol) were combined in acetonitrile (6.0 mL). After 1h, 4-methylmorpholine (0.100 mL, 0.909 mmol) was added, due to only trace conversion to the amide. After 4h, the mixture was concentrated in vacuo to ~1 mL, DMSO was added (1 mL) and the mixture was filtered. Purification of the filtrate by preparative mass-directed HPLC (25-75% acetonitrile:water, both containing 0.1% TFA) afforded 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(3-phenyl-prop-2-ynyl)-isobutyramide (165 mg; 46%) after partial evaporation in vacuo followed by lyophilization. LCMS (ESI): m/z = 393 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.22 (d, 1H, J = 15.6 Hz), 8.08 (t, 1H, J = 5.5 Hz), 7.86 (d, 2H, J = 8.7 Hz), 7.64 (d, 2H, J = 8.7 Hz), 7.37 (m, 5H), 6.90 (d, 1H, J = 15.6 Hz), 4.08 (d, 2H, J = 5.5 Hz), 1.51 (s, 6H); HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{21}N_2O_3S$, 393.1273; found, 393.1274.

N-Benzyl-2-[4-((E)-2-cyano-ethenesulfonyl)-phenyl]-N-methyl-isobutyramide (**4r**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), N-methyl-benzylamine, (34.6 µL, 0.268 mmol), EDCI (51.5 mg, 0.268 mmol) and HOBt (18.1 mg, 0.134 mmol) were dissolved in methylene chloride (7.5 mL) and the reaction mixture was allowed to stir overnight at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.2 mL of acetonitrile. Purification by mass-directed HPLC using a gradient of 25-

75% acetonitrile:water both containing 0.1% TFA as the eluting solvent afforded N-Benzyl-2-[4-((E)-2-cyano-ethenesulfonyl)-phenyl]-N-methyl-isobutyramide as a white foam (33 mg, 32%). LCMS (ESI): 383 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz, mixture of rotamers) δ 7.92-7.76 (m, 2H), 7.49 (d, 2H, J = 8.0 Hz), 7.40-7.01 (m, 6H), 6.55 (d, 1H, J = 14.7 Hz), 4.72-3.94 (m, 2H), 3.02-2.31 (m, 3H), 1.63 (s, 6H).

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(4-fluoro-benzyl)-N-methyl-isobutyramide (**4s**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), (4-fluoro-benzyl)-methyl-amine (37.4 mg, 0.268 mmol), EDCI (51.5 mg, 0.268 mmol) and HOBt (18.1 mg, 0.134 mmol) were dissolved in acetonitrile (1.0 mL) and the reaction mixture was allowed to stir for 2 hours at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.6 mL of DMSO. Purification by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent afforded 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(4-fluoro-benzyl)-N-methyl-isobutyramide (24 mg, 22%) as a white lyophilate. LCMS = 401 (M+H); HNMR (CDCl3, 400 MHz) δ 7.84 (d, 2H, J = 8.6 Hz), 7.46 (d, 2H, J = 8.6 Hz), 7.32-7.13 (m, 3H), 7.06-6.96 (m, 2H), 6.55 (d, 1H, J = 15.7 Hz), 4.68-3.79 (m, 2H), 3.00-2.24 (m, 3H), 1.61 (s, 6H).

(E)-3-[4-(1,1-Dimethyl-2-oxo-2-piperidin-1-yl-ethyl) -benzenesulfonyl]-acrylonitrile (**4t**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (75.0 mg, 0.268 mmol), piperidine (26.6 µL, 0.268 mmol), EDCI (51.5 mg, 0.268 mmol) and HOBt

(18.1 mg, 0.134 mmol) were dissolved in methylene chloride (7.5 mL) and the reaction mixture was allowed to stir overnight at room temperature. The reaction was concentrated under reduced pressure and the residue was taken up in 1.6 mL of DMSO. Purification by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent afforded (E)-3-[4-(1,1-Dimethyl-2-oxo-2-piperidin-1-yl-ethyl) -benzenesulfonyl]-acrylonitrile as a yellow oil (25 mg, 27%). LCMS = 347 (M+H); HNMR (CDCl3, 400 MHz) δ 7.88 (d, 2H, J = 8.4 Hz), 7.49 (d, 2H, J = 8.4 Hz), 7.22 (d, 1H, J = 15.6 Hz), 6.56 (d, 1H, J = 15.6 Hz), 3.26-2.64 (m, 3H), 1.93-1.68 (m, 1H), 1.57 (s, 6H), 1.56-1.44 (m, 4H), 1.25-0.95 (m, 2H).

(E)-3-{4-[1-Methyl-1-(5-phenylamino-[1,3,4]oxadiazol-2-yl)-ethyl]-benzenesulfonyl}-acrylonitrile (**5a**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (101 mg, 0.362 mmol), EDCI (211 mg, 1.10 mmol) and 4-phenyl-3-thiosemicarbazide (65 mg, 0.39 mmol) were combined in methylene chloride (3.0 mL) and stirred overnight. The mixture was concentrated in vacuo, taken up in DMSO and purified by mass-directed HPLC (25-75% acetonitrile:water, TFA modifier). Following lyophilization, (E)-3-{4-[1-Methyl-1-(5-phenylamino-[1,3,4]oxadiazol-2-yl)-ethyl]-benzenesulfonyl}-acrylonitrile trifluoroacetic acid salt (35 mg, 19%) was isolated as an off-white lyophilate. LCMS (ESI): 395 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.38 (s, 1H), 8.23 (d, 1H, J = 15.6 Hz), 7.91 (d, 2H, J = 8.0 Hz), 7.66 (d, 2H, J = 8.0 Hz), 7.52 (d, 2H, J = 7.8 Hz), 7.32 (m, 2H), 6.98 (m, 1H), 6.92 (d, 1H, J = 15.6 Hz), 1.77 (s, 6H). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 163.7, 160.1, 151.9, 149.0, 138.6, 136.1, 129.0,

128.6, 127.5, 121.7, 116.8, 114.6, 112.3, 39.9, 26.9; HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{19}N_4O_3S$, 395.1178; found, 395.1179.

(E)-3-{4-[1-(5-Ethylamino-[1,3,4]oxadiazol-2-yl)-1-methyl-ethyl]-benzenesulfonyl}-acrylonitrile (**5b**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (105 mg, 0.376 mmol), EDCI (241 mg, 1.26 mmol) and 4-ethyl-3-thiosemicarbazide (60.0 mg, 0.503 mmol) were combined in methylene chloride (3 mL) and stirred overnight. The mixture was concentrated in vacuo, taken up in DMSO and purified by mass-directed HPLC (25-75% acetonitrile:water, TFA modifier). Following lyophilization, (E)-3-{4-[1-(5-Ethylamino-[1,3,4]oxadiazol-2-yl)-1-methyl-ethyl]-benzenesulfonyl}-acrylonitrile trifluoroacetic acid salt (19 mg, 11%) was isolated as a beige lyophilate. LCMS (ESI): 347 $(M+H)^+$; ^{1}H-NMR (acetone-d6, 400 MHz) δ 7.94 (d, 2H, J = 8.1 Hz), 7.89 (d, 1H, J = 15.7 Hz), 7.66 (d, 2H, J = 8.1 Hz), 6.83 (d, 1H, J = 15.7 Hz), 6.53 (br s, 1H), 3.30 (q, 2H, J = 7.2 Hz), 1.76 (s, 6H), 1.20 (t, 3H, J = 7.2 Hz).

(E)-3-{4-[1-(5-Cyclohexyl-[1,3,4]oxadiazol-2-yl)-1-methyl-ethyl]-benzenesulfonyl}-acrylonitrile

(**5c**)

a) 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (131 mg, 0.469 mmol), cyclohexanecarboxylic acid hydrazide (72.0 mg, 0.506 mmol), EDCI (119 mg, 0.621 mmol) and HOBt (7.0 mg, 0.052 mmol) were combined in acetonitrile (3.0 mL) and stirred at room temperature. After 4h, the mixture was diluted with DMSO (0.4 mL), filtered and purified by mass-directed HPLC (25-85% acetonitrile:Water, TFA modifier) to afford Cyclohexanecarboxylic acid N'-{2-[4-

((E)-2-cyano-ethenesulfonyl)-phenyl]-2-methyl-propionyl}-hydrazide (108 mg, 57%) as a white lyophilate. LCMS (ESI): 404 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.52 (s, 1H), 9.39 (s, 1H), 8.24 (d, 1H, J = 15.6 Hz), 7.86 (d, 2H, J = 8.7 Hz), 7.73 (d, 2H, J = 8.7 Hz), 6.90 (d, 1H, J = 15.6 Hz), 2.15 (m, 1H), 1.6-1.7 (m, 5H), 1.50 (s, 6H), 1.1-1.4 (m, 5H); HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{26}N_3O_4S$, 426.1463; found, 426.1466.

b) Cyclohexanecarboxylic acid N'-{2-[4-((E)-2-cyano-ethenesulfonyl)-phenyl]-2-methyl-propionyl}-hydrazide (52 mg, 0.13 mmol) was dissolved in 1,4-dioxane (2.0 mL) and phosphoryl chloride (0.200 mL, 2.14 mmol) was added and the mixture was heated at 80 °C for 2h, then cooled on ice before being added to 5 g of ice. The mixture was diluted with satd. sodium bicarbonate (10 mL) and extracted with DCM (3X10 mL). The organic extracts were washed with 1:1 brine:satd. sodium bicarbonate (10 mL), dried over sodium sulfate, filtered and concentrated in vacuo to afford (E)-3-{4-[1-(5-Cyclohexyl-[1,3,4]oxadiazol-2-yl)-1-methyl-ethyl]-benzenesulfonyl}-acrylonitrile (38 mg, 76%) as a white foam. LCMS (ESI): 386 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.85 (d, 2H, J = 8.7 Hz), 7.51 (d, 2H, J = 8.7 Hz), 7.20 (d, 1H, J = 15.7 Hz), 6.55 (d, 1H, J = 15.7 Hz), 2.84 (tt, 1H, J = 3.7, 11.3 Hz), 2.03 (m, 2H), 1.84 (s, 6H), 1.79 (m, 2H), 1.71 (m, 2H), 1.24-1.40 (m, 4H).

(E)-3-{4-[1-Methyl-1-(5-phenyl-[1,3,4]oxadiazol-2-yl)-ethyl]-benzenesulfonyl}-acrylonitrile (**5d**)

a) 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (0.262 g, 0.938 mmol), benzhydrazide (0.160 g, 1.17 mmol), EDCI (0.232 g, 1.21 mmol) and HOBt (0.019 g, 0.14 mmol) were dissolved in acetonitrile (6.0 mL) and the reaction

mixture was allowed to stir at room temperature for 4h. The mixture was poured into ethyl acetate (60 mL), washed with 1N HCl (20 mL) and brine (20 mL), dried over sodium sulfate, filtered and concentrated in vacuo. Benzoic acid N'-{2-[4-((E)-2-cyano-ethenesulfonyl)-phenyl]-2-methyl-propionyl}-hydrazide was used in the next step without purification.

b) Benzoic acid N'-{2-[4-((E)-2-cyano-ethenesulfonyl)-phenyl]-2-methyl-propionyl}-hydrazide (324 mg, 0.815 mmol) was dissolved in 1,4-dioxane (12.0 mL) and phosphoryl chloride (0.50 mL, 5.36 mmol) was added and the mixture was heated at 90 °C for 2h, then cooled to room temperature and diluted with ethyl acetate (50 mL). The mixture was washed with satd. $NaHCO_3$ (25 mL) and brine (10 mL), dried over sodium sulfate, filtered and concentrated in vacuo. The residue was dissolved in DCM and applied to a silica gel cartridge (5 g) and purified (silica gel 24g, 0-50% ethyl acetate:hexanes) to afford a foam after concentration of product containing fractions. This foam was dissolved in acetonitrile and water, and then lyophilized to give (E)-3-{4-[1-Methyl-1-(5-phenyl-[1,3,4]oxadiazol-2-yl)-ethyl]-benzenesulfonyl}-acrylonitrile as a white solid (223 mg, 72%). LCMS (ESI): m/z = 380 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.22 (d, 1H, J = 15.7 Hz), 7.97 (m, 2H), 7.90 (d, 2H, J = 8.7 Hz), 7.70 (d, 2H, J = 8.7 Hz), 7.55-7.65 (m, 3H), 6.91 (d, 1H, J = 15.7 Hz), 1.86 (s, 6H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 170.5, 164.5, 151.6, 149.0, 136.2, 132.0, 129.4, 128.6, 127.5, 126.6, 123.3, 114.5, 112.3, 40.2, 27.1; HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{18}N_3O_3S$, 380.1069; found, 380.1073.

(E)-3-{4-[1-Methyl-1-(5-methyl-benzoxazol-2-yl)-ethyl]-benzenesulfonyl}-acrylonitrile (**6a**)

a) 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (124 mg, 0.444 mmol), 2-amino-4-methyl-phenol (61.0 mg, 0.495 mmol) and HOBt (6.0 mg, 0.044 mmol) were combined in 1,2-dichloroethane (4.0 mL, 51 mmol), and EDCI (105 mg, 0.548 mmol) was added. Stir at room temperature in a vial for 2h, then the mixture was concentrated in vacuo, taken up in DMSO and purified by mass-directed HPLC to afford 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(2-hydroxy-5-methyl-phenyl)-isobutyramide as an oil.

b) 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(2-hydroxy-5-methyl-phenyl)-isobutyramide (42 mg, 0.11 mmol) was dissolved in acetic acid (2.0 mL, 35 mmol) and heated at 105 °C for 3d. The mixture was concentrated in vacuo, taken up in DMSO and purified by mass-directed HPLC (25-85% acetonitrile:water, TFA modifier) to afford (E)-3-{4-[1-Methyl-1-(5-methyl-benzoxazol-2-yl)-ethyl]-benzenesulfonyl}-acrylonitrile trifluoroacetic acid salt (15 mg, 28%) as an off-white lyophilate. LCMS (ESI): 367 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.21 (d, 1H, J = 15.6 Hz), 7.88 (d, 2H, J = 8.4 Hz), 7.63 (d, 2H, J = 8.4 Hz), 7.56 (s, 1H), 7.52 (d, 1H, J = 8.3 Hz), 7.18 (d, 1H, J = 8.3 Hz), 6.90 (d, 1H, J = 15.6 Hz), 2.42 (s, 3H), 1.84 (s, 6H).

(E)-3-[4-(1-Benzoxazol-2-yl-1-methyl-ethyl)-benzenesulfonyl]-acrylonitrile (**6b**)

a) 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (0.336 g, 1.20 mmol), 2-aminophenol (0.234 g, 2.14 mmol), EDCI (0.298 g, 1.56 mmol) and HOBt (0.162 g, 1.20 mmol) were dissolved in acetonitrile (11.1 mL) and the reaction mixture was allowed to stir at room temperature for 20h. The mixture was poured into ethyl acetate (200 mL), washed with 1N HCl (25 mL) and brine (50 mL), dried over sodium sulfate, filtered and concentrated in vacuo. The residue was applied to

a silica gel cartridge (24g) as a DCM solution and purified on silica gel (40g, 5-50% ethyl acetate:hexanes) to give 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(2-hydroxy-phenyl)-isobutyramide as an off-white solid (213 mg, 48%). LCMS (ESI): m/z = 371 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.70 (s, 1H), 8.32 (s, 1H), 8.24 (d, 1H, J = 15.7 Hz), 7.91 (d, 2H, J = 8.6 Hz), 7.77 (d, 2H, J = 8.6 Hz),7.70 (dd, 1H, J = 1.5, 8.0 Hz), 6.88-6.96 (m, 2H), 6.82 (dd, 1H, J = 1.5, 8.0 Hz), 6.75 (dd, 1H, J = 1.2 Hz, 7.5 Hz), 1.62 (s, 6H).

b) 2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-(2-hydroxy-phenyl)-isobutyramide (99 mg, 0.27 mmol) and methanesulfonic acid (0.5 mL, 7.7 mmol) were heated in 1,4-dioxane (3.0 mL) in a vial at 90 °C for 24h. The mixture was cooled to room temperature, partitioned between ethyl acetate (60 mL) and satd. sodium bicarbonate (60 mL). After separation, the aqueous was extracted with ethyl acetate (30 mL), then the combined organics were washed with brine (50 mL), dried over sodium sulfate, filtered and concentrated in vacuo. The residue was dissolved in DCM and applied to a silica gel cartridge (5 g) followed by purification by chromatography (silica gel 40g, 0-40% ethyl acetate:hexanes) to afford (E)-3-[4-(1-Benzoxazol-2-yl-1-methyl-ethyl)-benzenesulfonyl]-acrylonitrile as a white solid (55% yield). LCMS (ESI): m/z = 353 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.22 (d, 1H, J = 15.6 Hz), 7.89 (d, 2H, J = 8.7 Hz), 7.78 (m, 1H), 7.66 (m, 3H), 7.38 (m, 2H), 6.90 (d, 1H, J = 15.6 Hz), 1.86 (s, 6H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 170.2, 152.3, 150.4, 149.0, 140.4, 136.0, 128.5, 127.6, 125.2, 124.5, 119.8, 114.6, 112.2, 110.9, 41.9, 27.3.

(E)-3-{4-[1-(1H-Benzimidazol-2-yl)-1-methyl-ethyl]-benzenesulfonyl}-acrylonitrile (**6c**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-2-methyl-propionic acid (112 mg, 0.401 mmol), 1,2-benzenediamine (51.0 mg, 0.472 mmol), EDCI (98 mg, 0.51 mmol) and HOBt (27 mg, 0.20 mmol) were combined in tetrahydrofuran (6.0 mL) and stirred at room temperature. After 1h, methylene chloride (1.0 mL) was added to aid in solubility. After stirring overnight, acetic acid (0.65 mL, 11 mmol) was added and the mixture was heated to 70 °C until complete cyclization of the aniline-amide. The mixture was concentrated in vacuo, taken up in DMSO and purified by mass-directed HPLC to afford (E)-3-{4-[1-(1H-Benzimidazol-2-yl)-1-methyl-ethyl]-benzenesulfonyl}-acrylonitrile trifluoroacetic acid salt (45 mg, 24%) as an off-white lyophilate. LCMS (ESI): 352 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.23 (d, 1H, J = 15.7 Hz), 7.90 (d, 2H, J = 8.2 Hz), 7.65 (m, 4H), 7.39 (br s, 2H), 6.91 (d, 1H, J = 15.7 Hz), 1.89 (s, 6H).

(E)-3-{4-[1-Methyl-1-(5-methyl-oxazol-2-yl)-ethyl]-benzenesulfonyl}-acrylonitrile (**7**)

2-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-N-prop-2-ynyl-isobutyramide (53 mg, 0.17 mmol) and gold(III) chloride (5.0 mg, 0.016 mmol) were combined in acetonitrile-d_3 (1.0 mL), and the rate of isomerization was monitored by ^{1}H-NMR . Once complete (4 days) the mixture was filtered and purified by mass-directed HPLC and lyophilized to afford an oil. After reconcentration from acetonitrile the residue remained an oil, which slowly turned purple. The mixture was redissolved in acetonitrile, treated with macroporous carbonate resin (3 mmol/g, 0.1 g) for 30

min and filtered to give a clear solution and purple solids which were captured on the filter. The filtrate was diluted with water and lyophilized to afford (E)-3-{4-[1-Methyl-1-(5-methyl-oxazol-2-yl)-ethyl]-benzenesulfonyl}-acrylonitrile as an off-white wax (16 mg, 30%). LCMS (ESI): 317 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.22 (d, 1H, J = 15.6 Hz), 7.86 (d, 2H, J = 8.6 Hz), 7.54 (d, 2H, J = 8.6 Hz), 6.91 (d, 1H, J = 15.6 Hz), 6.79 (s, 1H), 2.22 (s, 3H), 1.71 (s, 6H).

1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclopropanecarboxylic acid 4-fluoro-benzylamide (**10a**)

a) To a solution of 1-(4-bromophenyl)cyclopropanecarboxylic acid (1.0 g, 4.15 mmol) in anhydrous THF (50 ml) under nitrogen at – 80 °C (ether/dry ice), was added slowly phenyl lithium (1.8 M in toluene, 2.77 mL, 4.98 mmol). After 5 min, to this mixture, n-BuLi (2.5 M in hexane, 2.2 mL, 5.4 mmol) was added. A cloudy suspension was slowly formed. Twenty minutes after BuLi addition, a stream of sulfur dioxide was bubbled through the mixture for 10 min. The reaction mixture was then allowed to warm up to room temperature and the solvent was removed in vacuo. The sulfinate residue was dissolved in water (10 ml), acetic acid (5 ml), and MeOH (15 ml), followed by addition of 2-chloroacrylonitrile (0.54 g, 6.23 mmol). The resulting mixture was stirred at room temperature overnight. The organic solvents were removed and the residue was diluted with 20 ml of water. The solution was adjusted to pH5-6 with sat. K_2HPO_4 aq. solution, then extracted with dichloromethane (2x30 ml) and dried over $MgSO_4$. After filtration, the filtrate was stirred with triethylamine (1.16 mL, 8.3 mmol) for 1 h. The solution was washed with 10% aq citric acid and brine, then dried over $MgSO_4$. The final product was

purified by flash column chromatography (silica gel, dichloromethane/ethyl acetate, gradient) to give 1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclopropanecarboxylic acid (112 mg, 10%) as a white solid. LCMS (ESI): m/z = 232 (M-CO2H)-; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 12.59 (s, 1H), 8.24 (d, 1H, J = 16.6 Hz), 7.83 (d, 2H, J = 8.5 Hz), 7.66 (d, 2H, J = 8.5 Hz), 6.91 (d, 1H, J = 16.6 Hz), 1.51 (m, 2H), 1.22 (m, 2H).

b) 1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclopropanecarboxylic acid (51 mg, 0.18 mmol), 4-fluoro-benzylamine (24.2 μL, 0.213 mmol), EDCI (42.3 mg, 0.221 mmol) and HOBt (29 mg, 0.21 mmol) were combined in acetonitrile (1.0 mL) and the reaction mixture was allowed to stir at room temperature for 60 min. The reaction was diluted with 0.5 mL of DMSO, filtered and purified by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent to afford 1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclopropanecarboxylic acid 4-fluoro-benzylamide (49 mg; 69%) as a white foam. LCMS (ESI): m/z = 385 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.23 (d, 1H, J = 15.6 Hz), 7.87 (m, 2H), 7.66 (m, 3H), 7.20 (m, 2H), 7.11 (m, 2H), 6.90 (d, 1H, J = 15.6 Hz), 4.18 (d, 2H, J = 6.0 Hz), 1.42 (dd, 2H, J = 4.2, 7.0 Hz), 1.08 (dd, 2H, J = 4.2, 7.0 Hz). LCMS = 385 (M+H); HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{18}FN_2O_3S$, 385.1022; found, 385.1024.

1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclopropanecarboxylic acid cyclohexylamide (**10b**)

1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclopropanecarboxylic acid (37 mg, 0.13 mmol), cyclohexylamine (18 μL, 0.16 mmol), EDCI (32 mg, 0.17 mmol) and

HOBt (23 mg, 0.17 mmol) were combined in acetonitrile (1.0 mL) and the reaction mixture was allowed to stir at room temperature for 60 min. The reaction was diluted with 0.3 mL of DMSO, filtered and purified by mass-directed HPLC using a gradient of 25-75% acetonitrile:water both containing 0.1% TFA as the eluting solvent to afford 1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclopropanecarboxylic acid cyclohexylamide (26 mg; 54%) as a white foam. LCMS (ESI): m/z = 359 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.23 (d, 1H, J = 15.6 Hz), 7.84 (m, 2H), 7.59 (m, 2H), 7.20 (d, 1H, J = 8.1 Hz), 6.90 (d, 1H, J = 15.6 Hz), 3.54 (m, 1H), 1.63 (m, 4H), 1.53 (m, 1H), 1.37 (dd, 2H, J = 4.4, 7.0 Hz), 1.05 (dd, 2H, J = 4.4, 7.0 Hz), 1.02 (m, 1H). LCMS = 359 (M+H); HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{23}N_2O_3S$, 359.1429; found, 359.1423.

1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclobutanecarboxylic acid phenylamide (**10c**)

a) To a solution of 1-(4-bromophenyl)cyclobutanecarboxylic acid (5.1 g, 20 mmol) in anhydrous THF (150 ml) under nitrogen at – 80 °C (ether/dry ice), was added slowly phenyl lithium (1.8 M in toluene, 13.9 mL, 25 mmol). After 5 min, to this mixture, n-BuLi (2.5 M in hexane, 10.4 mL, 26 mmol) was added. A cloudy suspension was slowly formed. Twenty minutes after BuLi addition, a stream of sulfur dioxide was bubbled through the mixture for 15 min. The reaction mixture was then allowed to warm up to room temperature and the solvent was removed in vacuo. The sulfinate residue was dissolved in water (30 ml), acetic acid (16 ml), and MeOH (40 ml), followed by addition of 2-chloroacrylonitrile (2.6 g, 30 mmol). The resulting mixture was stirred at room temperature overnight. The organic solvents

were removed and the residue was diluted with 20 ml of water. The solution was adjusted to pH5-6 with sat. K_2HPO_4 aq. solution, then extracted with dichloromethane (2x100 ml), dried over $MgSO_4$. After filtration, the filtrate was stirred with triethylamine (5.6 mL, 40 mmol) for 1 h. The solution was washed with 10% aq citric acid and brine, dried over $MgSO_4$. The final product was purified by flash column chromatography (silica gel, dichloromethane/ethyl acetate, gradient) to give 1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclobutanecarboxylic acid (1.52 g, 26%) as a white solid. LCMS (ESI): m/z = 246 $(M-CO_2H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 12.66 (s, 1H), 8.23 (d, 1H, J = 15.7 Hz), 7.88 (d, 2H, J = 8.5 Hz), 7.58 (d, 2H, J = 8.5 Hz), 6.91 (d, 1H, J = 15.7 Hz), 2.75 (m, 2H), 2.45 (m, 2H), 2.01 (m, 1H), 1.81 (m, 1H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 175.5, 151.4, 149.1, 135.5, 128.2, 127.8, 114.6, 112.0, 52.0, 31.8, 16.1.

b) 1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclobutanecarboxylic acid (58 mg, 0.20 mmol), HOBt (6 mg, 0.04 mmol), EDCI (51 mg, 0.27 mmol) and aniline (21 µL, 0.23 mmol) in acetonitrile (0.5 mL) were stirred in a vial at room temperature for 1h. The mixture was diluted with DMSO (0.2 mL), filtered and purified by mass-directed HPLC to afford 1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclobutanecarboxylic acid phenylamide (34 mg; 47%). LCMS (ESI): m/z = 367 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.55 (s, 1H), 8.20 (d, 1H, J = 15.6 Hz), 7.91 (d, 2H, J = 8.6 Hz), 7.77 (d, 2H, J = 8.7 Hz), 7.58 (m, 2H), 7.27 (m, 2H), 7.02 (m, 1H), 6.90 (d, 1H, J = 15.6 Hz), 2.90 (M, 2H), 2.53 (m, 2H), 1.86 (m, 2H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 172.6, 151.6, 149.1, 139.0, 135.5, 128.5, 128.3, 127.6, 123.5, 119.9, 114.6, 112.0, 54.1, 32.2, 15.7.

1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclobutanecarboxylic acid 4-chloro-benzylamide (**10d**)

1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclobutanecarboxylic acid (62 mg, 0.21 mmol) , HOBt (6 mg, 0.04 mmol), EDCI (52 mg, 0.27 mmol) and p-chlorobenzylamine (31 µL, 0.25 mmol) in acetonitrile (1.0 mL) were stirred in a vial at room temperature for 4h. The mixture was concentrated in vacuo, diluted with DMSO (0.6 mL), filtered and purified by mass-directed HPLC to afford 1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclobutanecarboxylic acid 4-chloro-benzylamide (44 mg; 50%). LCMS (ESI): m/z = 367 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.37 (t, 1H, J = 6.0 Hz), 8.25 (d, 1H, J = 15.8 Hz), 7.89 (d, 2H, J = 8.5 Hz), 7.64 (d, 2H, J = 8.5 Hz), 7.28 (d, 2H, J = 8.5 Hz), 7.06 (d, 2H, J = 8.5 Hz), 6.92 (d, 1H, J = 15.8 Hz), 4.19 (d, 2H, J = 6.0 Hz), 2.78 (M, 2H), 2.43 (m, 2H), 1.84 (m, 2H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 173.8, 152.2, 149.2, 138.7, 135.4, 131.1, 128.5, 128.13, 128.08, 127.5, 114.6, 112.0, 53.3, 41.7, 32.1, 16.0.

1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclobutanecarboxylic acid cyclohexylamide (**10e**)

1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclobutanecarboxylic acid (58 mg, 0.20 mmol), HOBt (6 mg, 0.04 mmol), EDCI (51 mg, 0.27 mmol) and cyclohexylamine (26 µL, 0.23 mmol) in acetonitrile (1.0 mL) were stirred in a vial at room temperature. After 5h, the mixture was poured into ethyl acetate, washed with 1N HCl, brine, dried over sodium sulfate, filtered and conc. The residue was purified to afford 1-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-cyclobutanecarboxylic acid cyclohexylamide (32 mg; 43%). LCMS (ESI): m/z = 373 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ

8.23 (d, 1H, J = 15.6 Hz), 7.86 (d, 2H, J = 8.5 Hz), 7.64 (d, 2H, J = 8.5 Hz), 7.51 (d, 1H, J = 8.0 Hz), 6.90 (d, 1H, J = 15.6 Hz), 3.46 (m, 1H), 2.74 (M, 2H), 2.35 (m, 2H), 1.78 (m, 2H), 1.57 (m, 5H), 1.0-1.2 (m, 5H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 172.8, 152.6, 149.2, 135.1, 128.0, 127.4, 114.6, 111.9, 53.2, 48.1, 32.3, 32.1, 25.2, 24.8, 15.8.

4-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-tetrahydro-pyran-4-carboxylic acid phenylamide (**10f**)

a) To a solution of 1-(4-bromophenyl)tetrahydro-2H-pyran-4-carboxylic acid (2.85 g, 10 mmol) in anhydrous THF (100 ml) under nitrogen at – 80 °C (ether/dry ice), was added slowly phenyl lithium (1.8 M in toluene, 7 mL, 12.5 mmol). After 5 min, to this mixture, n-BuLi (2.5 M in hexane, 5.2 mL, 13 mmol) was added. A cloudy suspension was slowly formed. Twenty minutes after BuLi addition, a stream of sulfur dioxide was bubbled through the mixture for 15 min. The reaction mixture was then allowed to warm up to room temperature and the solvent was removed in vacuo. The sulfinate residue was dissolved in water (15 ml), acetic acid (8 ml), and MeOH (20 ml), followed by addition of 2-chloroacrylonitrile (1.3 g, 15 mmol). The resulting mixture was stirred at room temperature overnight. The organic solvents were removed and the residue was diluted with 20 ml of water. The solution was adjusted to pH5-6 with sat. K_2HPO_4 aq. solution, then extracted with dichloromethane (2x50 ml), dried over $MgSO_4$. After filtration, the filtrate was stirred with triethylamine (2.8 mL, 20 mmol) for 1 h. The solution was washed with 10% aq citric acid and brine, dried over $MgSO_4$. The final product was purified by flash column chromatography (silica gel, dichloromethane/ethyl acetate, gradient) to give 4-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-tetrahydro-pyran-4-carboxylic

acid (0.87 g, 27%) as a white solid. LCMS (ESI): m/z = 276 (M-CO2H)+; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 13.00 (s, 1H), 8.23 (d, 1H, J = 15.7 Hz), 7.91 (d, 2H, J = 8.7 Hz), 7.74 (d, 2H, J = 8.7 Hz), 6.92 (d, 1H, J = 15.7 Hz), 3.82 (m, 2H), 3.48 (m, 2H), 2.40 (m, 2H), 1.88 (m, 2H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 174.3, 150.2, 149.0, 136.1, 128.4, 127.6, 114.6, 112.2, 64.6, 48.5, 33.7.

b) 4-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-tetrahydro-pyran-4-carboxylic acid (67 mg, 0.21 mmol), aniline (29 µL, 0.32 mmol), HOBt (7.0 mg, 0.052 mmol) and EDCI (50.0 mg, 0.261 mmol) were combiend in acetonitrile (3.0 mL) and stirred at room temperature. After 24h, the mixture was concentrated in vacuo, dissolved in DMSO and purified by mass-directed HPLC (25-75% acetonitrile:water, 0.1% TFA) to give 4-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-tetrahydro-pyran-4-carboxylic acid phenylamide (23 mg, 28%). LCMS (ESI): m/z = 397 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.38 (s, 1H), 8.22 (d, 1H, J = 15.7 Hz), 7.93 (d, 2H, J = 8.7 Hz), 7.76 (d, 2H, J = 8.7 Hz), 7.54 (m, 2H), 7.28 (m, 2H), 7.06 (m, 1H), 6.91 (d, 1H, J = 15.7 Hz), 3.81 (m, 2H), 3.55 (m, 2H), 2.60 (m, 2H), 2.00 (m, 2H).

4-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-tetrahydro-pyran-4-carboxylic acid 4-chloro-benzylamide (**10g**)

4-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-tetrahydro-pyran-4-carboxylic acid (72 mg, 0.22 mmol), p-chlorobenzylamine (45 µL, 0.37 mmol), HOBt (9.0 mg, 0.067 mmol) and EDCI (57 mg, 0.30 mmol) were combined in acetonitrile (6.0 mL, 110 mmol) and stirred at room temperature. After 2h, the mixture was concentrated in vacuo, dissolved in DMSO and purified by mass-directed HPLC (25-75% acetonitrile:water, 0.1% TFA) to give 4-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-

tetrahydro-pyran-4-carboxylic acid 4-chloro-benzylamide (32 mg 32%). LCMS (ESI): m/z = 445 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.36 (t, 1H, J = 5.9 Hz), 8.26 (d, 1H, J = 15.6 Hz), 7.90 (d, 2H, J = 8.6 Hz), 7.69 (d, 2H, J = 8.6 Hz), 7.27 (d, 2H, J = 8.4 Hz), 7.03 (d, 2H, J = 8.4 Hz), 6.93 (d, 1H, J = 15.6 Hz), 4.21 (d, 2H, J = 5.9 Hz), 3.76 (m, 2H), 3.46 (m, 2H), 2.52 (m, 2H), 1.90 (m, 2H); HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{22}ClN_2O_4S$, 445.0989; found, 445.0996.

4-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-tetrahydro-pyran-4-carboxylic acid cyclohexylamide (**10h**)

4-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-tetrahydro-pyran-4-carboxylic acid (61 mg, 0.19 mmol), cyclohexylamine (33 µL, 0.29 mmol), HOBt (25.6 mg, 0.190 mmol) and EDCI (46 mg, 0.24 mmol) were combined in acetonitrile (6.0 mL) and stirred at room temperature. After 4h, the mixture was concentrated in vacuo, dissolved in DMSO and purified by mass-directed HPLC (25-75% acetonitrile:water, 0.1% TFA) to give 4-[4-((E)-2-Cyano-ethenesulfonyl)-phenyl]-tetrahydro-pyran-4-carboxylic acid cyclohexylamide (39 mg, 51%). LCMS (ESI): m/z = 403 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.23 (d, 1H, J = 15.6 Hz), 7.87 (d, 2H, J = 8.6 Hz), 7.66 (d, 2H, J = 8.6 Hz), 7.45 (d, 1H, 8.0 Hz), 6.91 (d, 1H, J = 15.7 Hz), 3.77 (m, 2H), 3.56 (m, 1H), 3.44 (m, 2H), 1.83 (m, 2H), 1.62 (m, 6H), 1.12 (m, 6H); ^{13}C-NMR: (DMSO-d_6, 100 MHz)

δ 170.6, 152.0, 149.1, 135.6, 128.2, 127.3, 114.6, 112.1, 64.5, 48.5, 48.3, 34.0, 32.0, 25.2, 24.8;

HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{27}N_2O_4S$, 403.1692; found, 403.1695.

[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-acetic acid (**12**)

To a solution of 2-(4-bromo-2-tert-butylphenoxy)acetic acid (2.86 g, 10 mmol) in anhydrous THF (100 ml) under nitrogen at – 80 °C (ether/dry ice), was added slowly phenyl lithium (1.8 M in toluene, 7 mL, 12.5 mmol). After 5 min, to this mixture, n-BuLi (2.5 M in hexane, 5.2 mL, 13 mmol) was added. A cloudy suspension was slowly formed. Twenty minutes after BuLi addition, a stream of sulfur dioxide was bubbled through the mixture for 15 min. The reaction mixture was then allowed to warm up to room temperature and the solvent was removed in vacuo. The sulfinate residue was dissolved in water (15 ml), acetic acid (8 ml), and MeOH (20 ml), followed by addition of 2-chloroacrylonitrile (1.3 g, 15 mmol). The resulting mixture was stirred at room temperature overnight. The organic solvents were removed and the residue was diluted with 20 ml of water. The solution was adjusted to pH5-6 with sat. K_2HPO_4 aq. solution, then extracted with dichloromethane (2x50 ml) and dried over $MgSO_4$. After filtration, the filtrate was stirred with triethylamine (2.8 mL, 20 mmol) for 1 h. The solution was washed with 10% aq citric acid and brine, then dried over $MgSO_4$. The final product was purified by flash column chromatography (silica gel, dichloromethane/ethyl acetate, gradient) to give [2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-acetic acid (1.03 g, 32%) as a white solid. LCMS (ESI): m/z = 322 (M-H)-; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 13.19 (s, 1H), 8.22 (d, 1H, J = 16.6 Hz), 7.74 (dd, 1H, J = 2.3, 8.7 Hz), 7.68 (d, 1H, J = 2.3 Hz), 7.18 (d, 1H, J = 8.7 Hz), 6.84 (d, 1H, J = 16.6 Hz), 4.90 (s, 2H), 1.40 (s, 9H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 169.3, 161.4, 149.8, 139.0, 128.6, 128.5, 126.4, 114.7, 113.3, 110.7, 64.9, 34.9, 29.0.

2-[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-N-cyclohexyl-acetamide (**13a**)

[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-acetic acid (57 mg, 0.18 mmol), EDCI (41.0 mg, 0.214 mmol), HOBt (5.1 mg, 0.038 mmol) and cyclohexylamine (25 µL, 0.22 mmol) in acetonitrile (0.70 mL) was stirred at room temperature for 5h, then was filtered, rinsing the vial with DMSO (0.3 mL) and the filtrate was purified by mass-directed HPLC (25-95% acetonitrile:water, both containing 0.1% TFA) to afford 2-[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-N-cyclohexyl-acetamide (27 mg; 38%) as an off white lyophilate. LCMS (ESI): m/z = 405 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.22 (d, 1H, J = 15.6 Hz), 7.94 (d, 1H, J = 7.8 Hz), 7.74 (dd, 1H, J = 2.4, 7.8 Hz), 7.67 (d, 1H, J = 2.4 Hz), 4.67 (s, 2H), 3.60 (m, 1H), 1.6-1.8 (m, 4H), 1.55 (m, 1H), 1.38 (s, 9H), 1.1-1.3 (m, 5H).

2-[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-N-(3-methoxy-phenyl)-acetamide (**13b**)

[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-acetic acid (81 mg, 0.25 mmol), EDCI (58.3 mg, 0.304 mmol), HOBt (22 mg, 0.16 mmol) and 3-methoxyaniline (35 µL, 0.31 mmol) in acetonitrile (1.0 mL) was stirred at room temperature for 5h, then was filtered, rinsing the vial with DMSO (0.3 mL) and the filtrate was purified by preparative HPLC (25-95% acetonitrile:water, both containing 0.1% TFA) to afford 2-[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-N-(3-methoxy-phenyl)-acetamide (41 mg; 38%) as a white lyophilate. LCMS (ESI): 429 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6,): δ 10.26 (s, 1H), 8.22 (d, 1H, J = 15.6 Hz), 7.77 (dd, 1H, J = 2.4, 8.6 Hz), 7.69 (d, 1H, J = 2.4 Hz), 7.30 (m, 1H), 7.23 (m, 1H),

7.17 (d, 1H, J = 8.8 Hz), 7.12 (m, 1H), 6.84 (d, 1H, J = 15.6 Hz), 6.67 (dd, 1H, J = 2.5, 8.2 Hz), 4.95 (s, 2H), 3.73 (s, 3H), 1.42 (s, 9H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 165.3, 161.8, 159.6, 149.8, 139.6, 139.0, 129.7, 128.7, 128.5, 126.4, 114.8, 113.3, 111.4, 110.7, 109.1, 104.9, 67.1, 55.0, 35.0, 29.1; HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{25}N_2O_5S$, 429.1484; found, 429.1491.

2-[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-N-(4-fluoro-benzyl)-acetamide (**13c**)

[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-acetic acid (104 mg, 0.322 mmol), EDCI (77 mg, 0.40 mmol), HOBt (51 mg, 0.38 mmol) and 4-fluoro-benzylamine (45 µL, 0.40 mmol) in acetonitrile (2.0 mL) was stirred at room temperature for 1h, then was added to a silica gel pre-load cartridge (5 g) and chromatographed (silica gel 12g, 0-20% ethyl acetate:hexanes). Some acetonitrile eluted with product as well as some HOBt. The fraction containing product was concentrated, diluted with acetonitrile and DCM (3 mL each) and treated with macroporous carbonate resin for 1h. After filtration and concentration, the residue was dissolved in acetonitrile, diluted with water and then lyophilized to afford 2-[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-N-(4-fluoro-benzyl)-acetamide (64 mg; 46%) as an off-white lyophilate. LCMS (ESI): 431 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6,): δ 8.63 (t, 1H, J = 5.9 Hz), 8.23 (d, 1H, J = 15.6 Hz), 7.74 (dd, 1H, J = 2.4, 8.7 Hz), 7.68 (d, 1H, J = 2.4 Hz), 7.32 (m, 2H), 7.1-7.2 (m, 3H), 6.84 (d, 1H, J = 15.6 Hz), 4.79 (s, 2H), 4.33 (d, 2H, J = 5.9 Hz), 1.38 (s, 9H).

2-[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-N-(2-hydroxy-5-methyl-phenyl)-acetamide (**13d**)

[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-acetic acid (186 mg, 0.575 mmol), EDCI (138 mg, 0.719 mmol), HOBt (85.5 mg, 0.633 mmol), and 2-amino-4-methyl-phenol (0.142 g, 1.15 mmol) were combined in acetonitrile (6.0 mL) and stirred in a vial for 3h. The mixture was then added to ethyl acetate (100 mL) and washed with 1N HCl (15 mL) and brine (20 mL). The organics were dried over sodium sulfate, diluted with hexane (20 mL) and filtered through 4 mL silica gel (hexane bed), rinsing with 60 mL 1:1 ethyl acetate:hexane. The filtrate was concentrated in vacuo, dissolved in DCM and applied to a silica gel cartridge (5 g) and purified (silica gel 24g, 0-50%ethyl acetate:hexanes) to afford 2-[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-N-(2-hydroxy-5-methyl-phenyl)-acetamide (0.195 g; 79%). LCMS (ESI): m/z = 429 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.80 (s, 1H), 9.18 (s, 1H), 8.23 (d, 1H, J = 15.6 Hz), 7.89 (s, 1H), 7.77 (dd, 1H, J = 2.4, 8.7 Hz), 7.71 (d, 1H, J = 2.4 Hz), 7.25 (d, 1H, J = 8.7 Hz), 6.84 (d, 1H, J = 15.6 Hz), 6.75 (m, 2H), 5.00 (s, 2H), 2.19 (s, 3H), 1.44 (s, 9H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 165.2, 161.3, 149.7, 144.5, 139.0, 128.8, 128.7, 127.6, 126.5, 125.5, 124.7, 121.1, 114.7, 114.7, 113.7, 110.8 , 67.5, 34.9, 29.2, 20.5; HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{25}N_2O_5S$, 429.1484; found, 429.1490.

(E)-3-[3-tert-Butyl-4-(5-methyl-benzoxazol-2-ylmethoxy)-benzenesulfonyl]-acrylonitrile (**14**)

2-[2-tert-Butyl-4-((E)-2-cyano-ethenesulfonyl)-phenoxy]-N-(2-hydroxy-5-methyl-phenyl)-acetamide (0.178 g, 0.415 mmol) was heated in 1,4-dioxane (6.0 mL) with methanesulfonic acid (1.0 mL, 15 mmol) at 90 °C for 8h. The mixture was cooled to room temperature, stirred for 48 h, then was treated with additional

methanesulfonic acid (1 mL) and heated for 3h at 90 °C. The mixture was cooled and added into ethyl acetate (80 mL) and satd. $NaHCO_3$ (40 mL). The layers were separated and the organics washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated in vacuo. The residue was dissolved in DCM, applied to a silica gel cartridge (5 g) and purified (silica gel 40g, 0-40% ethyl acetate:hexanes) to afford (E)-3-[3-tert-Butyl-4-(5-methyl-benzoxazol-2-ylmethoxy)-benzenesulfonyl]-acrylonitrile (86 mg; 50%). LCMS (ESI): m/z = 411 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.23 (d, 1H, J = 15.7 Hz), 7.80 (dd, 1H, J = 2.4, 8.7 Hz), 7.72 (d, 1H, J = 2.4 Hz), 7.64 (d, 1H, J = 8.4 Hz), 7.61 (m, 1H), 7.46 (d, 1H, J = 8.7 Hz), 7.26 (dd, 1H, J = 1.2, 8.4 Hz), 6.85 (d, 1H, J = 15.7 Hz), 5.67 (s, 2H), 2.43 (s, 3H), 1.39 (s, 9H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 161.2, 161.0, 149.7, 148.6, 140.5, 139.2, 134.3, 129.1, 128.7, 126.8, 126.5, 119.9, 114.7, 113.9, 110.9, 110.5, 62.8, 34.9, 29.0, 20.9; HRMS-TOF-MS-ES+ (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{23}N_2O_4S$, 411.1379; found, 411.1382.

(E)-3-(3-tert-Butyl-benzenesulfonyl)-acrylonitrile (**13e**)

To a solution of 3-tert-butyl-bromobenzene (2.13 g, 10 mmol) in anhydrous THF (50 ml) under nitrogen at – 80 °C (ether/dry ice), was added slowly n-BuLi (2.5 M in hexane, 11 mmol). A cloudy suspension was slowly formed. Twenty minutes after BuLi addition, a stream of sulfur dioxide was bubbled through the mixture for 15 min. The reaction mixture was then allowed to warm up to room temperature and the solvent was removed under reduced pressure. The sulfinate residue was dissolved in water (15 ml), acetic acid (8 ml), and MeOH (20 ml), followed by addition of 2-chloroacrylonitrile (18 mmol). The resulting mixture was stirred at room temperature overnight. The organic solvents were removed in vacuo and the

residue was diluted with 20 ml of water. The solution was adjusted to pH5-6 with sat. K_2HPO_4 aq. solution, then extracted with dichloromethane (2x50 ml), dried over $MgSO_4$. After filtration, the filtrate was stirred with triethylamine (20 mmol) for 1 h. The solution was washed with 10% aq. citric acid and brine then dried over $MgSO_4$. The crude product was purified by flash column chromatography (silica gel, dichloromethane/ethyl acetate, gradient) to give (E)-3-(3-tert-Butyl-benzenesulfonyl)-acrylonitrile (0.76 g, 31%) as a white solid. LCMS (ESI): 250 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.89 (s, 1H), 7.76 (d, 1H, J = 7.7 Hz), 7.70 (d, 1H, J = 7.9 Hz), 7.55 (m, 1H), 7.23 (d, 1H, J = 15.9 Hz), 6.55 (d, 1H, J = 15.9 Hz), 1.37 (s, 9H).

(E)-3-(2-tert-Butyl-quinoline-6-sulfonyl)-acrylonitrile (**17**)

a) Potassium hydroxide (0.322 g, 5.74 mmol) and water (1.3 mL) were added to a mixture of 2-amino-5-bromo-benzaldehyde (0.933 g, 4.66 mmol) and 3,3-dimethyl-2-butanone (0.700 mL, 5.60 mmol) at room temperature. After 5 min, the mixture was heated at reflux for 3h. The mixture was diluted with water (40 mL) then extracted with DCM (40 mL, 2X20 mL). The organic extract was washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated in vacuo. The residue was purified (DCM load onto 24 g loading cartridge, then silica gel 40g, 0-20% ethyl acetate:hexanes) to afford 6-Bromo-2-tert-butyl-quinoline (0.97 g; 64%) as a yellow oil with small crystals forming.

b) To a solution of 6-bromo-2-tert-butylquinoline (1 g, 3.8 mmol) in anhydrous THF (50 ml) under nitrogen at – 80 °C (ether/dry ice), was added slowly n-BuLi (2.5 M in hexane, 2 mL, 4.94 mmol). A cloudy suspension was slowly formed. Twenty minutes

after BuLi addition, a stream of sulfur dioxide was bubbled through the mixture for 15 min. The reaction mixture was then allowed to warm up to room temperature, the solvent was removed under reduced pressure. The sulfinate residue was dissolved in water (10 ml), acetic acid (5 ml), and MeOH (15 ml), followed by addition of 2-chloroacrylonitrile (0.54 g, 6.23 mmol). The resulting mixture was stirred at room temperature overnight. The organic solvents were removed and the residue was diluted with 20 ml of water. The solution was adjusted to pH5-6 with sat. K_2HPO_4 aq. solution, then extracted with dichloromethane (2x50 ml) and dried over $MgSO_4$. After filtration, the filtrate was stirred with triethylamine (1.16 mL, 8.3 mmol) for 1 h. The solution was washed with 10% aq citric acid and brine, then dried over $MgSO_4$. The final product was purified by flash column chromatography (silica gel, dichloromethane/ethyl acetate, gradient) to give (E)-3-(2-tert-Butyl-quinoline-6-sulfonyl)-acrylonitrile (0.68 g, 59%) as a white solid. LCMS (ESI): m/z = 301 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.68 (d, 1H, J = 2.1 Hz), 8.61 (d, 1H, J = 8.8 Hz), 8.30 (d, 1H, J = 15.7 Hz), 8.21 (d, 1H, J = 8.9 H), 8.07 (dd, 1H, J = 2.2, 8.9 Hz), 7.92 (d, 1H, J = 8.8 Hz), 6.96 (d, 1H, J = 15.7 Hz), 1.43 (s, 9H).

2-[5-((E)-2-Cyano-ethenesulfonyl)-pyridin-2-yl]-N-cyclohexyl-isobutyramide (**18**)

a) To a suspension of 2-(5-bromopyridin-2-yl)-2-methylpropanoic acid (1.22 g, 5 mmol) 25 in 25 mL of DMF was added triethylamine (2.1 mL, 15 mmol) and cyclohexylamine (0.86 mL, 7.5 mmol) and followed by propylphosphonic anhydride (50% solution in DMF) (8.9 mL, 15 mmol) at room temperature under nitrogen. The resulting mixture was stirred at room temperature for 2h. The mixture was concentrated in vacuo, dissolved in DCM (100 mL) and brine (50 mL), separated and

the organic phase was concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, dichloromethane/acetone, gradient) to give 2-(5-Bromo-pyridin-2-yl)-N-cyclohexylisobutyramide (0.83 g, 51%) as a white solid.

b) To a solution of 2-(5-Bromo-pyridin-2-yl)-N-cyclohexyl-isobutyramide (0.8 g, 3.3 mmol) in anhydrous THF (1 00 ml) under nitrogen at -80 °C (ether/dry ice), was added slowly phenyl lithium in toluene 1.8 M (2.3 mL, 4.1 mmol). After 5 min, to this mixture, n-BuLi (2.5 Min hexane) (1.7 mL, 4.3 mmol) was added. A cloudy suspension was slowly formed. Twenty minutes after BuLi addition, a stream of sulfur dioxide was bubbled through the mixture for 10 min. The reaction mixture was then allowed to warm up to room temperature and the solvent was removed in vacuo. The sulfinate residue was dissolved in water (10 ml), acetic acid (5 ml), and MeOH (15 ml), followed by addition of 2-chloroacrylonitrile (0.43 g, 5.0 mmol). The resulting mixture was stirred at room temperature overnight. The organic solvents were removed and the residue was diluted with 20 ml of water. The solution was adjusted to pH5-6 with sat. K_2HP0_4 aq. solution, then extracted with dichloromethane (2x30 ml) and dried over MgS04. After filtration, the filtrate was stirred with triethylamine (0.92 mL, 6.6 mmol) for 1 h. The solution was washed with 10% aq citric acid and brine, then dried over $MgSO_4$. The final product was purified by flash column chromatography (silica gel, dichloromethane/Ethyl acetate, gradient) to give 2-[5-((E)-2-Cyano-ethenesulfonyl)-pyridin-2-yl]-N-cyclohexylisobutyramide (182 mg, 15%) as a white solid. LCMS (ESI): m/z = 362

(M+H)+; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.97 (dd, 1H, J = 0.6, 2.4 Hz), 8.28 (d, 1H, J = 15.7 Hz), 8.24 (dd, 1H, J = 2.4, 8.5 Hz), 7.62 (dd, 1H, J = 0.6, 8.5 Hz), 7.22 (d, 1H, J = 8.0 Hz), 6.97(d, 1H, J = 15.7 Hz), 3.55 (m, 1H), 1.66 (m, 4H), 1.53 (m, 1H), 1.49 (s, 6H), 1.0-1.2 (m, 5H).

Reagents for Biological Studies

D-Luciferin was purchased from Xenogen. Ketamine and xylazine HCl were purchased from the University of Utah Pharmacy. Steady-Glo Luciferase Assay kit was purchased from Promega. Buthionine-sulfoximine and N-acetylcysteine were purchased from Sigma. A glutathione assay kit (Trevigen) was used per the manufacturer's instructions to evaluate the concentration of GSH in the cancer cell lysates with or without **1** or its analogs.

Cell cultures and transduction

BxPC-3 and SU.86 pancreatic cancer cell lines were maintained in the Kuwada lab as previously described.[19] SKOV3 and A2780 ovarian cancer cell lines were purchased from American Type Culture Collection. SKOV3 cells were cultured in McCoy's 5A and A2780 cells in DMEM, both supplemented with 10% fetal bovine serum, glutamine, penicillin and streptomycin. All cells were cultured at 37˚C in a humidified incubator in a 5% CO2 atmosphere. The cancer cell lines were transduced with a retrovirus containing pLuc-puro, a constitutive luciferase reporter (a kind gift from Dr. Stephen Lessnick, University of Utah) and selected in G418-containing media. These cells were used in all in vitro and in vivo assays.

MiaPaCa-2 cells were transduced with a red fluorescent protein construct as previously described.[38]

Co-culture assay

An *in vitro* model of peritoneal tumor implantation was utilized in which pancreatic or ovarian cancer cells in suspension were allowed to adhere to confluent monolayers of mesothelial cells. Briefly, the vehicle or analogs were added to the cancer cell suspensions and then the mixture were overlaid on the mesothelial cell monolayers. After a 4-hour co-culture, the apoptotic cancer cells unable to adhere to the mesothelial cells were washed away from the wells, only the adherent and surviving cells were detected by bioluminescence in the presence of substrate (D-luciferin). The luminescence of cancer cells correlates with the number of surviving and adherent cancer cells present.

The effect of buthionine sulfoximine (BSO) on cancer cell viability was tested using the co-culture model. HT29 cells were pretreated with H_2O or various concentrations of BSO for 24 hours, or, with *N*-acetylcysteine (NAC) (680μM) for 30 minutes. The cancer cells were transiently suspended with trypsin then overlayed onto confluent monolayers of human mesothelial cells in 96-well plates in the presence of **1** and BSO or NAC for 5 hours. The remainder of the co-culture procedure was performed as described above.

Statistical analysis

Values are expressed as the mean ± Standard Error of the Mean (SEM). The significance of the difference between the control and each experimental test condition was analyzed by unpaired Student's t-test and a value of $p < 0.05$ was considered statistically significant.

Luciferase expressing murine peritoneal carcinomatosis model

The animal studies were approved by and performed in accordance with the University of Hawai'i Institutional Animal Care and Use Committee. Athymic 5-week-old female *nu/nu* mice were randomized to intraperitoneal (IP) treatment with either **1** (5mg/kg), test compounds (5mg/kg) or an equal volume of vehicle (80% polyethylene glycol 400 : 20% ethanol) 4 hours prior to IP injection of 1.5-2×10^6 suspended A2780 or SKOV3 cells. Cell viability was assessed prior to injection. The mice continued to receive vehicle or test compound (5mg/kg) IP three times a week for 14 days total. A mixture of D-luciferin (1.0 mg/20 mg mouse), ketamine HCl (2.0 mg/20 mg mouse) and xylazine HCl (0.20 mg/20 mg mouse) in PBS (pH 7.4) was injected IP just prior to bioluminescent imaging in order to anesthetize the mice and provide substrate for the luciferase expressing cancer cells. The peritoneal tumor implants were imaged with a Xenogen bioluminescent imaging system.

Red fluorescent protein stably expressing MIA PaCa-2cell line in vivo model

All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Utah Health Sciences Center. A total of 180

male nude mice (NCr-Nu/Nu _6 to 8 wk old; National Cancer Institute, Frederick, MD) were used in this experiment and maintained in a barrier facility on HEPA-filtered racks. Animals were individually identified using numbered ear tags. All experiments were conducted in a biological laminar flow hood, and all surgical procedures were conducted with strict adherence to aseptic technique.

The mice were anesthetized in an isoflurane chamber and anesthetic was continued on the operating table using a nose cone. The mice were prepped with 10% povidone-iodine on the left flank and draped in a sterile fashion. Using the Universal S3B operating microscope (Carl Zeiss, Inc., Thornwood, NY) for visualization, a small (1 cm) left subcostal incision was made, and the tail of the pancreas exteriorized gently. Then 1 X 10^6 pancreatic cancer cells were suspended in 0.125 mL of Extracel (Advanced BioMatrix) and loaded in a 1 mL syringe. Using a 25 gauge needle, the cells/sECM (soluble extracellular matrix) solution was injected into the tail of the pancreas. All mice were injected with MiaPaCa-2/RFP cells. Once hemostasis was confirmed, the tail of the pancreas was returned into the abdomen and the wound was closed as a single layer using interrupted 5-0 silk sutures (US Surgical, Norwalk, CT). The mice were re-anesthetized at 5 weeks after injection and underwent resection surgery. Resection involved midline laparotomy with mobilization of the tumor, ligation of the mid body of the pancreas proximal to the tumor using 4-0 silk suture and pancreatic transection distal to the ligature. Intraoperative RFP imaging was performed to verify that the mice had no evidence of disease after their resection, and single layer closure performed. Necropsy was performed 8 wk following pancreatic resection or sham surgery. RFP imaging was used to identify

locally recurrent and metastatic disease. Any mouse exhibiting ascites, cachexia, lethargy, or failure to thrive underwent necropsy at an earlier time point. At necropsy, laparotomy and sternotomy were performed to identify recurrent and metastatic disease. All organ sites of local recurrence and metastases were recorded.

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FIGURE LEGENDS

Figure 1 - Analogs of **1**. The diagram shows the strategies for modifiying the parent compound **1** to generate more effective anti-metastatic drugs.

Figure 2 - Apoptotic effects of test compounds. A2780 ovarian cancer cells were suspended and allowed to adhere in the presence of vehicle (DMSO) or test compound (5 μM in DMSO) for 4 hours. The cells were then collected, lysed and normalized for total protein concentration. The lysates were used to generate immunoblots of cleaved PARP and cleaved Caspase 3 which are indicators of apoptosis. An immunoblot of actin served as a loading control. A sulfide analog (**21**), which lacked potency in the co-culture assay, was used as a negative control.

Figure 3 – **1** and analogs of **1** were tested for their ability to bind glutathione (GSH) *in vitro* (**A**) and in HT-29 cancer cells *in vivo* (**B**). In this assay, the sulfhydryl group of glutathione reacts with DTNB (5,5'-dithiobis-2-nitrobenzoic acid, Ellman's reagent) and produces a yellow-colored 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB (between GSH and TNB) that is concomitantly produced, is reduced by Glutathione Reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to the concentration of GSH in the sample.

HT-29 cancer cells that stably expressed Firefly luciferase were incubated with **1** and analogs of **1** with vehicle (H20) or 680μM *N*-acetylcysteine (NAC) for 30

minutes. The HT-29 cells were then transiently suspended and co-cultured on mesothelial cell monolayers for 5 hours. The surviving HT-29 cells were detected by luciferase activity in a luminometer (**C**). Results are expressed as the mean light output and error bars represent standard errors of the mean.

HT-29 cells, that stably expressed Firefly luciferase, were treated with buthionine sulfoximine (BSO) for 24 hours. The cells were lysed and levels of cellular GSH determined (**D**) as in **3A-B** above. The effect of 24-hour pre-incubation of BSO on cancer cell viability was tested using the co-culture model (**E**) as in **3C**.

The structures of compounds **22** and **23** are shown (**F**).

Figure 4 - 1 and test compounds inhibit peritoneal carcinomatosis by of ovarian cancer cells. The mice were pre-treated intraperitoneally (IP) with 5mg/kg active analog or vehicle. Four hours later, 1.5 X 10^6 A2780-luciferase or 2 X 10^6 SKOV3-luciferase ovarian cancer cells were injected into each mouse IP. The treatment with vehicle or 5mg/kg active analog was continued three times a week for 14 days total. The intraperitoneal tumor implants were detected and quantified using a bioluminescence imager following IP D-luciferin administration. The graphs show abdominal bioluminescent output of the mice inoculated with A2780-luc cells (**3A**) or SKOV-3-luc (**3B**) cells. The columns represent the average tumor burden (bioluminescence) for the mice in each treatment arm and the error bars, the standard error of the means. Figures **3C** and **3D** show the imaging data for A2780-luc cells when treated with vehicle or benzoxazole **6b** on day 14 respectively.

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Abbreviations Used:

c-FLIP - cellular - FLICE Inhibitory Protein; IP – Intraperitoneal; ITCH - itchy E3 ubiquitin protein ligase; RFP – Red Fluorescent Protein

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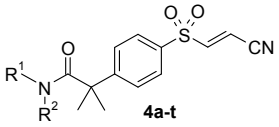
TABLES

Table 1. Biological data for **1** versus amide analogs from Series 1



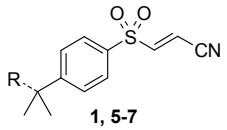
4a-t

Cmpd	R1	R2	Efficacy of compounds by co-culture assay			
			SU.86	BxPC-3	A2780	SKOV3
1	-	-	0.13±0.01	0.07±0.03	0.04±0.00	0.32±0.02
4a	Ph	H	0.52±0.02	0.22±0.02	0.17±0.00	0.55±0.04
4b	Bn	H	0.21±0.03	0.16±0.02	0.10±0.00	0.52±0.02
4c	$PhCH_2CH_2$	H	0.29±0.02	0.15±0.02	0.10±0.01	0.56±0.1
4d	(S)-$PhCH(CH_3)$	H	0.32±0.01	0.27±0.03	0.13±0.00	0.67±0.03
4e	(R)-$PhCH(CH_3)$	H	0.21±0.01	0.21±0.03	0.10±0.02	0.64±0.05
4f	4-$CH_3SO_2C_6H_4CH_2$	H	1.09±0.09	0.99±0.03	0.95±0.04	0.78±0.05
4g	4-$H_2NSO_2C_6H_4CH_2$	H	1.11±0.03	1.00±0.09	1.00±0.07	0.96±0.03
4h	2-$MeOC_6H_4CH_2$	H	0.21±0.00	0.07±0.01	0.01±0.00	0.27±0.00
4i	3-$MeOC_6H_4CH_2$	H	0.30±0.04	0.09±0.02	0.03±0.01	0.25±0.02
4j	4-$CH_3O(CH_2)_2O$-$CH_2C_6H_4$	H	0.66±0.08	0.40±0.04	0.26±0.05	0.60±0.06
4k	4-$FC_6H_4CH_2$	H	0.18±0.02	0.23±0.01	0.11±0.01	0.60±0.03
4l	3-$FC_6H_4CH_2$	H	0.24±0.02	0.17±0.01	0.05±0.01	0.30±0.02

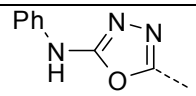
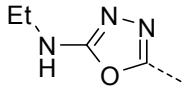
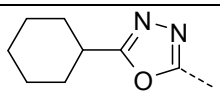
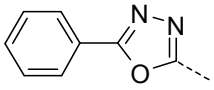
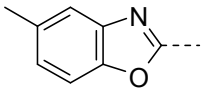
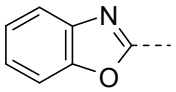
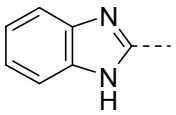
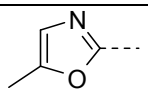
4m	2-$FC_6H_4CH_2$	H	0.20±0.02	0.15±0.01	0.03±0.01	0.30±0.01
4n	4-(pyrazol-1-yl)-$C_6H_4CH_2$	H	0.49±0.02	0.48±0.03	0.34±0.02	0.84±0.05
4o	F_2CHCH_2	H	0.81±0.02	0.82±0.03	0.86±0.03	1.13±0.01
4p	(*S*)-3-amino-caprolactam	H	0.97±0.04	0.82±0.05	0.84±0.01	0.85±0.01
4q	$PhC{\equiv}C\text{-}CH_2$	H	0.50±0.03	0.14±0.01	0.07±0.01	0.40±0.01
4r	Bn	Me	0.24±0.02	0.12±0.02	0.06±0.00	0.50±0.03
4s	4-$FC_6H_4CH_2$	Me	0.23±0.01	0.11±0.02	0.02±0.01	0.22±0.02
4t	$-(CH_2)_5-$		0.51±0.00	0.25±0.00	0.08±0.01	0.51±0.06

[a]The final concentration of all compounds was defined at 10 μM. Luminescence of cancer cells = living cell number. Efficacy = Luminescence of cancer cells treated with analog/luminescence of cancer cells treated with DMSO; the data are presented as a mean±SEM, n=3.

Table 2. Biological data for heterocyclic amide isosteres from Series 2

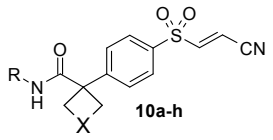


1, 5-7

Cmpd	R	Efficacy of compounds by co-culture assay			
		SU.86	BxPC-3	A2780	SKOV3
1	Me	0.13±0.01	0.07±0.03	0.04±0.00	0.32±0.02
5a		0.62±0.01	0.39±0.03	0.22±0.00	0.65±0.02
5b		0.96±0.02	1.02±0.02	0.86±0.01	1.12±0.03
5c		0.27±0.02	0.13±0.02	0.02±0.00	0.28±0.04
5d		0.45±0.05	0.24±0.01	0.06±0.02	0.25±0.02
6a		0.20±0.03	0.09±0.01	0.10±0.01	0.17±0.10
6b		0.29±0.02	0.24±0.04	0.01±0.00	0.17±0.03
6c		1.04±0.03	1.01±0.06	1.07±0.01	1.10±0.03
7		0.37±0.04	0.09±0.00	0.03±0.00	0.26±0.02

[a]The final concentration of all compounds was defined at 10 μM. Luminescence of cancer cells = living cell number. Efficacy = Luminescence of cancer cells treated with analog/luminescence of cancer cells treated with DMSO; the data are presented as a mean±SEM, n=3.

Table 3. Biological data for cyclic amide analogs from Series 3

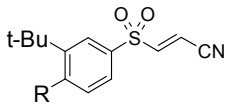


10a-h

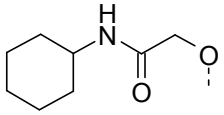
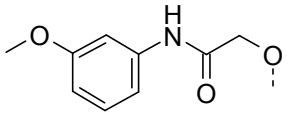
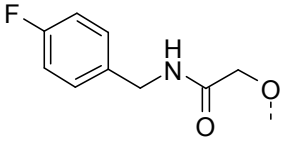
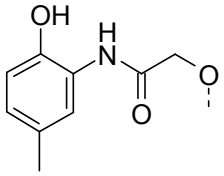
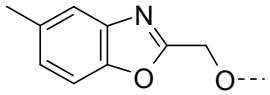
Cmpd	R, X	Efficacy of compounds by co-culture assay			
		SU.86	BxPC-3	A2780	SKOV3
10a	4-fluorobenzyl X = direct bond	0.64±0.04	0.34±0.02	0.06±0.01	0.43±0.02
10b	cyclohexyl X = direct bond	0.34±0.04	0.28±0.04	0.09±0.01	0.23±0.02
10c	phenyl X = CH_2	0.35±0.01	0.12±0.02	0.17±0.03	0.39±0.05
10d	4-chlorobenzyl X = CH_2	0.36±0.02	0.21±0.01	0.13±0.01	0.30±0.02
10e	cyclohexyl X = CH_2	0.32±0.03	0.18±0.04	0.09±0.01	0.33±0.1
10f	phenyl X = CH_2OCH_2	0.92±0.03	0.85±0.04	0.87±0.02	0.97±0.06
10g	4-chlorobenzyl X = CH_2OCH_2	0.66±0.04	0.30±0.02	0.46±0.02	0.71±0.05
10h	cyclohexyl X = CH_2OCH_2	1.00±0.09	0.67±0.06	1.12±0.08	0.88±0.06

[a]The final concentration of all compounds was defined at 10 μM. Luminescence of cancer cells = living cell number. Efficacy = Luminescence of cancer cells treated with analog/luminescence of cancer cells treated with DMSO; the data are presented as a mean±SEM, n=3.

Table 4. Biological data for cyclic phenyl ether series

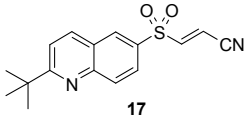
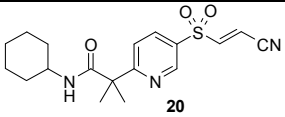


12, 13a-e, 14

Cmpd	R	Efficacy of compounds by co-culture assay			
		SU.86	BxPC-3	A2780	SKOV3
12	HO_2CCH_2O-	0.97±0.05	0.81±0.04	1.05±0.04	0.92±0.01
13a		0.21±0.02	0.09±0.03	0.01±0.00	0.19±0.02
13b		0.37±0.01	0.17±0.02	0.03±0.01	0.29±0.02
13c		0.28±0.03	0.10±0.01	0.02±0.00	0.23±0.02
13d		0.38±0.02	0.10±0.00	0.05±0.01	0.24±0.03
14		0.56±0.05	0.24±0.04	0.09±0.00	0.36±0.05
13e	H[b]	0.13±0.01	0.08±0.03	0.10±0.01	0.35±0.01

[a]The final concentration of all compounds was defined at 10 μM. Luminescence of cancer cells = living cell number. Efficacy = Luminescence of cancer cells treated with analog/luminescence of cancer cells treated with DMSO; the data are presented as a mean±SEM, n=3. [b]Prepared from 3-bromo-*t*-butylbenzene

Table 5. Biological data for heteroaryl series

Cmpd	Efficacy of compounds by co-culture assay			
	SU.86	BxPC-3	A2780	SKOV3
 17	0.45±0.01	0.22±0.01	0.04±0.01	0.35±0.02
 20	0.61±0.06	0.16±0.03	0.16±0.02	0.58±0.04

[a]The final concentration of all compounds was defined at 10 μM. Luminescence of cancer cells = living cell number. Efficacy = Luminescence of cancer cells treated with analog/luminescence of cancer cells treated with DMSO; the data are presented as a mean±SEM, n=3.

Table 6. Dose-response data for active compounds.

Cmpd	EC_{50} (μM)[a] by co-culture assay[19]			
	SU.86	BxPC-3	A2780	SKOV3
1	5.9±0.2	3.7±0.1	2.6±0.1	5.0±0.4
4b	7.4±0.2	3.7±0.0	3.0±0.1	8.7±0.8
5d	6.2±0.3	3.1±0.1	2.6±0.1	5.7±0.3
6a	7.3±0.1	3.5±0.1	2.1±0.1	5.6±0.4
6b	6.5±0.1	3.1±0.1	2.2±0.1	5.4±0.4
10e	8.0±0.6	3.6±0.2	3.1±0.1	7.4±0.6
13a	6.6±0.1	3.4±0.2	2.6±0.0	6.1±0.1
13d	8.0±0.3	3.8±0.2	2.4±0.2	6.6±0.1

[a]EC_{50} values are reported as the average of three separate determinations.

Table 7. In vivo efficacy of select analogs in a pancreatic resection model

ID	n	Local Recurrence	Metastases	Total Recurrence	%-Local Recurrence	%-Metastases	%-Total Recurrence
1	16	5	5	6	31	31	38
4b	18	1	1	1	6	6	6
5d	11	5	0	5	45	0	45
6a	10	4	1	5	40	10	50
10e	10	3	2	4	30	20	40
Vehicle	15	9	6	10	60	40	67

FIGURES

Figure 1

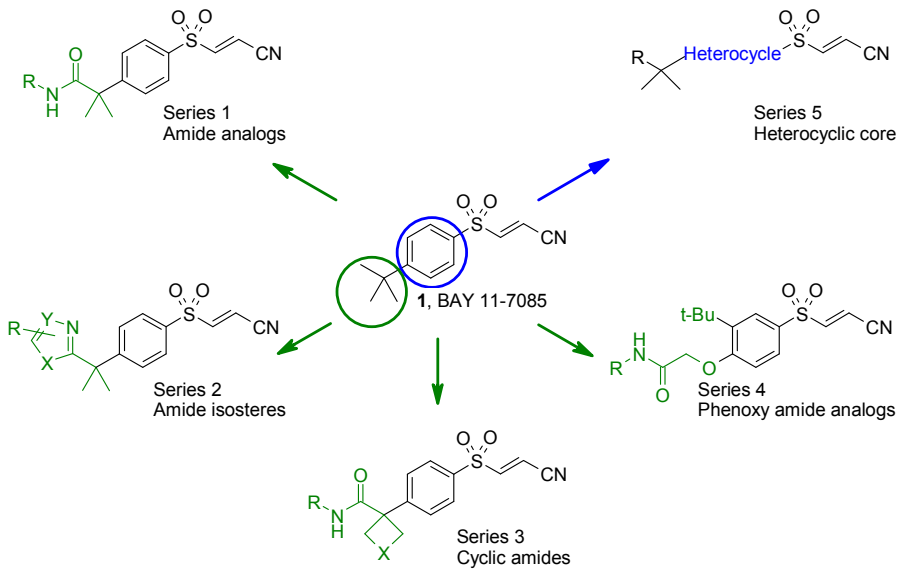


Figure 2

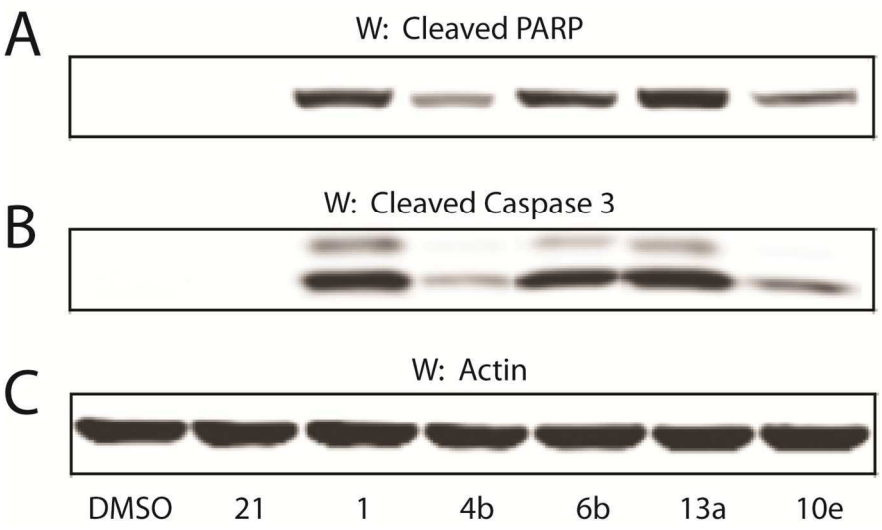

A
W: Cleaved PARP
B
W: Cleaved Caspase 3
C
W: Actin
DMSO
21
1
4b
6b
13a
10e

Figure 3

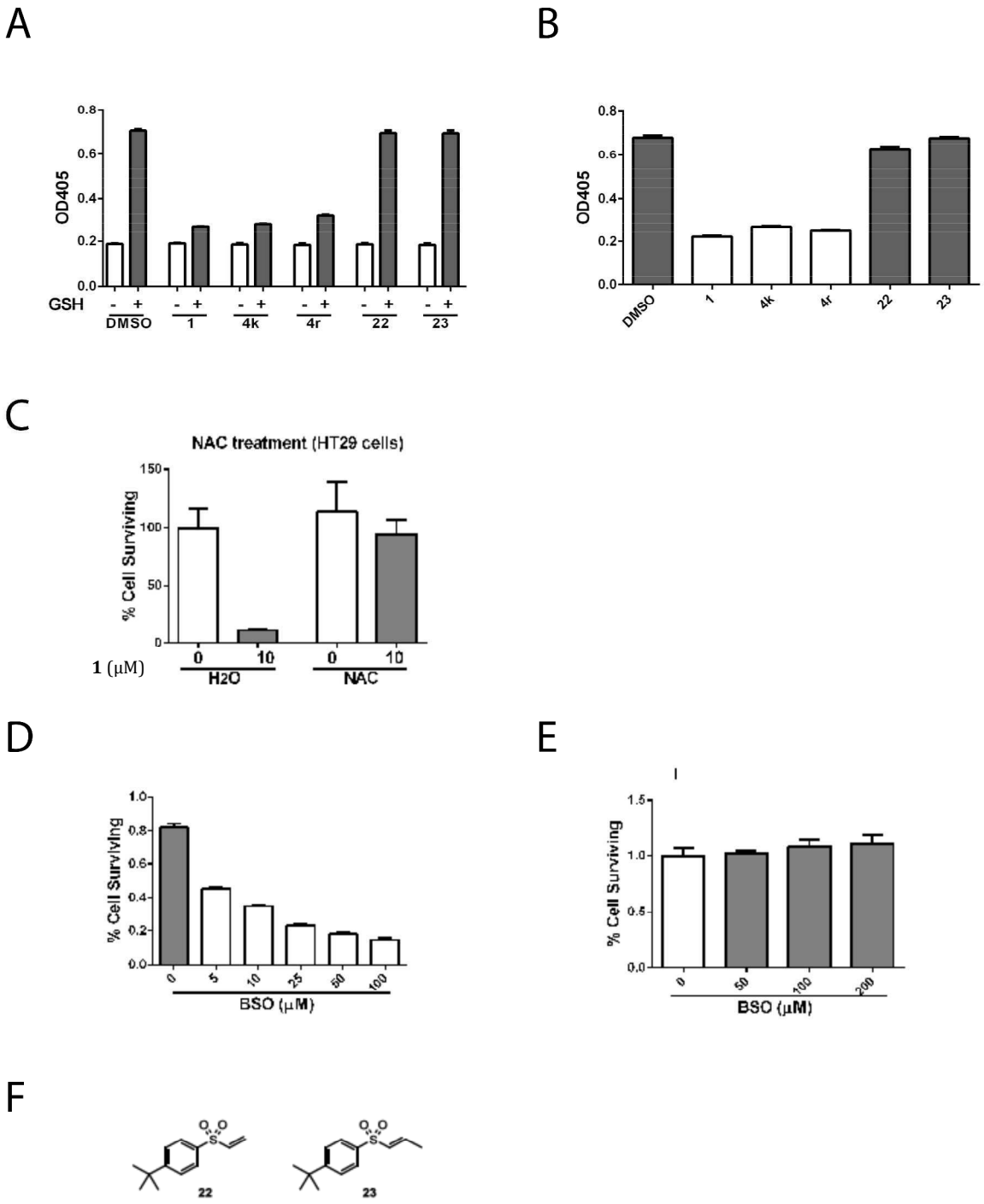

A
OD405
GSH
DMSO
1
4k
4r
22
23
B
OD405
DMSO
1
4k
4r
22
23
C
NAC treatment (HT29 cells)
% Cell Surviving
1 (μM)
H2O
NAC
D
% Cell Surviving
BSO (μM)
E
% Cell Surviving
BSO (μM)
F
22
23

FIGURE 4

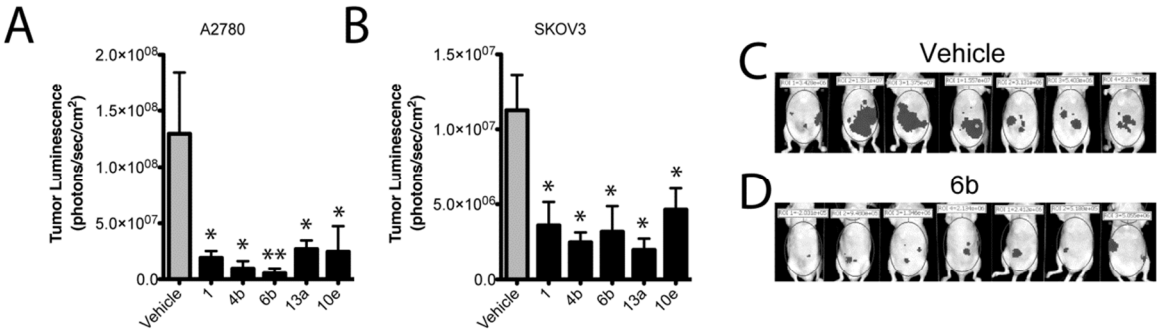

A
A2780
Tumor Luminescence (photons/sec/cm²)
2.0×10⁰⁸
1.5×10⁰⁸
1.0×10⁰⁸
5.0×10⁰⁷
0.0
Vehicle
1
4b
6b
13a
10e
B
SKOV3
Tumor Luminescence (photons/sec/cm²)
1.5×10⁰⁷
1.0×10⁰⁷
5.0×10⁰⁶
0.0
Vehicle
1
4b
6b
13a
10e
C
Vehicle
D
6b

TABLE OF CONTENTS GRAPHIC: